NordiQC

Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, September 19th – 21st 2016

Optimization of antibodies, selection, protocols and controls

Unknown primary tumour - I

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IHC – Protocols and controls for UPT I

1' panel	Recommendable clones (conc.)	Less successful clones (conc.)	RTU "plug and play" giving optimal result
CK-PAN	mAb AE1/AE3 mAb AE1/AE3/5D3 mAb BS5	mAb MNF116 mAb C-11 mAb Lu-5 mAb KL1*	Dako: mAb AE1/AE3 VMS: mAb AE1/AE3/PCK26
CD45	mAb 2B11+PD7/26 mAb X1699		Dako: mAb 2B11+PD7/26 Leica: mAb X1699
S100(B)	pAbs (e.g. Z0311)	mAb 15E2E2	Dako: pAb (GA504)
VIM	mAb V9 mAb 3B4 rmAb SP20		Dako: mAb V9 VMS: mAb V9

* Discontinued

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1' panel	Positive tissue control HE	Positive tissue control LE	Negative tissue control NE
CK-PAN	Liver: Epithelial cells of bile ducts Tonsil: Squamous epithelial cells	Liver: Hepatocytes Tonsil: Squamous epithelial cells	Liver: Stroma Tonsil: Lymphocytes
CD45	Tonsil: T- and B-cells	Liver: Kupffer cells	Tonsil: Epithelial cells Liver: Hepatocytes
S100(B)	Appendix: Nerves	Tonsil: Germinal centre dendritic cells*	Appendix: Epithelial cells
VIM	Appendix: Endothelial cells	Appendix: Intra-epithelial T-cells	Appendix: Epithelial cells

* pAb reacting with S100 A1, most likely

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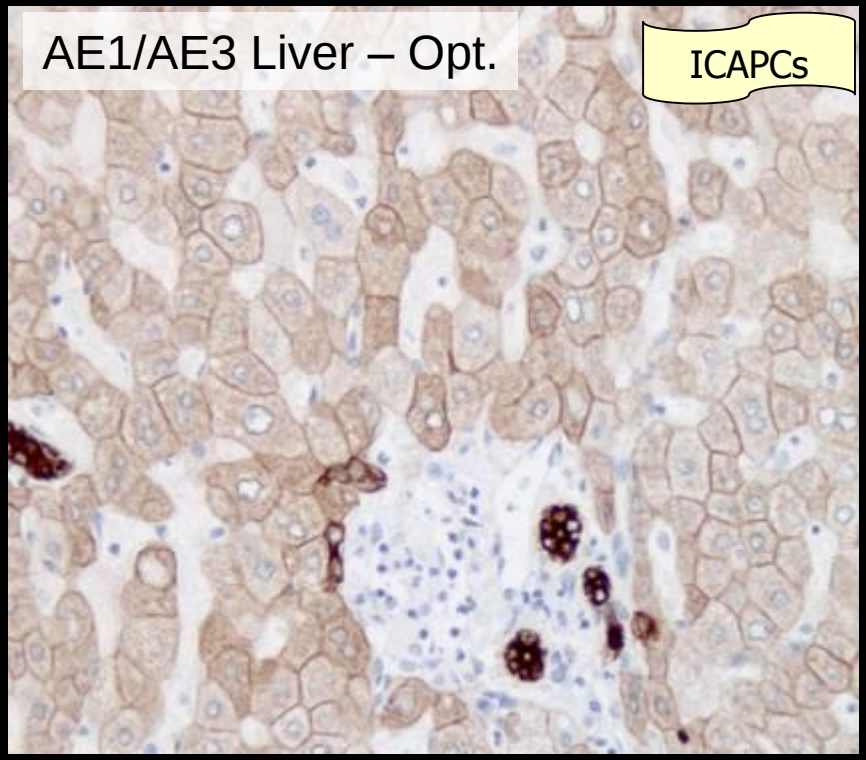
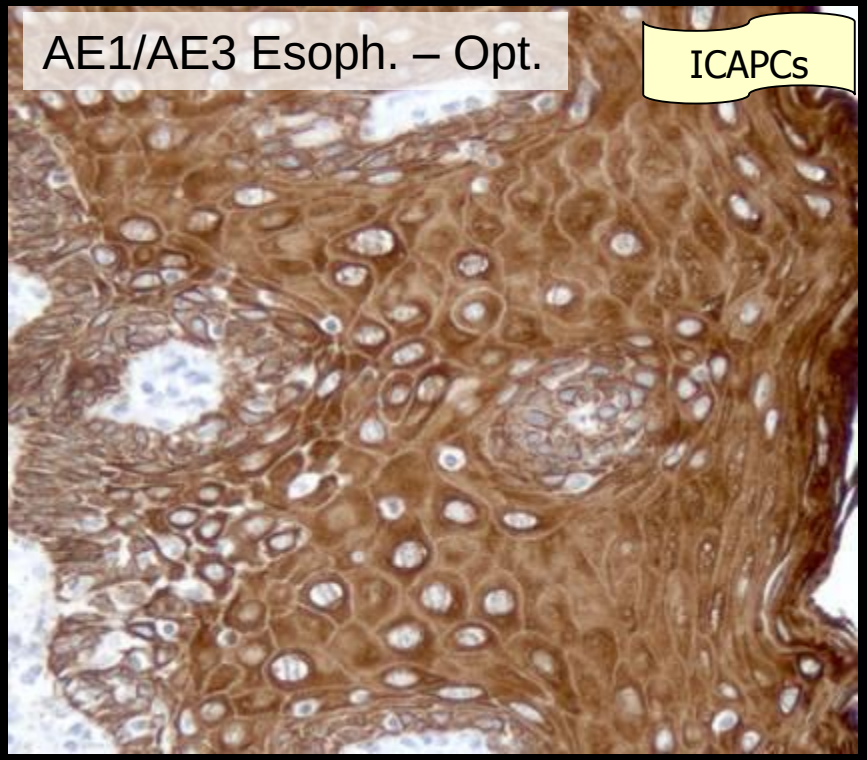
CK LMW types
AE1/AE3: 7, **8**, 19



A strong, distinct cytoplasmic staining reaction of all bile ductal epithelial cells and at least a moderate cytoplasmic staining reaction withmembrane accentuation of the vast majority of hepatocytes.

CK HMW types
AE1/AE3: 1, 4, **5**, 10, **14**



A strong, distinct cytoplasmic staining reaction of virtually all squamous epithelial cells throughout all cell layers.

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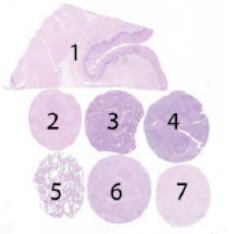
Assessment Run 47 2016

Pan Cytokeratin (CK-PAN)

Material
The slide to be stained for CK-PAN comprised:
1. Esophagus, 2. Liver, 3. Small cell lung carcinoma (SCLC), 4. Tonsil,
5. Lung adenocarcinoma, 6. Lung squamous cell carcinoma, 7. Renal clear cell carcinoma (RCC).



Criteria for assessing a CK-PAN staining as optimal were:

- A strong, distinct cytoplasmic staining reaction of all bile ductal epithelial cells and at least a moderate cytoplasmic staining reaction with membrane accentuation of the vast majority of hepatocytes.
- A strong, distinct cytoplasmic staining reaction of all squamous epithelial cells throughout all cell layers in the esophagus.
- A strong, distinct cytoplasmic staining reaction of the majority of neoplastic cells in the lung adenocarcinoma and squamous cell carcinoma.
- An at least moderate, distinct cytoplasmic, dot-like staining reaction of the majority of neoplastic cells in the SCLC.
- An at least weak to moderate, distinct cytoplasmic and membranous staining reaction of the majority of neoplastic cells in the RCC.

All tissues were fixed in 10% neutral buffered formalin.

Participation

Number of laboratories registered for CK-PAN, run 47	298
Number of laboratories returning slides	276 (93%)

Results
276 laboratories participated in this assessment. One laboratory used an inappropriate antibody (CK-HMW). Of the remaining 275 laboratories, 72% achieved a sufficient mark. Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:

- Too low concentration of the primary antibody
- Insufficient HIER – too short efficient heating time and/or use of non-alkaline HIER buffers
- Inappropriate epitope retrieval
- Less successful primary antibodies.

Performance history
This was the eighth NordiQC assessment of CK-PAN. The overall pass rate was slightly improved compared to previous runs performed, as shown in table 2.

Table 2. **Proportion of sufficient results for CK-PAN in the eight NordiQC runs performed**

	Run 8 2003	Run 15 2005	Run 20 2008	Run 24 2008	Run 30 2010	Run 36 2012	Run 41 2014	Run 47 2016
Participants, n=	72	85	103	123	168	202	233	275
Sufficient results	53%	58%	62%	60%	65%	65%	67%	72%

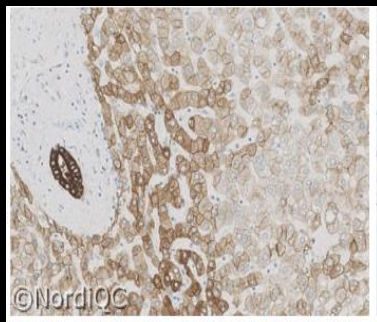


Fig. 1a. Optimal staining for CK-Pan of the liver based on HIER and the mAb clone cocktail AE1/AE3. The majority of the hepatocytes show a distinct, moderate to strong staining with membrane enhancement, while the columnar epithelial cells of the bile duct show a strong cytoplasmic staining. Compare with Figs. 2a-4a, same protocol.

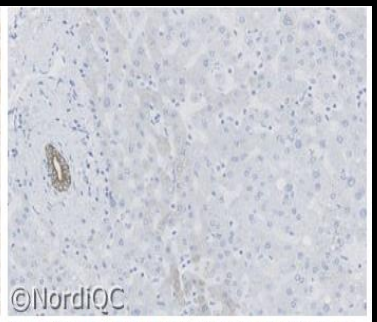


Fig. 1b. Insufficient CK-Pan staining of the liver, using an efficient HIER and Ab clone KL1 but applying the Ab in a too low concentration - same field as in Fig. 1a. Only the epithelial cells of the bile duct are demonstrated, while the hepatocytes are unstained. Compare with Figs. 2b-4b, same protocol.

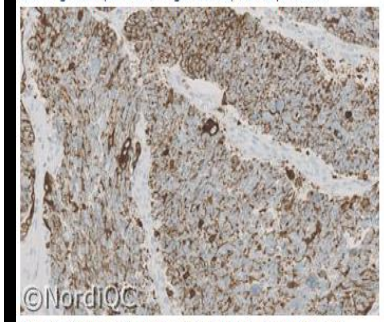


Fig. 2a. Optimal CK-pan staining of the small cell lung carcinoma using same protocol as in Figs. 1a, 3a and 4a. The majority of the neoplastic cells show a moderate, distinct dot-like cytoplasmic staining.

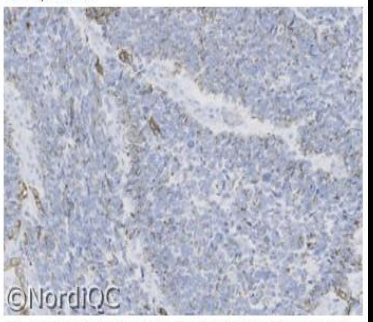


Fig. 2b. Insufficient CK-Pan staining of the small cell lung carcinoma using same protocol as in Figs. 1b, 3b and 4b - same field as in Fig. 2a. Only scattered neoplastic cells show a weak staining reaction. Also compare with Figs. 3b. & 4b., same protocol.

Too weak or false negative result is most commonly observed in the insufficient results.

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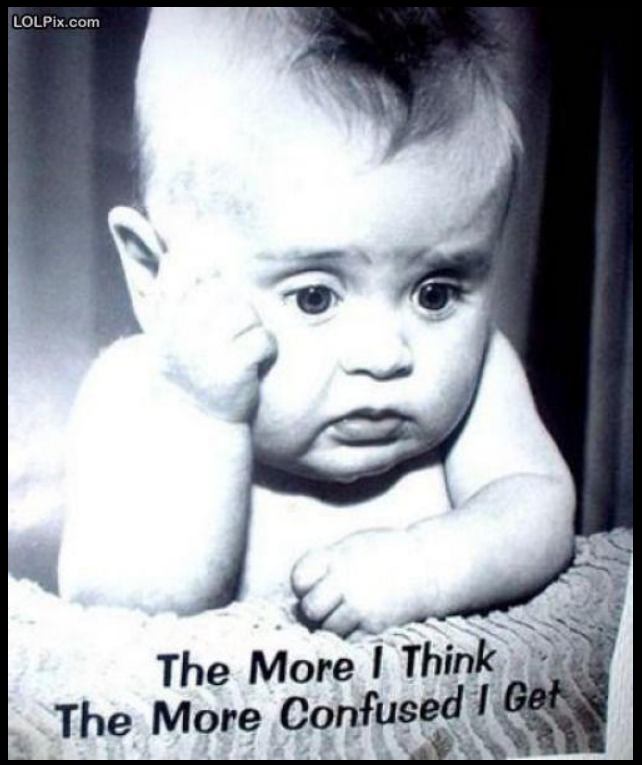
Table 1. **Antibodies and assessment marks for CK-PAN, run 47**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone cocktail **AE1/AE3**	85	Dako/Agilent	33	28	17	7	72%	83%
	11	Thermo/NeoMarkers	2	3	4	2	45%	100%
	5	Cell Marque	0	3	0	2	60%	-
	5	Leica/Novocastra	1	0	3	1	20%	50%
	3	Biocare	1	2	0	0	-	-
	2	Zytomed	0	2	0	0	-	-
	1	Biosystems	1	0	0	0	-	-
	1	Genemed	0	1	0	0	-	-
	1	Gennova	0	1	0	0	-	-
	1	Immunologic	0	0	1	0	-	-
	1	Millipore	1	0	0	0	-	-
	1	Monosan	0	0	0	0	-	-
mAb clone cocktail **AE1/AE3/ks 13.2**	1	Linaris	0	0	0	1	-	-
mAb clone cocktail **AE1/AE3/5D3**	2 1	Biocare Zytomed	1	2	0	0	-	-
mAb clone cocktail **PAN CK Ab-2**	1	Thermo/NeoMarkers	0	1	0	0	-	-
mAb clone **BS5**	1 1	Monosan Nordic Biosite	2	0	0	0	-	-
mAb clone **C-11**	1	Leica/Novocastra	0	0	0	1	-	-
mAb clone **Lu-5**	2	Immunologic	0	0	1	1	-	-
mAb clone **MNF116**	7	Dako/Agilent	0	0	4	3	0%	-
mAb clone **OSCAR**	1 1	Signet "In-house"	0	2	0	0	-	-
Unknown	3		1	1	0	1	-	-
"Laboratory made" antibody cocktails								
mAb clone cocktail **AE1/AE3/5D3**	2	Leica/Novocastra & Milipore	1	1	0	0	-	-
mAb clone cocktail **AE1/AE3/5D3**	1	Leica/Novocastra	1	0	0	0	-	-
mAb clone cocktail **AE1AE3/CAM5.2**	1	Dako/Agilent & BD	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone cocktail **AE1/AE3 IR053**	36	Dako/Agilent	28	5	2	1	92%	95%
mAb clone cocktail **AE1/AE3 GA053**	19	Dako/Agilent	18	0	1	0	95%	100%
mAb clone cocktail **AE1/AE3 313M-18**	3	Cell Marque	0	1	0	2	-	-
mAb clone cocktail **AE1/AE3 MAD 001000QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone cocktail **AE1/AE3 Kit-0009**	1	Maixin	1	0	0	0	-	-
mAb clone cocktail **AE1/AE3 PA0909**	5	Leica/Novocastra	0	1	3	1	20%	-
mAb clone cocktail **AE1/AE3 RTU-AE1/AE3**	2	Leica/Novocastra	0	0	2	0	-	-
mAb clone cocktail **AE1/AE3/5D3 IP162**	2	Biocare	1	1	0	0	-	-
mAb clone cocktail **AE1/AE3/PCK26 760-2135/2595**	62	Ventana/Roche	37	8	5	12	73%	96%
rmAb clone cocktail **EP24/EP67/B22.1/B23.1 MAD-000680QD**	2	Master Diagnostica	0	2	0	0	-	-
Total	275		132	65	43	35	-	
Proportion			48%	24%	16%	12%	72%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Clone/Retrieval/Titre/Control

Too many choices
Misleading datasheets



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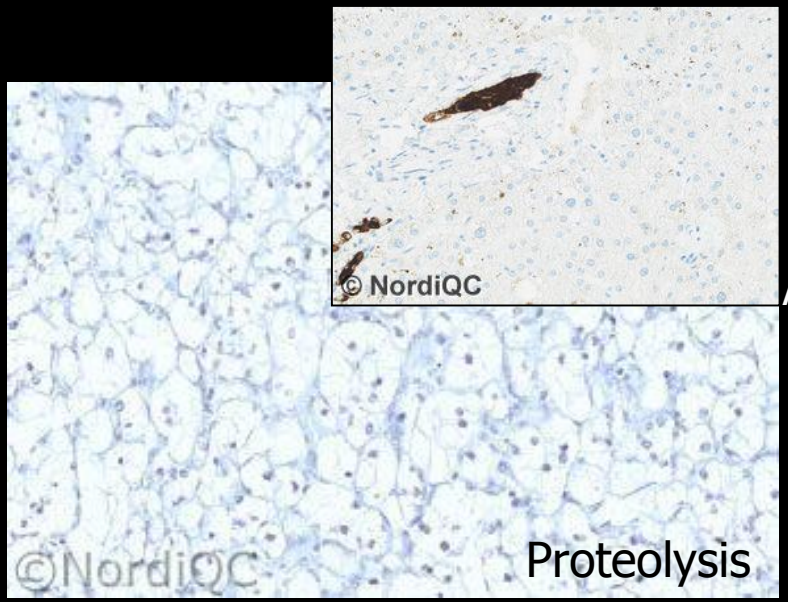
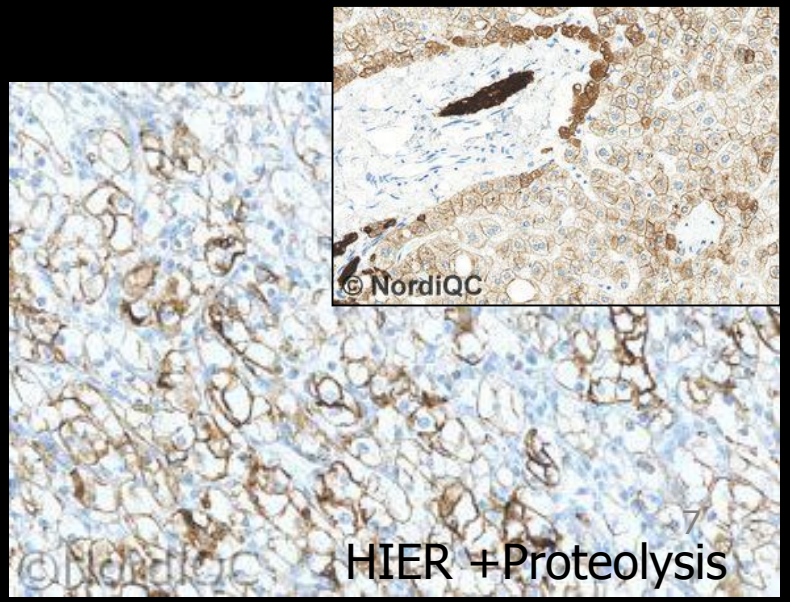
Clone/Retrieval/Titre/Control

Table 4. **Pass rates for antibody cocktails combined with epitope retrieval methods in seven NordiQC runs**

Pass rate for run 15, 20, 24, 30, 36, 41 & 47								
	Total		HIER		Proteolysis		HIER + proteolysis	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb AE1/AE3	752	542 (72%)	693	535 (77%)	44	5 (11%)	5	2 (40%)
mAb AE1/AE3/5D3	37	34 (92%)	36	34 (94%)	1	0	0	0
mAb AE1/AE3/PCK26	176	105 (60%)	25	13 (48%)	34	0	117	92 (79%)
mAb MNF116	91	30 (33%)	40	9 (23%)	47	21 (45%)	4	2 (50%)



RCC
AE1/AE3/PCK26



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Participants, n=	72	85	103	123	168	202	233	275
Sufficient results	53%	58%	62%	60%	65%	65%	67%	72%

Too many choices

Misleading data sheets

Wrong control material used

AE1/AE3 : Optimal results only obtained by HIER in NordiQC runs

Dako: RTU – HIER
Leica: RTU – Proteolysis
Thermo:

Conc: Proteolysis or HIER
Conc: HIER
Conc: HIER Quanto – Proteolysis UltraVision

……

AE1/AE3/PCK26: Optimal results mainly obtained by HIER+protelysis in NordiQC runs

VMS: RTU - Proteolysis

Till 2015

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STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on the VENTANA BenchMark ULTRA, BenchMark XT and BenchMark GX automated slide stainers in combination with VENTANA detection kits and accessories. Refer to Table 1 for recommended staining protocols.

This antibody has been optimized for specific incubation times, but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instruments Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for Anti-Pan Keratin (AE1/AE3/PCK26) with *ultra*View Universal DAB Detection Kit on a BenchMark ULTRA instrument, BenchMark XT instrument or BenchMark GX instrument.

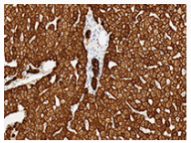
Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Mild
Enzyme (Protease)	Protease 3, 4 minutes
Antibody (Primary)	BenchMark ULTRA instrument 8 minutes, 36°C BenchMark XT instrument 8 minutes, 37°C BenchMark GX instrument 4 minutes, 37°C
ultraBlock	*VENTANA Antibody Diluent with Casein, 4 minutes
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

*Use of VENTANA Antibody Diluent with Casein (Cat. No. 760-219/06440002001) at the ultraBlock step is recommended to reduce staining on smooth muscle.

Home > Products > Product Catalog > Primary Antibodies
> Pan Keratin (AE1/AE3/PCK26) Primary Antibody

Print Share

anti-Pan Keratin (AE1/AE3/PCK26) Primary Antibody



Catalog Number:	760-2135
Ordering Code:	05266840001
Quantity:	250 tests
Controls:	Intestine, Liver
Isotypes:	IgG_1
Clone Name:	AE1/AE3 & PCK26
Species:	Mouse
Localization:	Cytoplasmic
Regulatory Status:	IVD

Related Links
Cytokeratin (Pan) MSDS/SDS
Package Inserts

This antibody is intended for in vitro diagnostic use. Ventana Medical Systems, Inc. (Ventana) anti-Pan Keratin (AE1/AE3/PCK26) Primary Antibody may be used to aid in the identification of normal and abnormal epithelial cells and to determine the lineage of poorly differentiated malignant tumors. The keratins are a group of intermediate filament proteins that occur in normal and neoplastic cells of epithelial origin. Nineteen human cytokeratins are known which are divided into acidic and basic subfamilies. They occur in pairs in epithelial tissues, the composition of pairs varying with the epithelial cell type, stage of differentiation, cellular growth environment, and disease state. This pan keratin cocktail recognizes most of the acidic and all of the basic cytokeratins, making it a useful stain for nearly all epithelial tissues and their tumors. Anti-Pan Keratin (AE1/AE3/PCK26) specifically binds to antigens located in the cytoplasm of simple and complex epithelial cells. The antibody is intended for laboratory use to qualitatively stain cytokeratins in sections of formalin fixed, paraffin embedded tissue on a Ventana automated slide stainer. Anti-Pan Keratin (AE1/AE3/PCK26) contains a mouse monoclonal antibody cocktail raised against an epitope found on human epidermal keratins as reported by Woodcock-Mitchell, et al.1 This antibody cocktail reacts with the 56.5kD, 50kD, 50'kD, 48kD, and 40kD cytokeratins of the acidic subfamily and 65-67kD, 64kD, 59kD, 58kD, 56kD, and 52kD cytokeratins of the basic subfamily.1,2,3,4,5 Unexpected antigen expression or loss of expression may occur, especially in neoplasms. Occasionally stromal elements surrounding heavily stained tissue and or cells will show immunoreactivity. The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests. Caution: U.S. Federal law restricts this device to sale by or on the order of a physician.

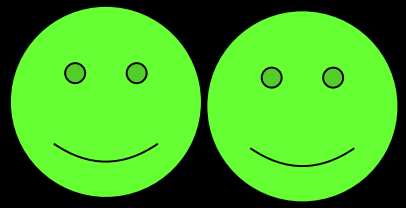


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Table 1. Abs and assessment marks for CK-PAN, run 36

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone cocktail **AE1/AE3**	73	Dako	32	19	8	14	70 %	70 %
	14	Thermo/NeoMarkers	2	5	3	4	50 %	100 %
	7	Leica/Novocastra	0	1	1	5	14 %	-
	2	Biocare	0	1	0	1	-	-
	2	Cell Marque	1	1	0	0	-	-
	2	Chemicon	0	1	1	0	-	-
	1	Biogenex	0	1	0	0	-	-
	1	ID Labs	0	1	0	0	-	-
	1	Progen	0	0	0	1	-	-
	1	Zytomed	0	1	0	0	-	-
mAb clone cocktail **AE1/AE3 + 5D3**	5	Biocare	2	3	0	0	100 %	100 %
mAb clone cocktail **AE1/AE3 + DC10**	1	Leica/Novocastra (home-made cocktail)	0	1	0	0	-	-
mAb clone **KL1**	5 1	Beckman Coulter AbD Serotec	3	2	0	1	83 %	100 %
mAb clone **Lu-5**	1 1	Immunologic BMA Biomedicals	0	0	0	2	-	-
mAb **MNF116**	13 1	Dako Abcam	0	4	3	7	29%	-
mAb cocktail **MNF116+DC10+ AE1/AE3+CAM5.2**	1	Dako/BD (home-made coctail)	1	0	0	0	-	-
mAb clone **OSCAR**	1	Covance	1	0	0	0	-	-
mAb clone cocktail **PAN CK Ab-2**	1	Thermo/NeoMarkers	0	0	1	0	-	-

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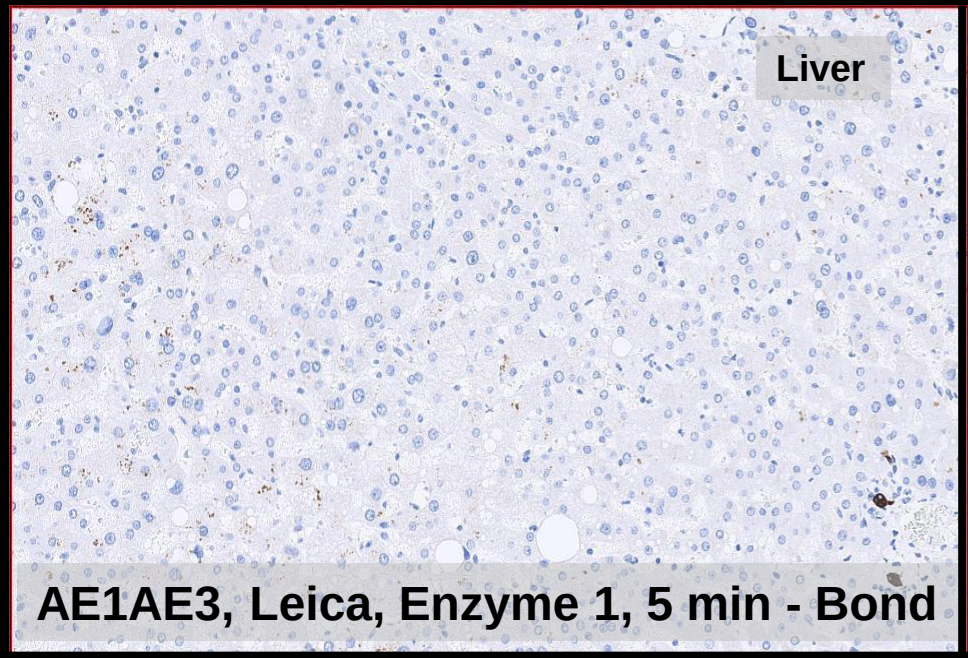
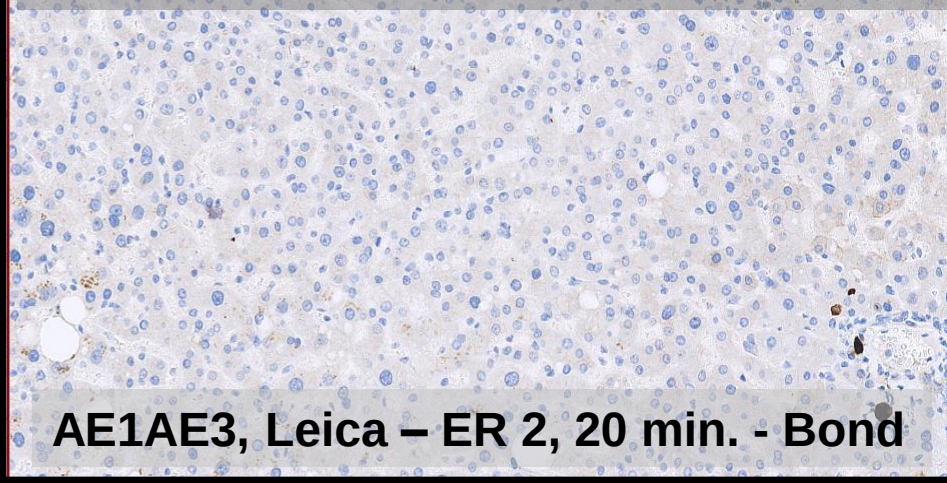


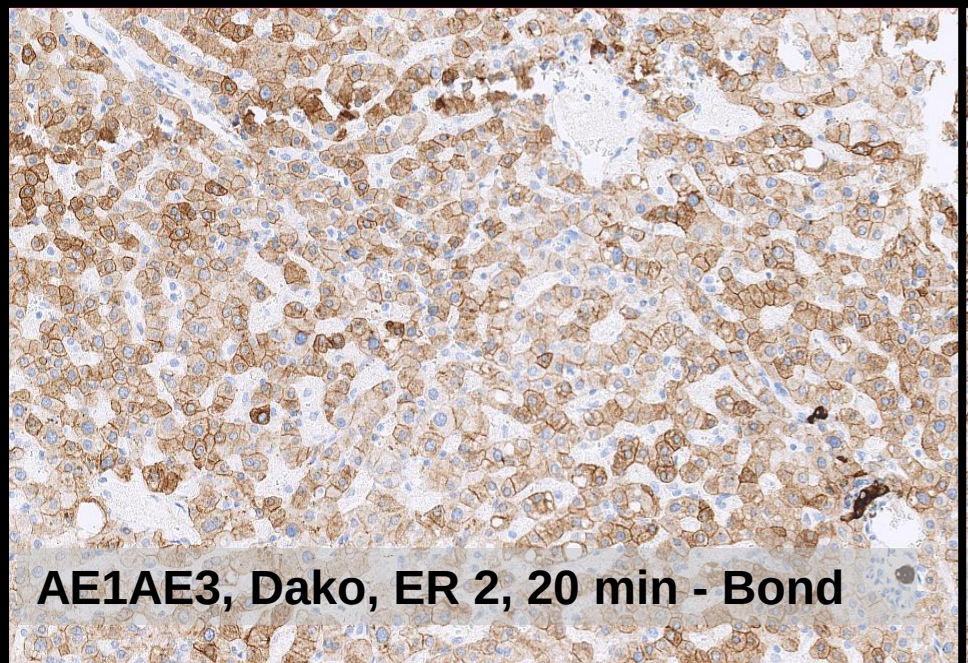
AE1AE3, Leica, Enzyme 1, 5 min - Bond

Dako: AE1 (CK19) + AE3 (CK8) 1:3?

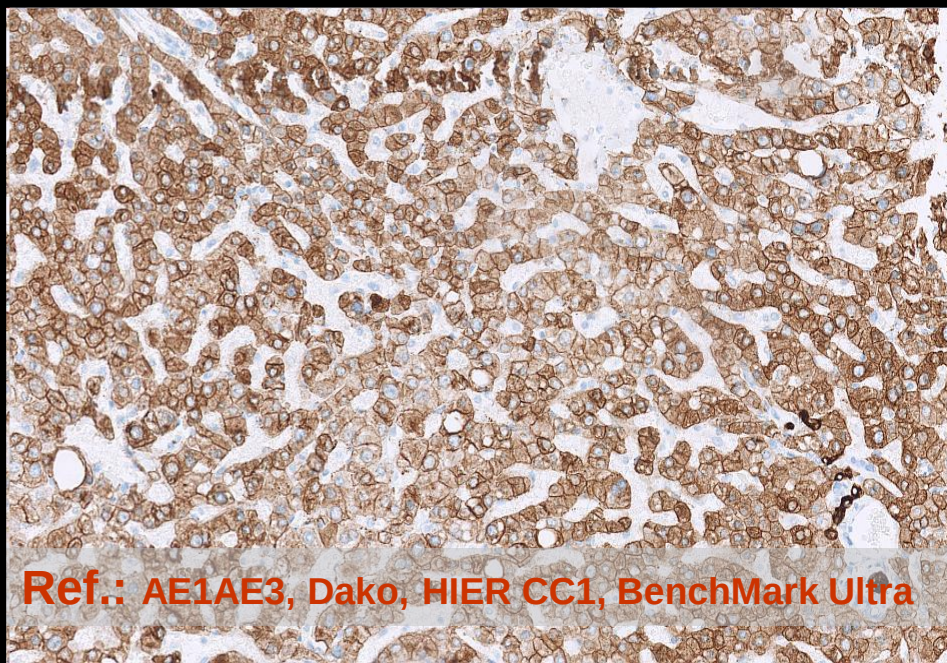
Leica: AE1 (CK19) + AE3 (CK8) 1:10?



AE1AE3, Leica – ER 2, 20 min. - Bond



AE1AE3, Dako, ER 2, 20 min - Bond



Ref.: AE1AE3, Dako, HIER CC1, BenchMark Ultra

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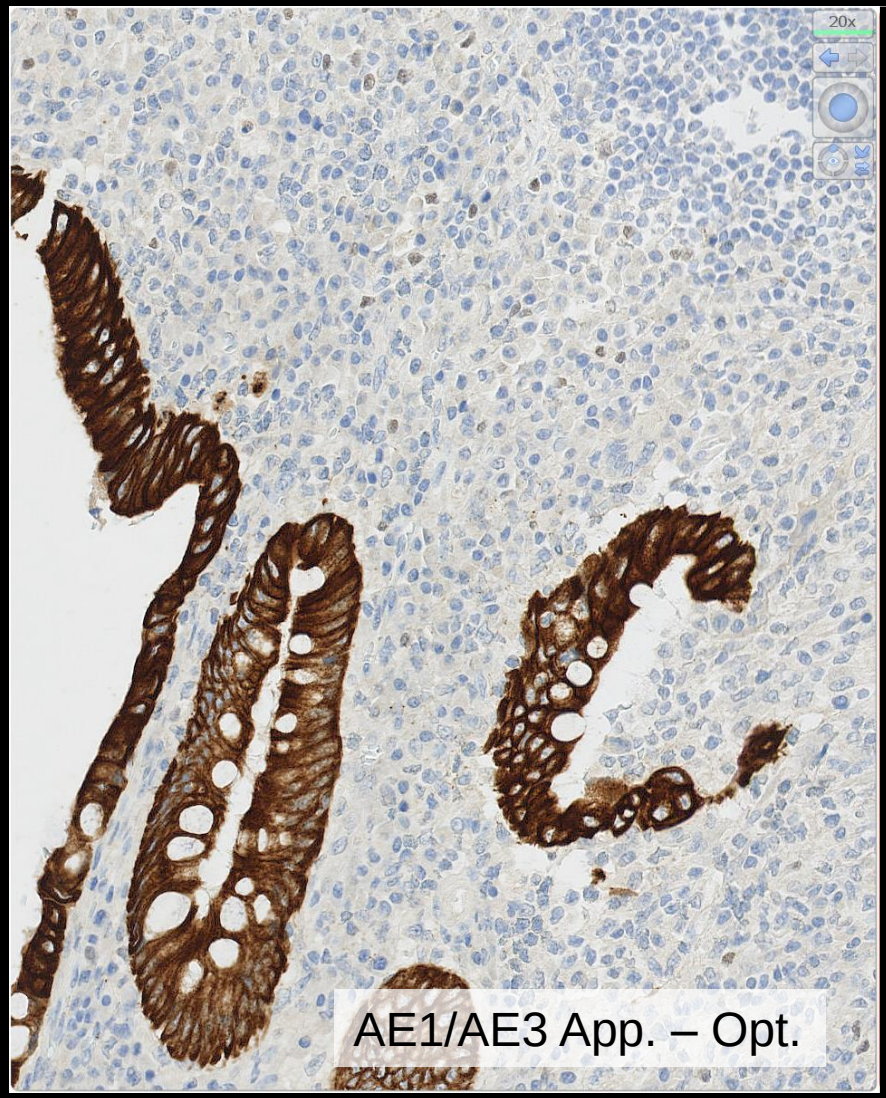
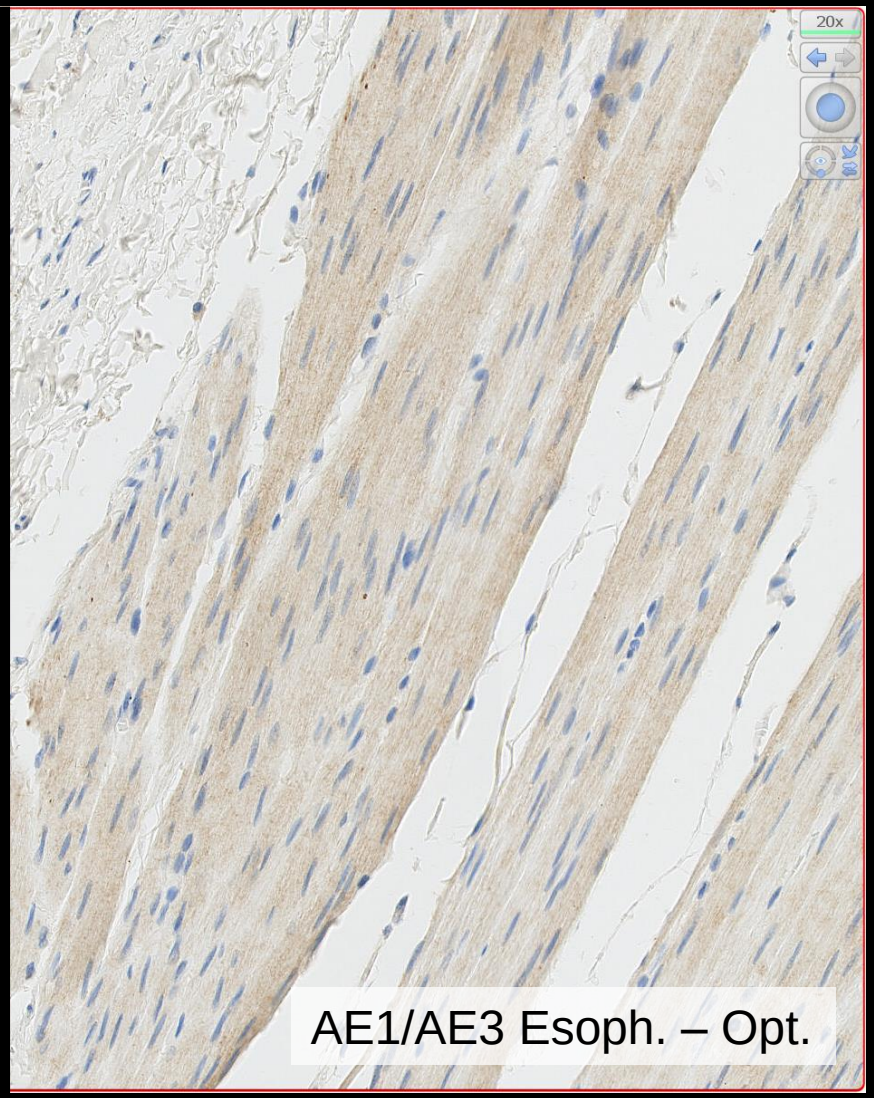


AE1/AE3 App. – Opt.



AE1/AE3 Esoph. – Opt.

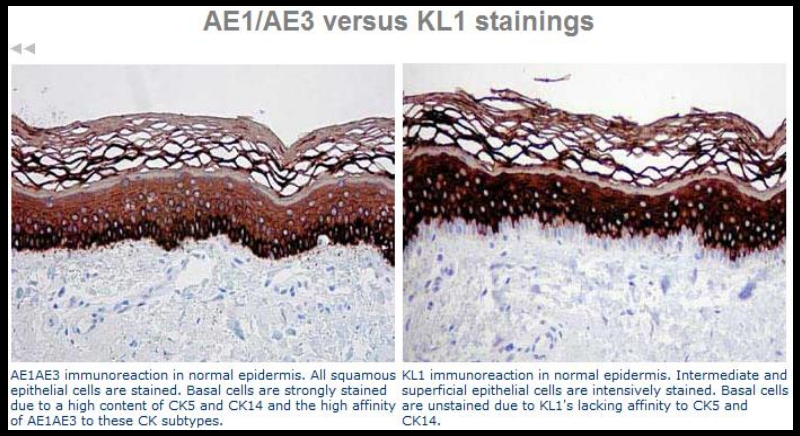
Nuclear staining reaction and cytoplasmic staining reaction in smooth muscle cells can be seen

IHC – Protocols and controls for UPT I

UPT I: CK-PAN

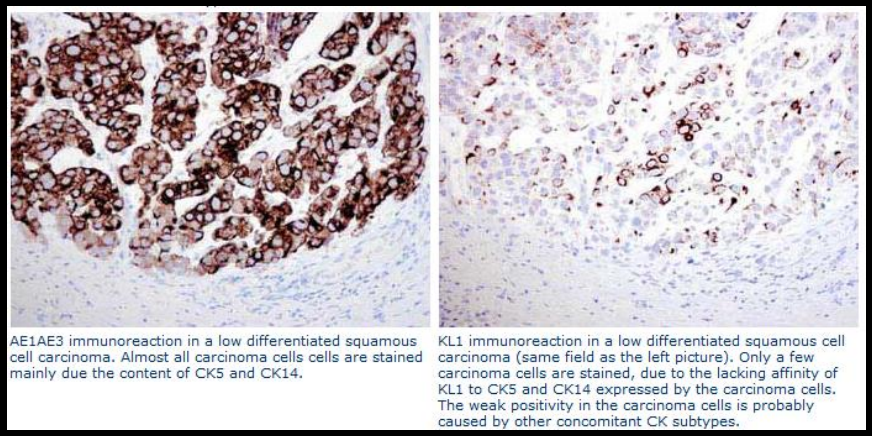
Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb AE1AE3	HIER High	1:25-200	2- & 3-step	Dako	2-step
mAb AE1AE3+5D3	HIER High	1:100-300	2- & 3-step	-	-
mAb BS5	HIER High	1:100-300	2- & 3-step	-	-
mAb AE1AE3+PCK26	HIER High +P3	-	-	Ventana	2 & 3-step



AE1AE3 immunoreaction in normal epidermis. All squamous epithelial cells are stained. Basal cells are strongly stained due to a high content of CK5 and CK14 and the high affinity of AE1AE3 to these CK subtypes.

KL1 immunoreaction in normal epidermis. Intermediate and superficial epithelial cells are intensively stained. Basal cells are unstained due to KL1's lacking affinity to CK5 and CK14.

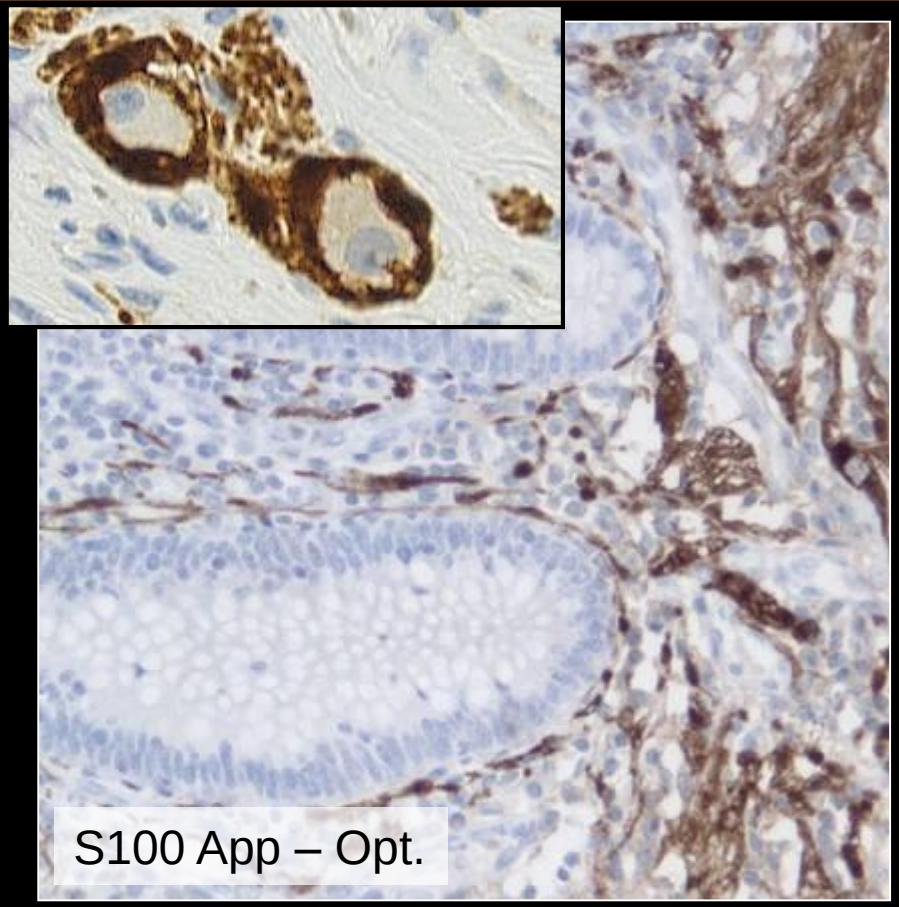


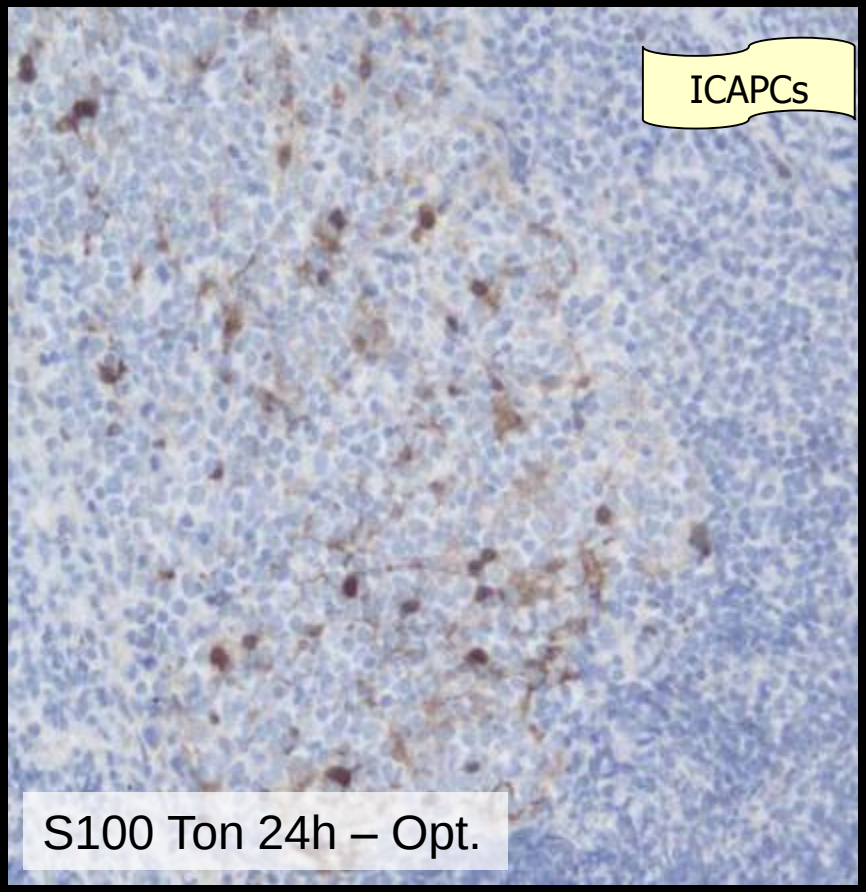
AE1AE3 immunoreaction in a low differentiated squamous cell carcinoma. Almost all carcinoma cells cells are stained mainly due the content of CK5 and CK14.

KL1 immunoreaction in a low differentiated squamous cell carcinoma (same field as the left picture). Only a few carcinoma cells are stained, due to the lacking affinity of KL1 to CK5 and CK14 expressed by the carcinoma cells. The weak positivity in the carcinoma cells is probably caused by other concomitant CK subtypes.

* Pruned from the market 2015….

IHC – Protocols and controls for UPT I

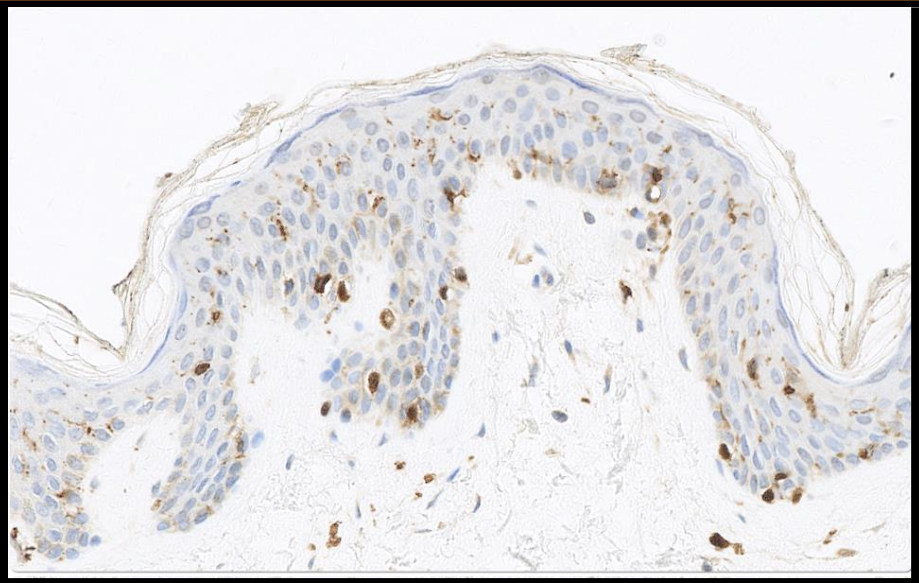


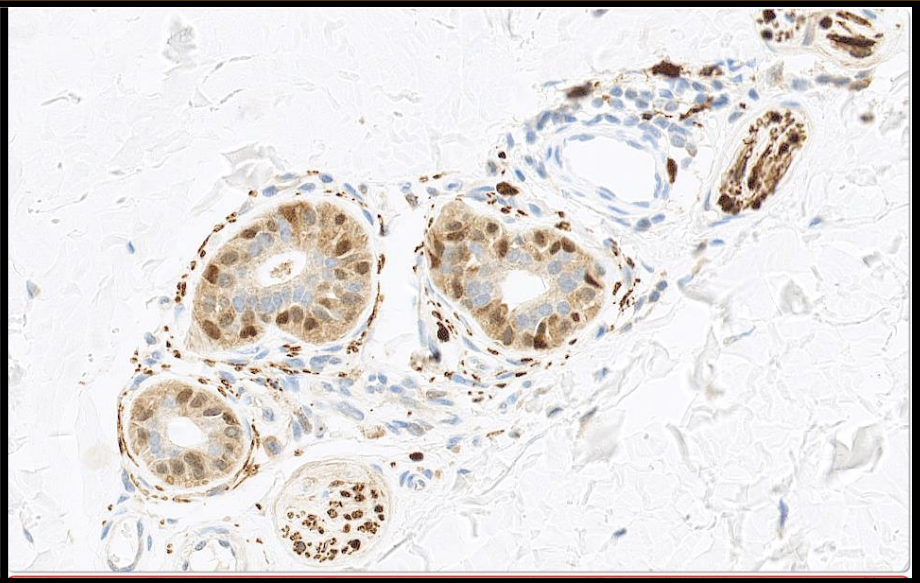


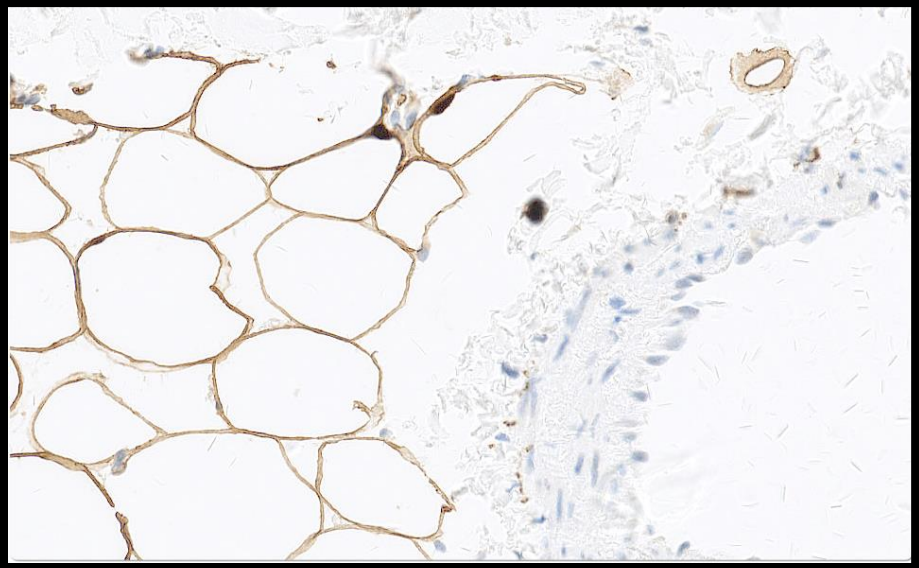
A strong, distinct nuclear and cytoplasmic staining reaction of the macrophages in lamina propria, the Schwann cells of the peripheral nerve fibres and the ganglionic satellite cells in the muscularis propria and submucosa in the appendix. The epithelial cells and muscle cells should be negative.

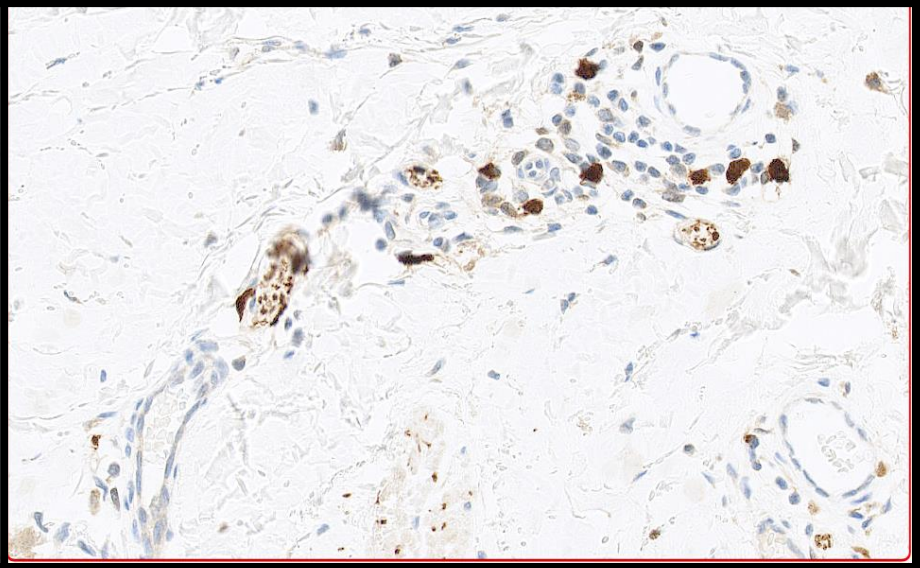
An at least weak but distinct nuclear and cytoplasmic staining reaction of the follicular dendritic cells in the germinal centres (most likely due to reaction to S100A and thus mainly seen for pAbs to S100).

IHC – Protocols and controls for UPT I







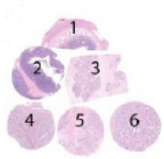


Skin: Pos.: myoepithelial cells, lipocytes, Langerhans cells
Neg.: lymphocytes, squamous epithelial cells, smooth muscle cells

IHC – Protocols and controls for UPT I

Assessment Run 45 2015
S-100 protein (S100)

Material
The slide to be stained for S100 comprised:



1. Appendix, 2. Tonsil, 3. Breast hyperplasia, 4-5. Malignant melanoma, 6. Colon adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing S100 staining as optimal included:

- A strong, distinct nuclear and cytoplasmic staining reaction of the vast majority of macrophages in lamina propria, Schwann cells of peripheral nerve fibres and ganglionic satellite cells in the muscularis propria and submucosa in the appendix.
- A moderate to strong, distinct nuclear and cytoplasmic staining reaction of the vast majority of myoepithelial cells in the breast, and no more than a moderate reaction in the epithelial cells.
- A weak to moderate, distinct nuclear and cytoplasmic staining reaction of the majority of neoplastic cells of the melanoma (core 4).
- A strong, distinct nuclear and cytoplasmic staining reaction of all neoplastic cells of the melanoma (core 5).
- A moderate to strong, distinct nuclear and cytoplasmic staining reaction of adipocytes and macrophages in all specimens.
- No staining of other cells. Especially all neoplastic cells in the colon adenocarcinoma, squamous epithelial cells in tonsil, smooth muscle cells and columnar epithelial cells in the appendix should be negative

In addition, for the polyclonal antibodies (Abs) Z0311 (Dako), NCL-L-S100p (Leica) and 760-2523 (Ventana), a weak cytoplasmic and nuclear staining reaction of the follicular dendritic cells in the germinal centres of the tonsil and the Peyer's plaques in the appendix was expected and accepted.

Participation

Number of laboratories registered for S100, run 45	296
Number of laboratories returning slides	251 (85%)

Results
251 laboratories participated in this assessment. 169 (68%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Insufficient HIER (too low temperature and/or too short heating time)
- Proteolytic pre-treatment or omission of epitope retrieval
- Low sensitive detection systems
- Unexplained technical issues

Performance history
This was the fourth NordiQC assessment of S100. The overall pass rate was relatively low and comparable with the result obtained in run 34, 2012 (see table 2).

Table 2: **Proportion of sufficient results for S100 in the four NordiQC runs performed**

	Run 7 2003	Run 20 2007	Run 34 2012	Run 45 2015
Participants, n=	63	106	200	251
Sufficient results	71%	75%	64%	68%

IHC – Protocols and controls for UPT I

Table 1: **Antibodies and assessment marks for S100, run 45**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **4C4.9**	2 1 1 1	Immunologic Thermo/NeoMarkers Zytomed Systems Unknown	0	4	1	0	80%	-
mAb clone **15E2E2**	1	Biogenex	0	0	0	1	-	-
mAb clone **S1/61/69**	1	Leica/Novocastra	0	0	0	1	-	-
mAb clone **15E2E2+4C4.9**	2	BioCare	0	0	2	0	-	-
pAb **Z0311**	123	Dako	56	39	24	4	77%	88%
pAb **NCL-L-S100p**	9	Leica/Novocastra	2	2	3	2	44%	100%
Ready-To-Use systems								
mAb clone **4C4.9 790-2914**	24	Ventana	2	8	9	5	42%	100%
mAb clone **4C4.9 330M-18**	1	Cell Marque	0	0	1	0	-	-
mAb clone **4C4.9 MAD-001221QD**	2	Master Diagnostica	1	0	1	0	-	-
mAb clone **4C4.9 MON-RTU1191**	1	Monosan/Sanbio	0	0	1	0	-	-
mAb clone **15E2E2 AM058-5M**	1	Biogenex	0	0	1	0	-	-
mAb clone **16/F5 MAB-0697**	1	Maixin	0	1	0	0	-	-
pAb **IR504**	34	Dako	3	27	4	0	88%	95%
pAb **GA504**	13	Dako	5	6	2	0	85%	90%
pAb **760-2523**	26	Ventana	1	11	12	2	46%	100%
pAb **PA0900**	5	Leica/Novocastra	0	1	3	1	20%	-
pAb **PP021**	1	BioCare	0	0	1	0	-	-
pAb **E031**	1	Linaris	0	0	1	0		
Total	251		70	99	66	16	-	
Proportion			28%	40%	26%	6%	68%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

68 % sufficient

If using pAb Z0311 a titre of 1:300-4.000 &

HIER:
87 % sufficient
60 % optimal

Prot. / omission:
50% sufficient.
7% optimal.

Typically false negative, too weak and/or impaired morphology

IHC – Controls and CSQI for UPT I

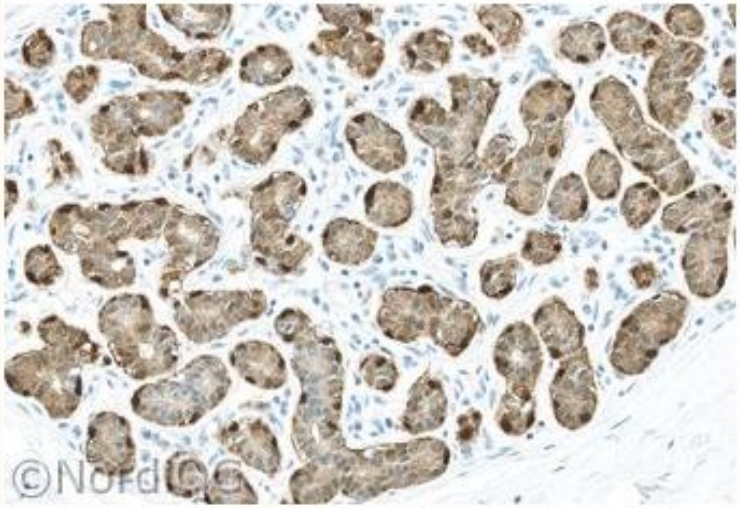


Fig. 3a. Optimal S100 staining of the breast hyperplasia using same protocol as in Figs. 1a & 2a. The myoepithelial cells show a moderate to strong cytoplasmic and nuclear staining reaction. A weaker staining of the secretory cells is seen, but no background staining is seen.

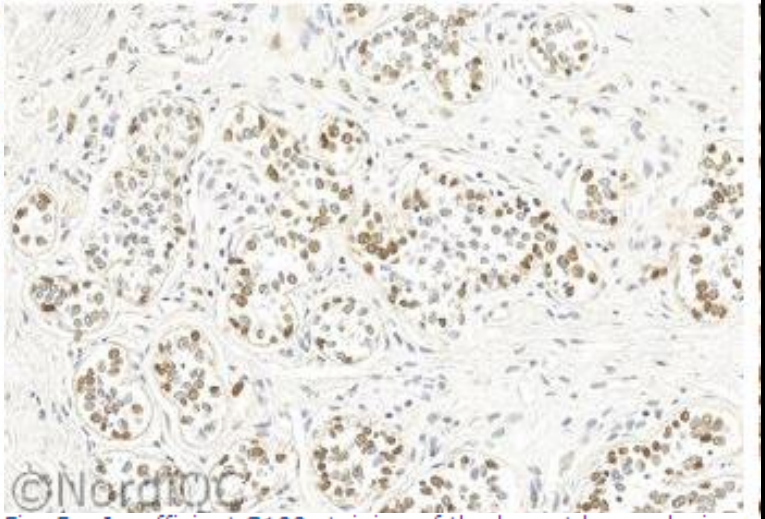


Fig. 5a. Insufficient S100 staining of the breast hyperplasia using proteolytic pre-treatment. The cytoplasmic compartment of both the myoepithelial cells and the glandular epithelial cells is digested and only the moderately stained nuclei are left. Also compare with Fig. 5b, same protocol.

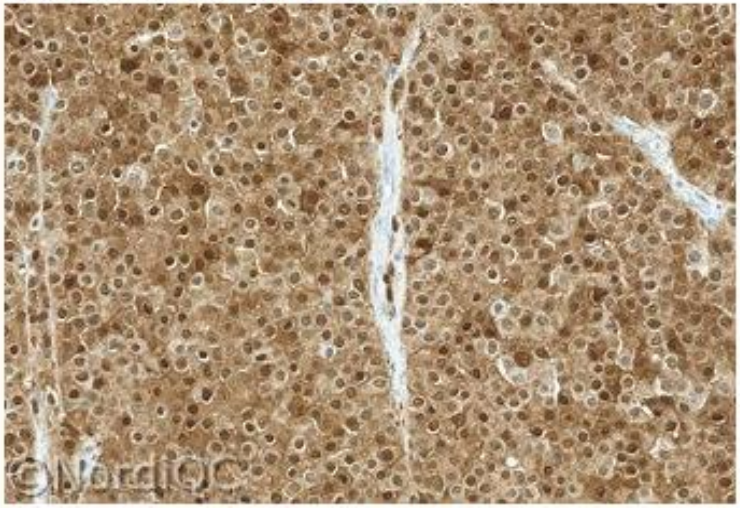


Fig. 4a. Optimal S100 staining of the malignant melanoma using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic and nuclear staining reaction.

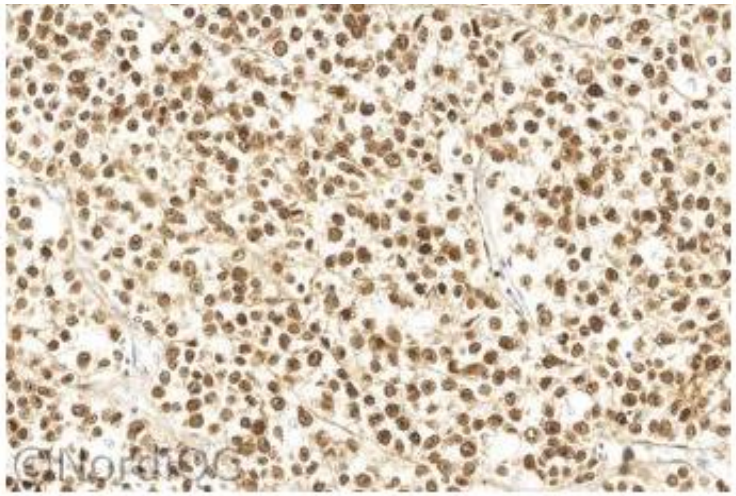
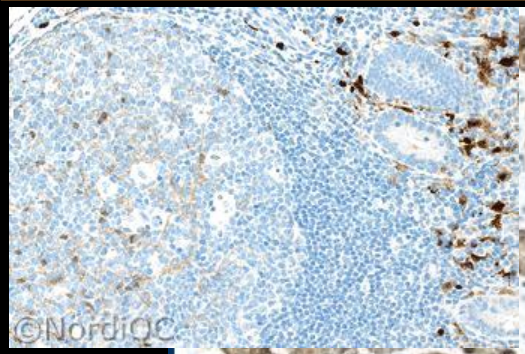


Fig. 5b. Insufficient S100 staining of the malignant melanoma using proteolytic pre-treatment, same protocol as in Fig. 5a. The cytoplasmic compartment is digested and only the moderately stained nuclei are left.

Proteolysis can provide impaired morphology

IHC – Controls and CSQI for UPT I



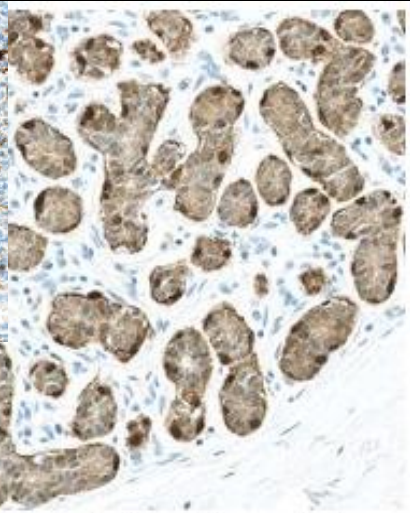
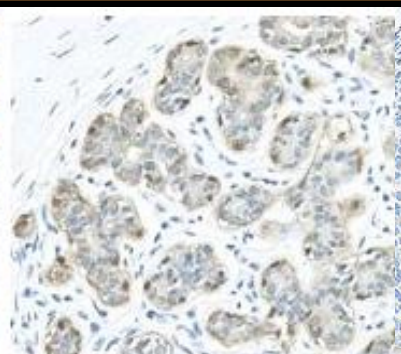


Fig. 3a. Optimal S100 staining of the breast hyperplasia using same protocol as in Figs. 1a & 2a. The myoepithelial cells show a moderate to strong cytoplasmic and nuclear staining reaction. A weaker staining of the secretory cells is seen, but no background staining is seen.



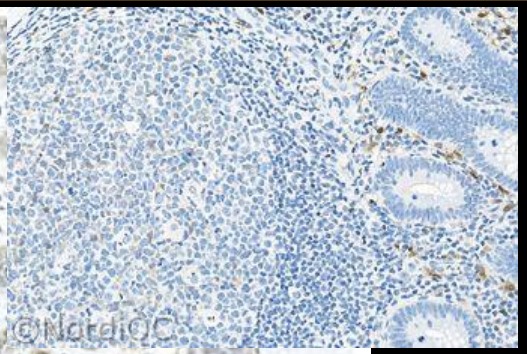


Fig. 3b. Insufficient S100 staining of the breast hyperplasia using same protocol as in Figs. 1b & 2b., same field as in Fig. 3a. The myoepithelial cells show a weak and equivocal staining reaction also compare with Fig. 4b, same protocol.

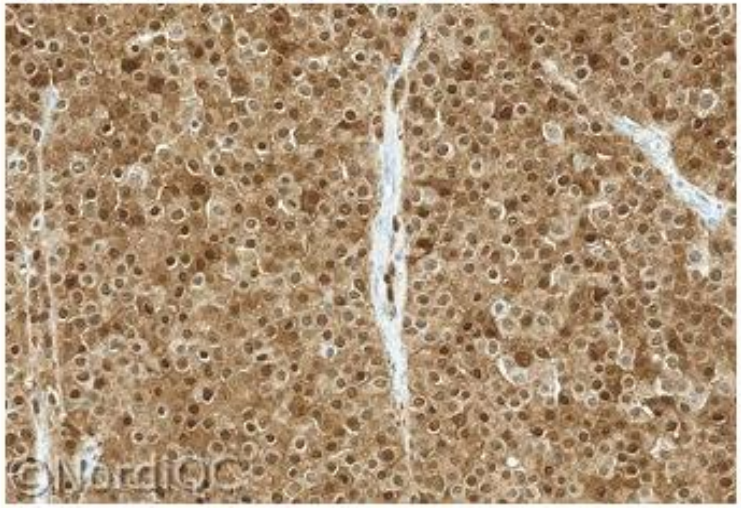


Fig. 4a. Optimal S100 staining of the malignant melanoma using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic and nuclear staining reaction.

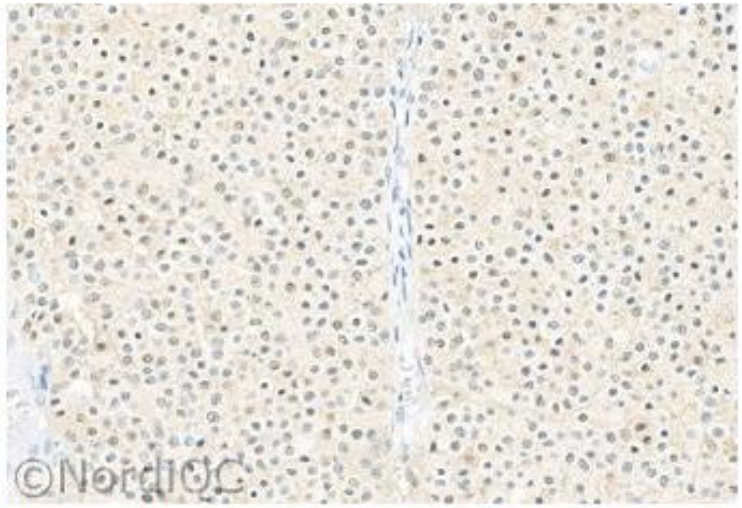


Fig. 4b. Insufficient S100 staining of the malignant melanoma using same protocol as in Figs. 1b – 3b., same field as in Fig. 4a. The neoplastic cells show a weak and equivocal staining reaction.

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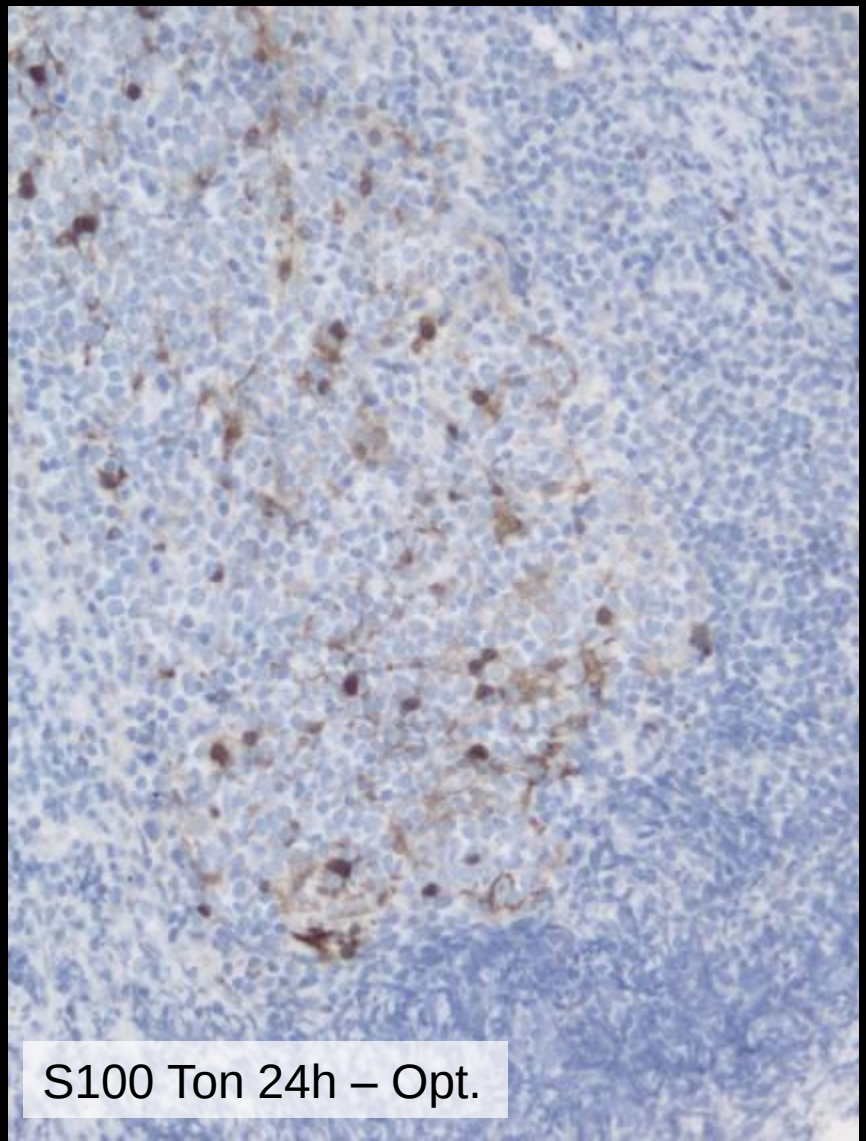
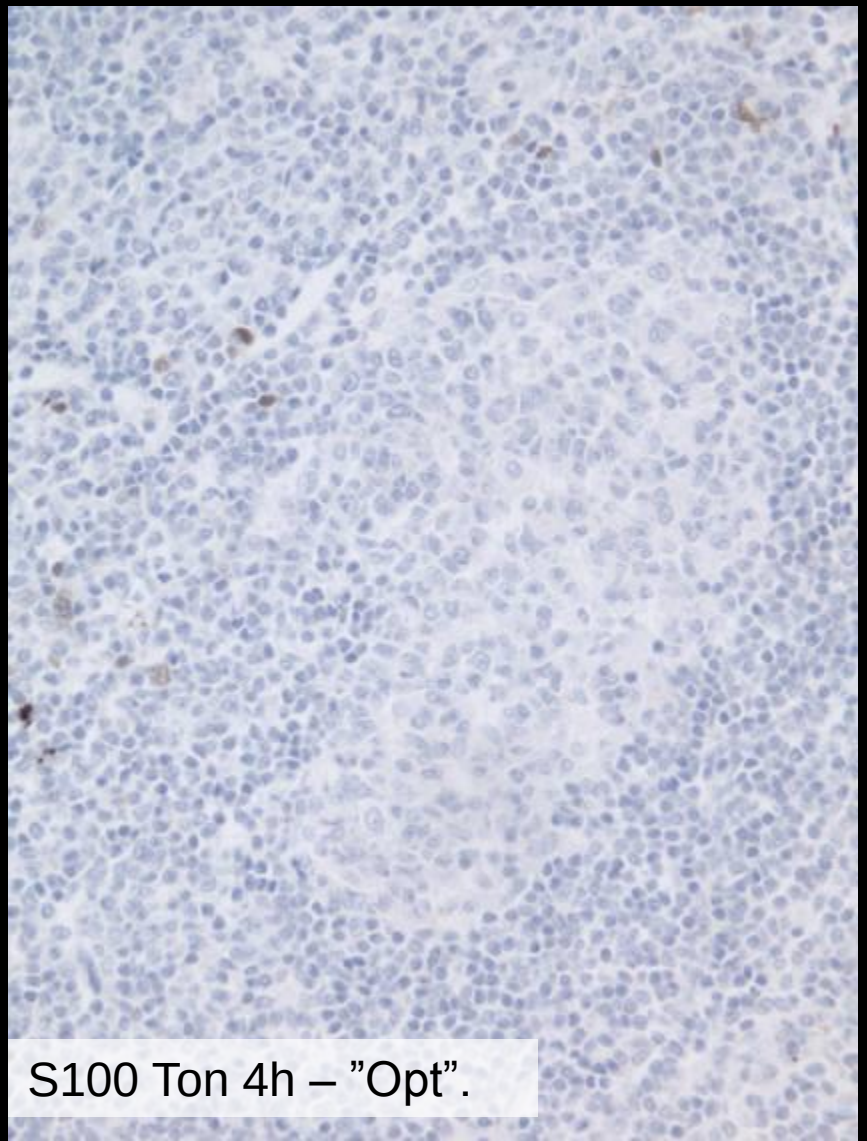
S100 = Soluble in 100% ethanol/alcohol….!



S100 Ton 24h – Opt.



S100 Ton 4h – ”Opt”.

IHC – Protocols and controls for UPT I

NordiQC

Nordic immunohistochemical Quality Control

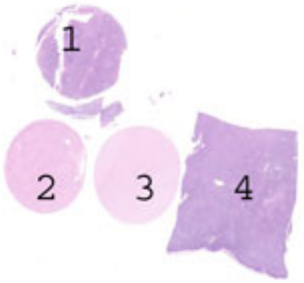
Home ■ Participation ■ Assessments ■ Epitopes ■ Protocols ■ Techniques ■ Links

Recommended CD45 protocols ■ Recommended CD45 control tissue

Assessment Run 37 2013

CD45 (Leucocyte Common Antigen, LCA)

The slide to be stained for CD45 comprised:



1. Tonsil, 2. Liver, 3. Brain, 4. B-CLL

All tissues were fixed in 10 % neutral buffered formalin.

Criteria for assessing a CD45 staining as optimal included:

- A moderate to strong, distinct, predominantly membranous staining reaction of all lymphocytes in all four tissues tested. In the tonsil both the B- and T-cells should be distinctively demonstrated.
- An at least weak to moderate, distinct cytoplasmic staining reaction of the Kupffer cells in the liver and the microglial cells of the brain.
- An at least weak to moderate, predominantly membranous staining reaction of virtually all the neoplastic cells of the B-CLL
- No staining of squamous epithelial cells in the tonsil or hepatocytes in the liver.

214 laboratories participated in this assessment, but 9 participants used an inappropriate antibody (CD45R0 and CD45RA). Of the remaining 205 laboratories 82% achieved a sufficient mark (optimal or good). Antibodies (Abs) used and marks given are summarized in table 1.

Table 3: Proportion of sufficient CD45 results in the two NordiQC runs performed

	Run 15 2005	Run 37 2013
Participants, n=	80	205
Sufficient results	86 %	82 %

IHC – Protocols and controls for UPT I

Table 1. Abs and assessment marks for CD45, run 37

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clones **2B11+PD7/26**	111 1 1	Dako Diagnostic Biosystems Zytomed	64	29	16	4	82 %	85 %
mAb clones **MEM28/ MEM56/MEM55**	1	Invitrogen	0	1	0	0	-	-
mAb clones **PD7/26/16+2B11**	3	Thermo/Neomarkers	0	1	2	0	-	-
mAb clone **X16/99**	9	Leica/Novocastra	6	2	0	1	89 %	100 %
rmAb clone **EP68**	1	Epitomics	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clones **2B11+PD7/26 IS/IR751**	31	Dako	29	2	0	0	100 %	100 %
mAb clones **2B11+PD7/26 760-4279**	14	Ventana/Cell Marque	4	6	4	0	71 %	100 %
mAb clones **2B11+PD7/26 148M-98**	2	Cell Marque	2	0	0	0	-	-
mAb clones **2B11+PD7/26 N1514**	1	Dako	1	0	0	0	-	-
mAb clones **2B11+PD7/26 E005**	1	Linaris	0	0	1	0	-	-
mAb clones **2B11+PD7/26 MAD-004010QD**	1	Master Diagnostica	0	1	0	0	-	-
mAb clones **PD7/26/16+2B11 PM-016**	1	Biocare	0	1	0	0	-	-
mAb clone **RP2/18 760-2505**	21	Ventana	3	11	7	0	67 %	80 %
mAb clone **X16/99 PA0042**	6	Leica	6	0	0	0	100 %	100 %
Total	205		115	54	30	6	-	
Proportion			56 %	26 %	15 %	3 %	82 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

HIER
Conc.
Control

IHC – Protocols and controls for UPT I

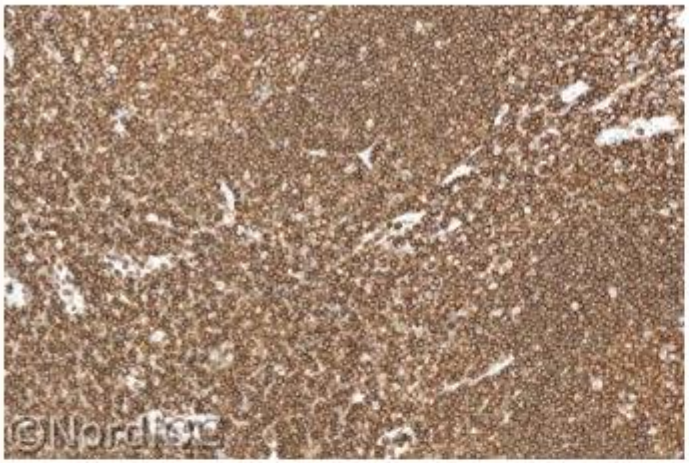


Fig. 1a. Optimal CD45 staining of the tonsil using the mAb clones 2B11+PD7/26 optimally calibrated and with HIER. Virtually all the B- and T-lymphocytes show a strong and distinct membranous staining reaction. No background staining is seen.
Also compare with Figs. 2a – 4a, same protocol.

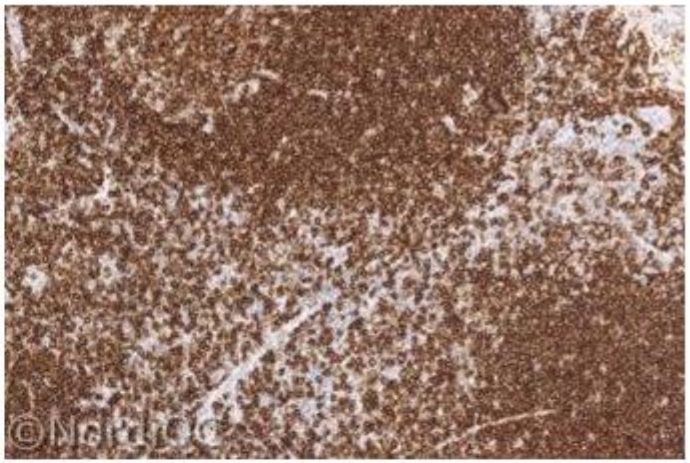


Fig. 1b. Staining for CD45 of the tonsil using the mAb clones 2B11+PD7/26 by protocol settings giving a too low sensitivity (too low concentration of the primary Ab) - same field as in Fig. 1a.
The vast majority of the B- and T-lymphocytes are demonstrated. However also compare with Figs. 2b – 4b, same protocol.

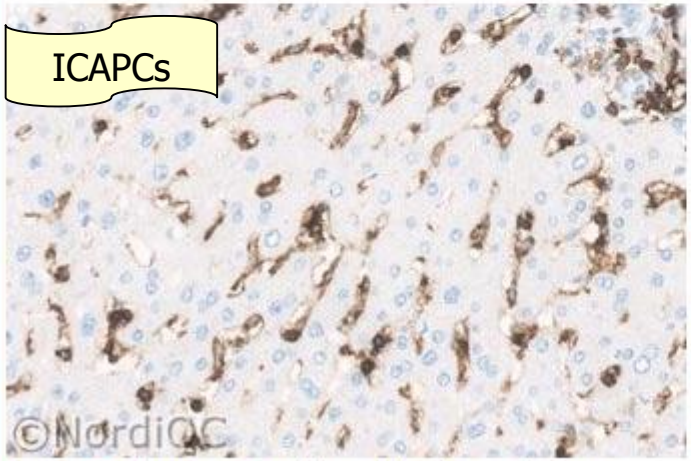


Fig. 2a. Optimal CD45 staining of the liver using same protocol as in Fig. 1a. The lymphocytes show a strong staining reaction, while the Kupffer cells display a weak to moderate staining reaction. The liver cells are negative and no background staining is seen.

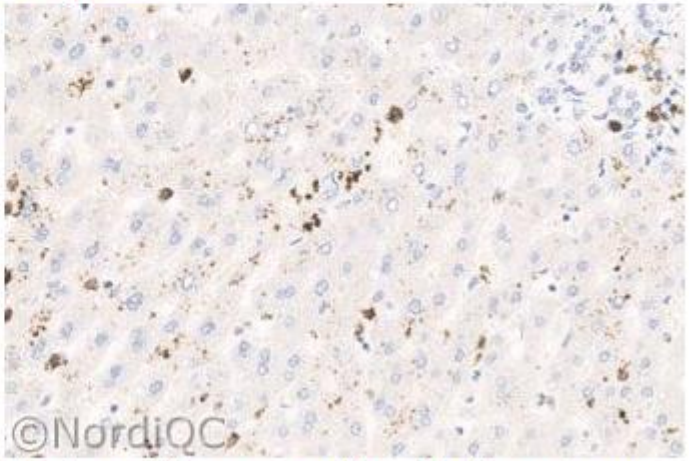


Fig. 2b. Insufficient CD45 staining of the liver using same protocol as in Fig. 1b – same field as in Fig. 2a. Only lymphocytes are demonstrated and the Kupffer cells with a low CD45 expression are false negative.

IHC – Protocols and controls for UPT I

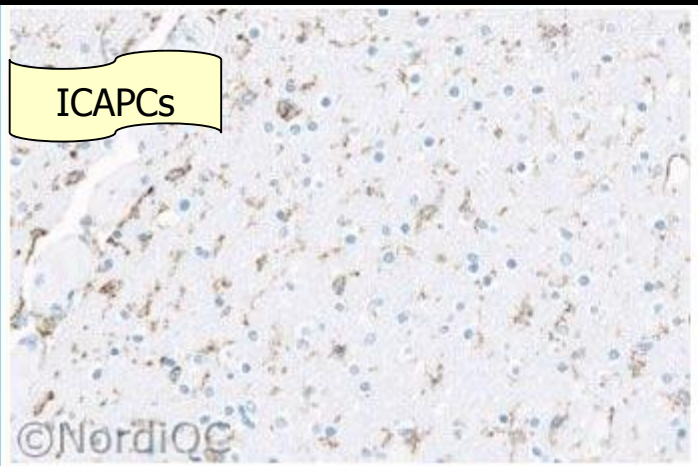


Fig. 3a. Optimal CD45 staining of the brain using same protocol as in Figs. 1a & 2a. The microglial cells with a low CD45 expression are distinctively demonstrated, no background staining is seen.

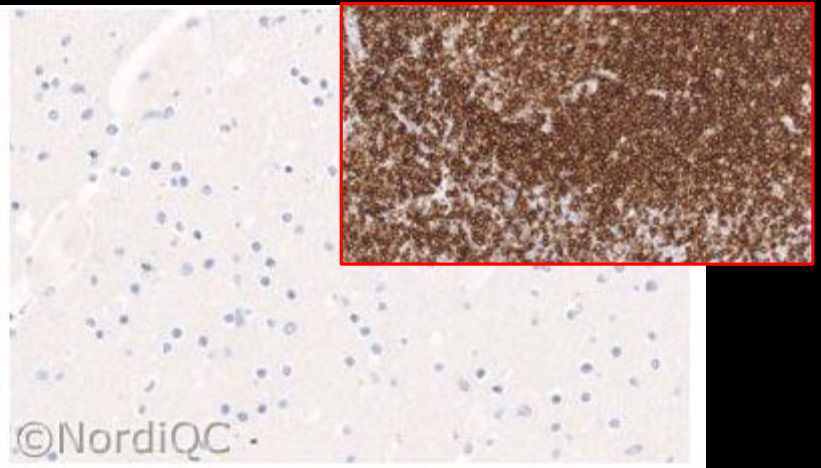


Fig. 3b. Insufficient CD45 staining of the brain using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a. The microglial cells are false negative.

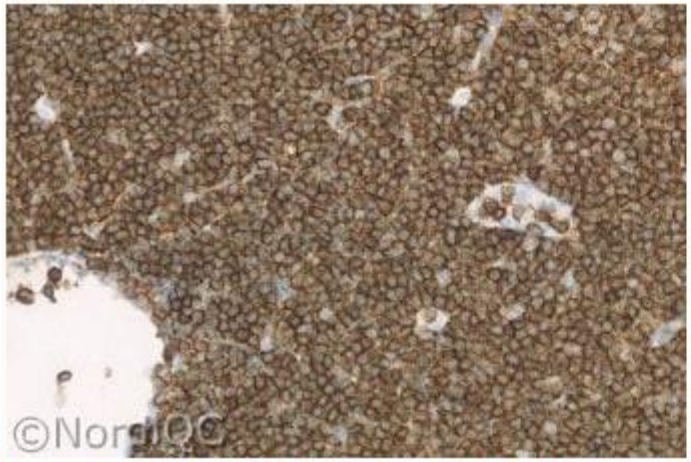


Fig. 4a. Optimal CD45 LCA staining of the B-CLL using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a moderate to strong and distinct membranous staining reaction. No background staining is seen.

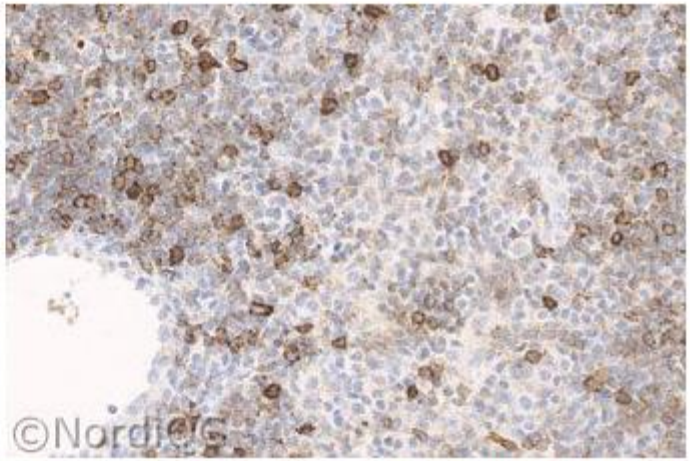


Fig. 4b. Insufficient CD45 LCA staining of the B-CLL using same protocol as in Figs. 1b - 3b. – same field as in Fig. 4a.

The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 4a.

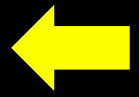
IHC – Protocols and controls for UPT I

Table 1. Abs and assessment marks for CD45, run 37

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clones **2B11+PD7/26**	111 1 1	Dako Diagnostic Biosystems Zytomed	64	29	16	4	82 %	85 %
mAb clones **MEM28/ MEM56/MEM55**	1	Invitrogen	0	1	0	0	-	-
mAb clones **PD7/26/16+2B11**	3	Thermo/Neomarkers	0	1	2	0	-	-
mAb clone **X16/99**	9	Leica/Novocastra	6	2	0	1	89 %	100 %
rmAb clone **EP68**	1	Epitomics	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clones **2B11+PD7/26 IS/IR751**	31	Dako	29	2	0	0	100 %	100 %
mAb clones **2B11+PD7/26 760-4279**	14	Ventana/Cell Marque	4	6	4	0	71 %	100 %
mAb clones **2B11+PD7/26 148M-98**	2	Cell Marque	2	0	0	0	-	-
mAb clones **2B11+PD7/26 N1514**	1	Dako	1	0	0	0	-	-
mAb clones **2B11+PD7/26 E005**	1	Linaris	0	0	1	0	-	-
mAb clones **2B11+PD7/26 MAD-004010QD**	1	Master Diagnostica	0	1	0	0	-	-
mAb clones **PD7/26/16+2B11 PM-016**	1	Biocare	0	1	0	0	-	-
mAb clone **RP2/18 760-2505**	21	Ventana	3	11	7	0	67 %	80 %
mAb clone **X16/99 PA0042**	6	Leica	6	0	0	0	100 %	100 %
Total	205		115	54	30	6	-	
Proportion			56 %	26 %	15 %	3 %	82 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

HIER
Conc.
Control



IHC – Protocols and controls for UPT I

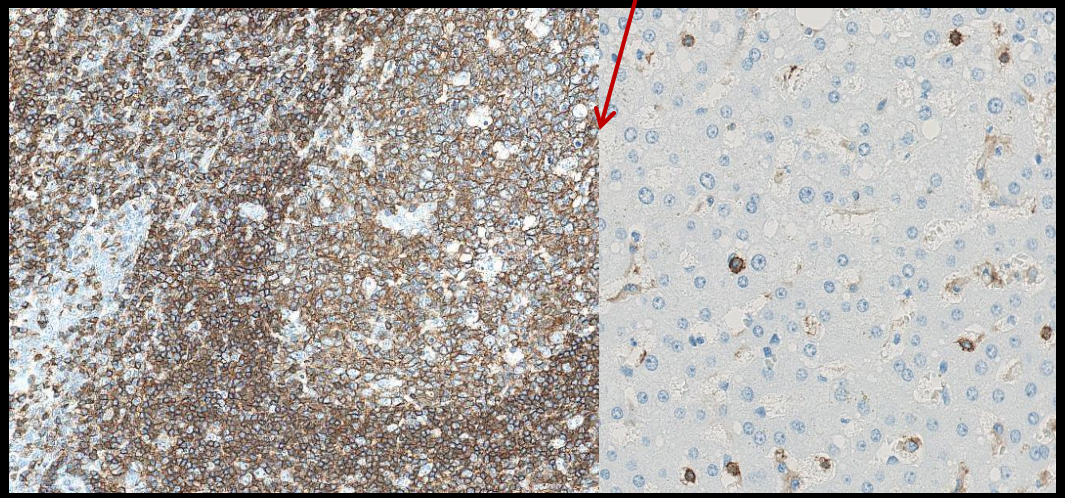
Table 1. Recommended Staining Protocols for CONFIRM anti-CD45, LCA (RP2/18)

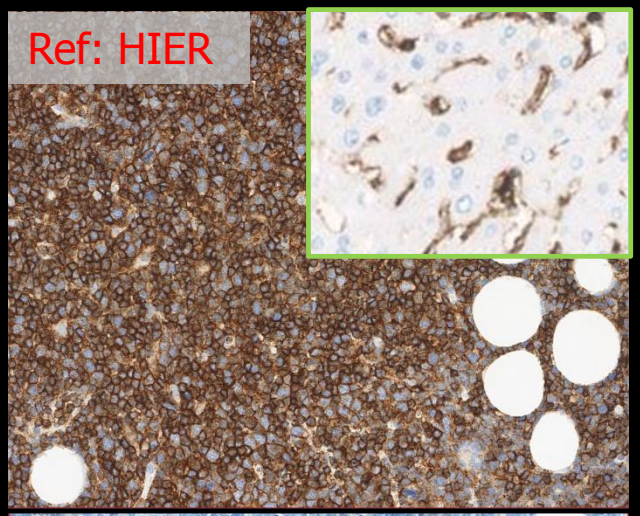
Procedure Type	Platform or Method	
	NexES IHC	BenchMark Series
Deparaffinization	Off Line	Selected
Cell Conditioning (Antigen Unmasking)	None required	None required
Enzyme (Protease)	None required	None required
Antibody (Primary)	Approximately 16 minutes, 37° C	Approximately 16 minutes, 37° C
A/B Block (Biotin Blocking)	Optional	Optional
Amplify (Amplification)	Optional	Optional
Counterstain (Hematoxylin)	Hematoxylin II, 2 to 4 minutes	Hematoxylin II, 2 to 4 minutes
Post Counterstain	Bluing, 2 to 4 minutes	Bluing, 2 to 4 minutes

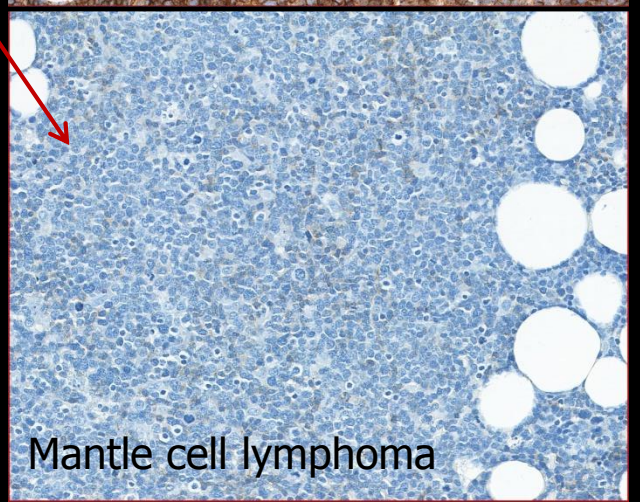
VENTANA®

CONFIRM™ anti-CD45, LCA (RP2/18) Primary Antibody

Catalog Number 760-2505







IHC – Protocols and controls for UPT I

CD45R0

T-cells

mAb
UCHL1

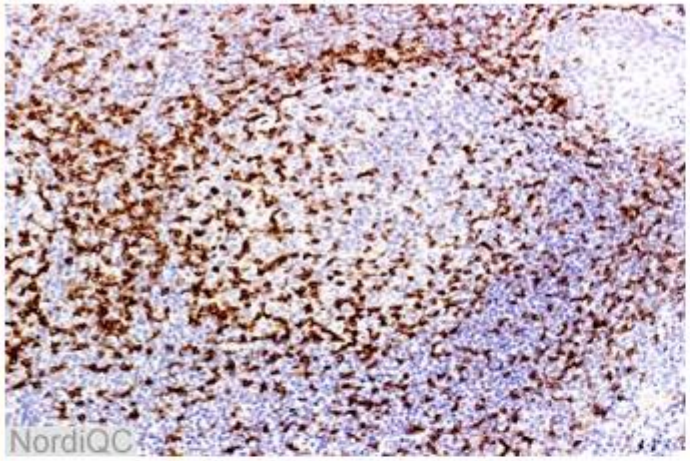


Fig. 4a. Staining for CD45 in the tonsil using an inappropriate antibody to CD45R0. Only the T-cells are demonstrated while the B-cells in germinal center and mantle zone are negative.

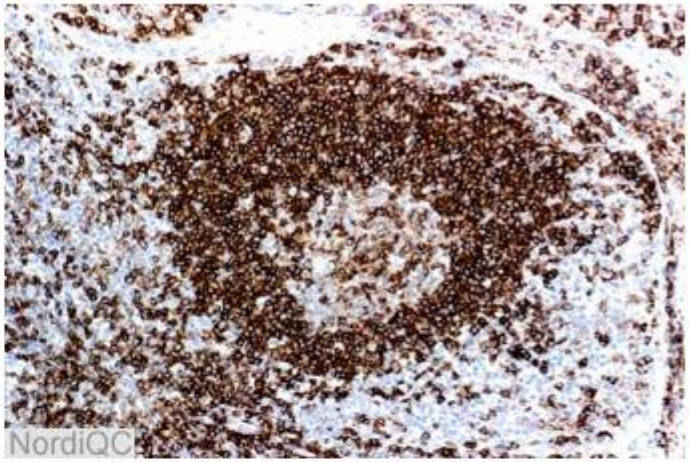


Fig. 4b. Staining for CD45 in the tonsil using an inappropriate antibody to CD45RA. The majority of B-cells are demonstrated while the T-cells are negative.

CD45RA

B-cells

mAb
4KB5

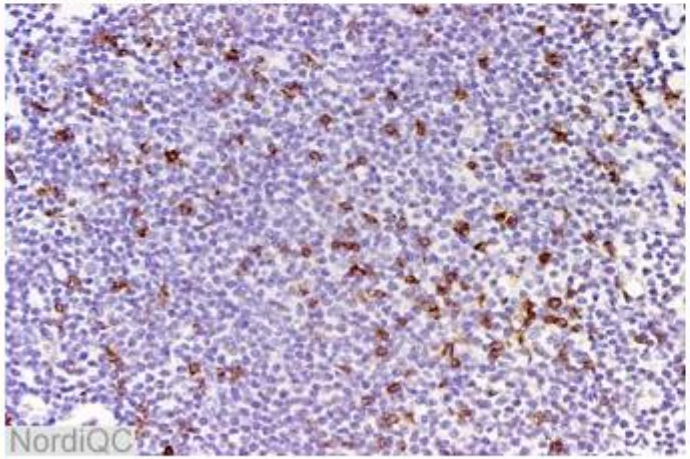


Fig. 5a. Staining for CD45 in the CLL using an inappropriate antibody to CD45R0. The neoplastic cells are negative and only the normal T-cells are stained.

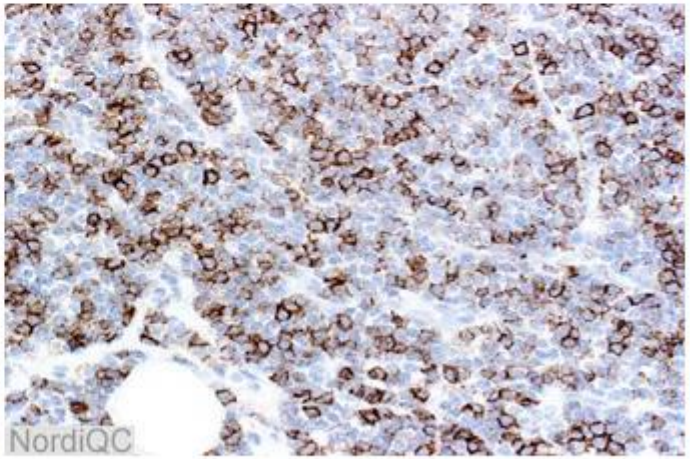
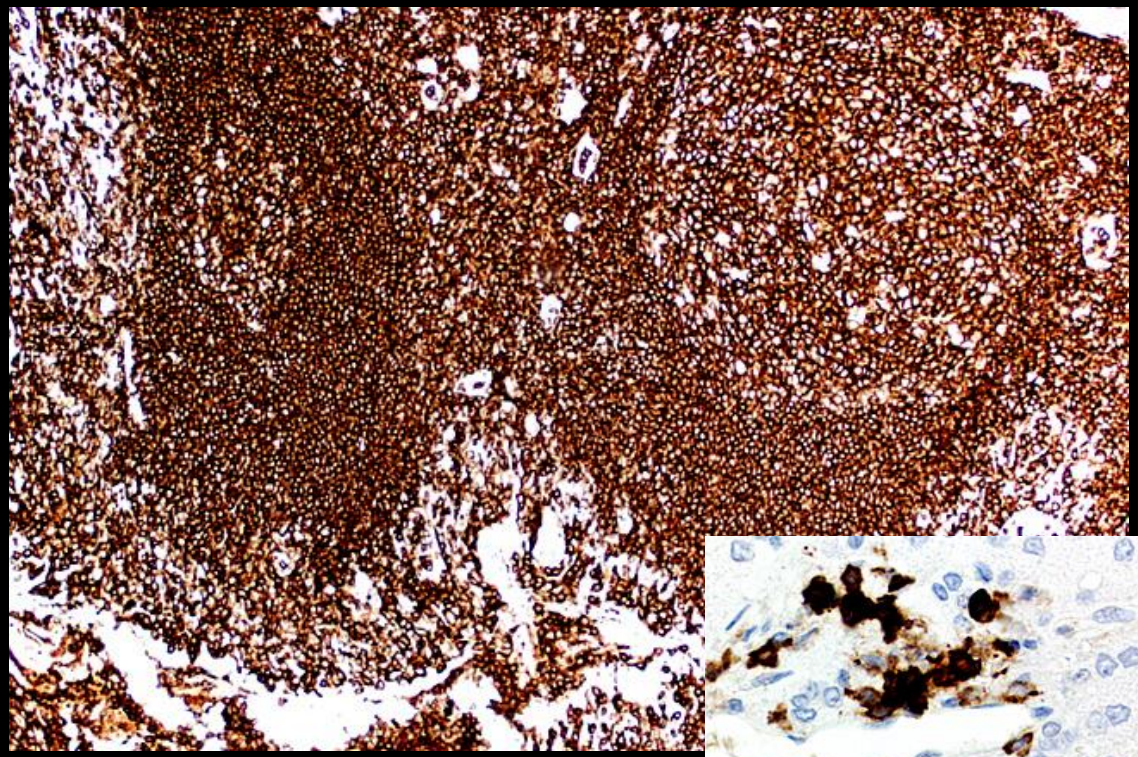


Fig. 5b. Staining for CD45 in the CLL using an inappropriate antibody to CD45RA. The majority of the neoplastic cells are stained. However, compare with Fig. 4b - the T-cells are not demonstrated. T-cell lymphomas will not be identified with CD45RA.

IHC – Protocols and controls for UPT I

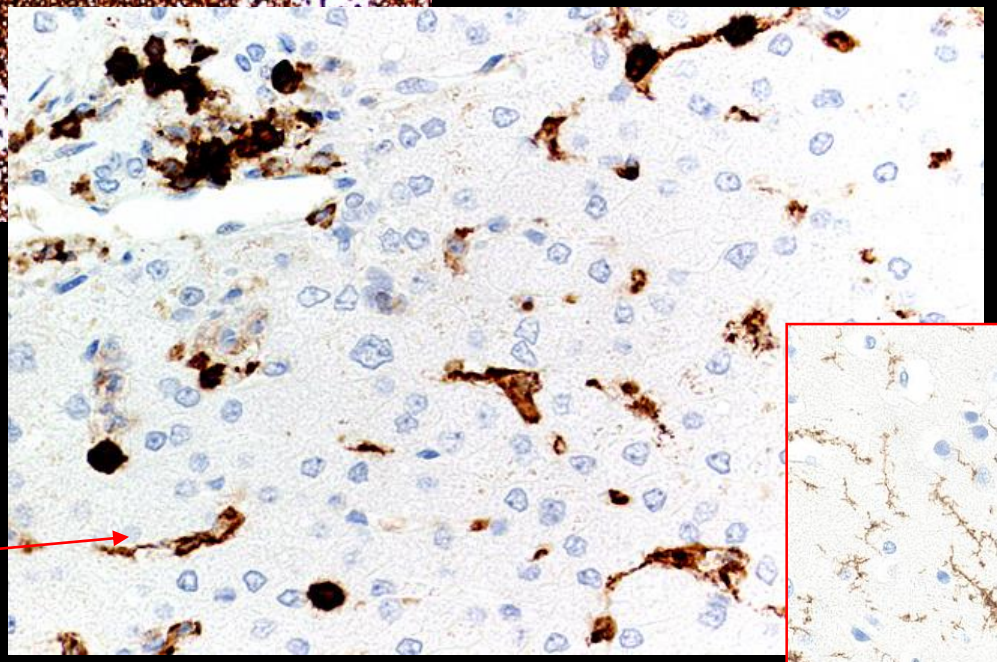


CD45

Tonsil (right ab)

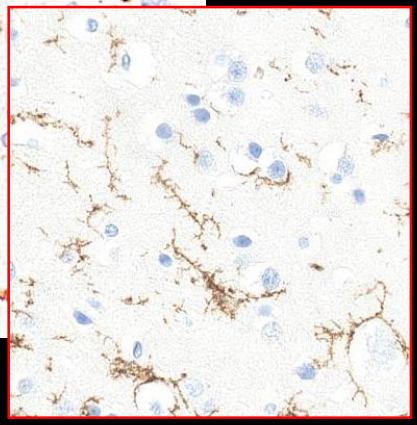
Liver (calibration)

Brain



ICAPCs:

Kupffer cell
(macrophages)



IHC – Protocols and controls for UPT I

UPT I: CD45 (LCA)
Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb 2B11 + PD7/26	HIER	1:100-1.000	2- & 3-step	Dako Ventana	2-step 3-step
mAb X16/99	HIER Ci, TRS low	1:25-50	3-step	Leica	3-step
mAb RP2/18	HIER High	-	-	Ventana	3-step

IHC – Protocols and controls for UPT I

NordiQC

Nordic immunohistochemical Quality Control

Home ■ Participation ■ Assessments ■ Epitopes ■ Protocols ■ Techniques ■ Links

Recommended VIM protocols ■ Recommended VIM control tissue

Assessment Run 30 2010

Table 1. Abs and assessment marks for VIM, run 30

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **V9**	48 7 7 5 1 1 1 1 1	Dako BioGenex Novocastra/ Leica NeoMarkers Cell Marque BioCare Monosan Zymed Zytomed	45	17	6	4	86 %	91 %
mAb clone **Vim 3B4**	31 1 1	Dako APR Progen	8	16	9	0	73 %	94 %
rmAb clone **SP20**	4 1	NeoMarkers Master Diagnostica	2	0	3	0	20 %	100 %
Ready-To-Use Abs								
mAb clone **V9, 790-2917**	30	Ventana	11	19	0	0	100 %	100 %
mAb clone **V9, IR630**	13	Dako	12	1	0	0	100 %	100 %
mAb clone **V9, AM074-5M**	2	BioGenex	1	1	0	0	-	-
mAb clone **V9, PM048**	1	Biocare	0	1	0	0	-	-
mAb clone **V9, 347M-18**	3	Cell Marque	0	1	1	1	-	-
mAb clone **V9, N1521**	1	Dako	0	1	0	0	-	-
mAb clone **Vim 3B4, 760-2512**	3	Ventana	0	0	3	0	-	-
mAb clone **SRL, PA0033**	1	Novocastra/Leica	0	0	0	1	-	-
Total	164		79	57	22	6	-	-
Proportion			48 %	35 %	13 %	4 %	83 %	-

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

83 % suffcient

+ HIER !

V9 sup. to 3B4

SP20 good alternative

IHC – Protocols and controls for UPT I

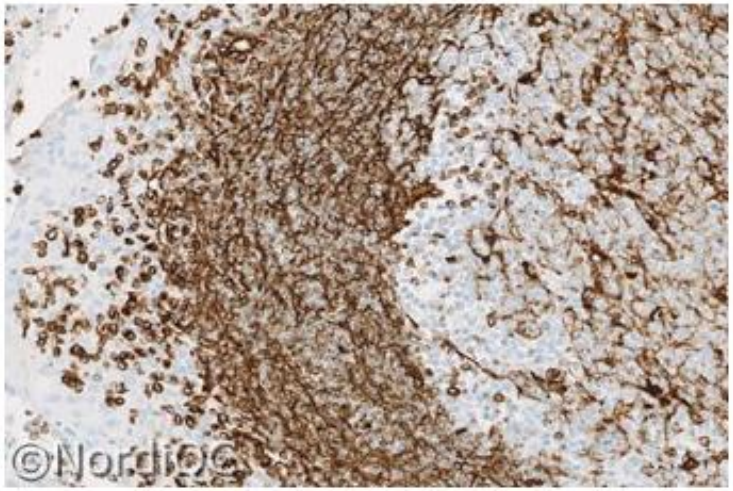


Fig. 1a. Optimal VIM staining of the tonsil using the mAb clone V9 carefully calibrated after HIER. The intraepithelial lymphocytes, the mantle zone B-cells and the germinal centre macrophages show a strong and distinct staining. No staining is is seen in the squamous epithelial cells.

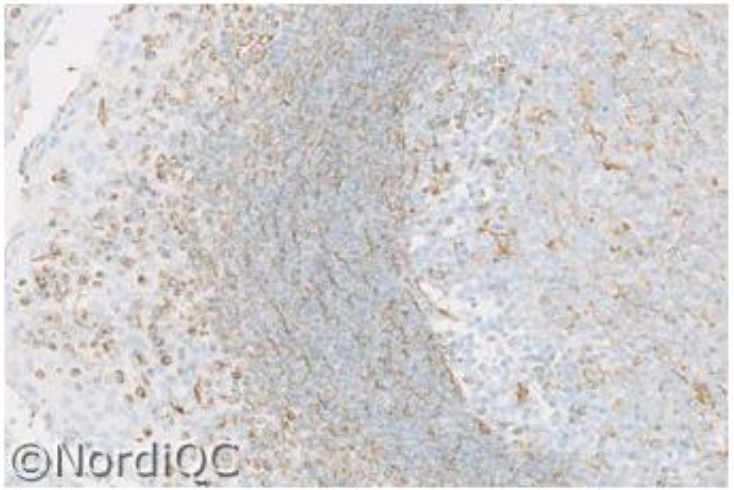


Fig. 1b. Insufficient VIM staining of the tonsil, using the mAb clone V9 in a too low concentration – same field as in Fig. 1a. Both the proportion and intensity of the demonstrated cells is significantly reduced. Also compare with Figs. 2b & 3b – same protocol.

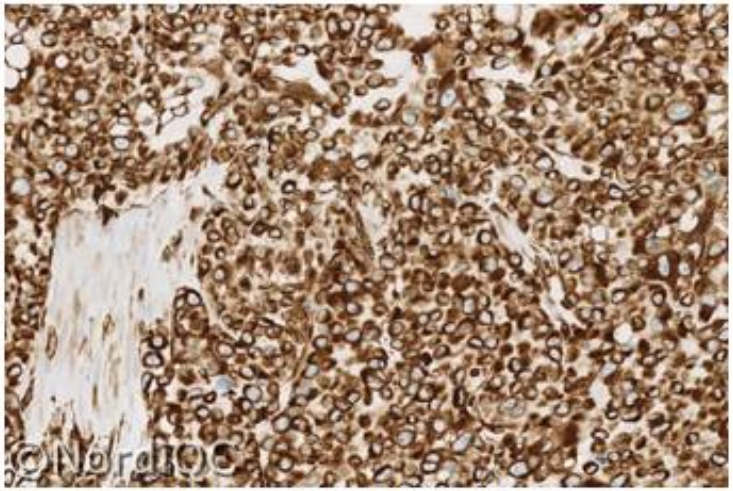


Fig. 2a. Optimal VIM staining of the melanoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining.

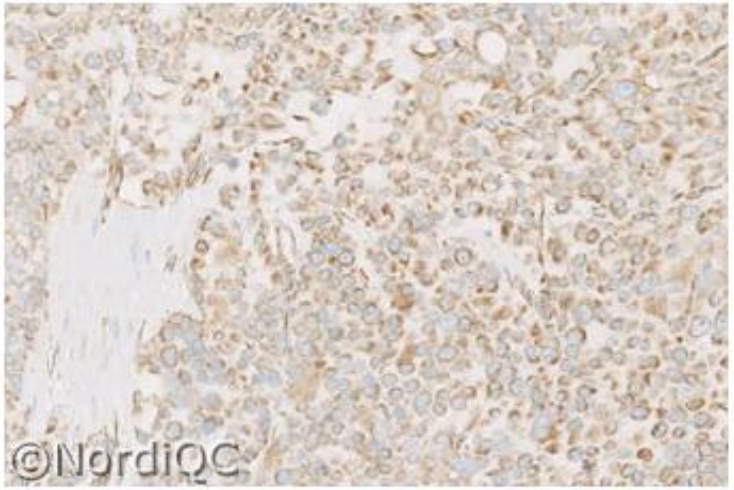


Fig. 2b. Insufficient VIM staining of the melanoma using same protocol as in Fig. 1b - same field as in Fig. 2a. The majority of the neoplastic cells only show a weak or equivocal staining. Also compare with Fig. 3b - same protocol.

V9 too dilute

IHC – Protocols and controls for UPT I

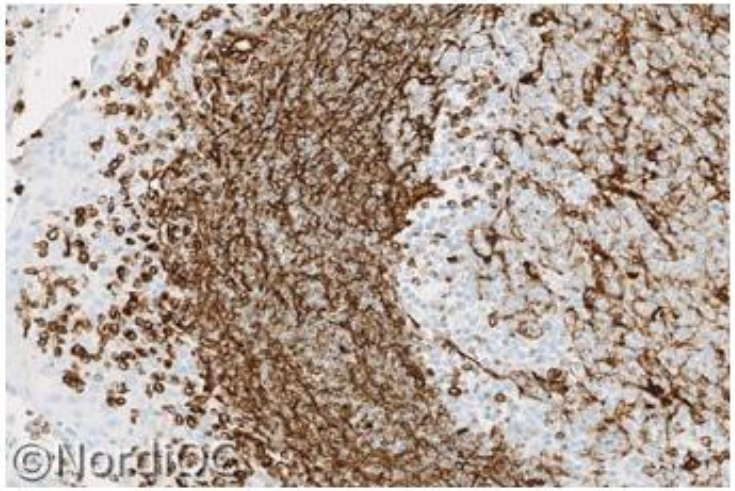


Fig. 1a. Optimal VIM staining of the tonsil using the mAb clone V9 carefully calibrated after HIER. The intraepithelial lymphocytes, the mantle zone B-cells and the germinal centre macrophages show a strong and distinct staining. No staining is is seen in the squamous epithelial cells.

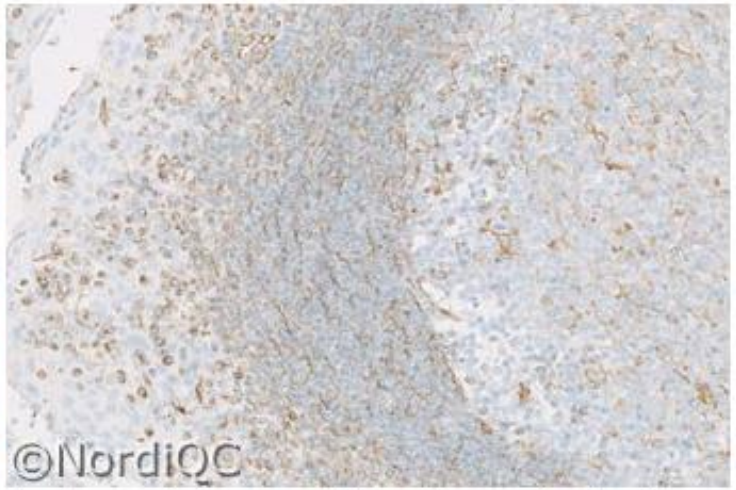


Fig. 1b. Insufficient VIM staining of the tonsil, using the mAb clone V9 in a too low concentration – same field as in Fig. 1a. Both the proportion and intensity of the demonstrated cells is significantly reduced. Also compare with Figs. 2b & 3b – same protocol.

V9 too dilute

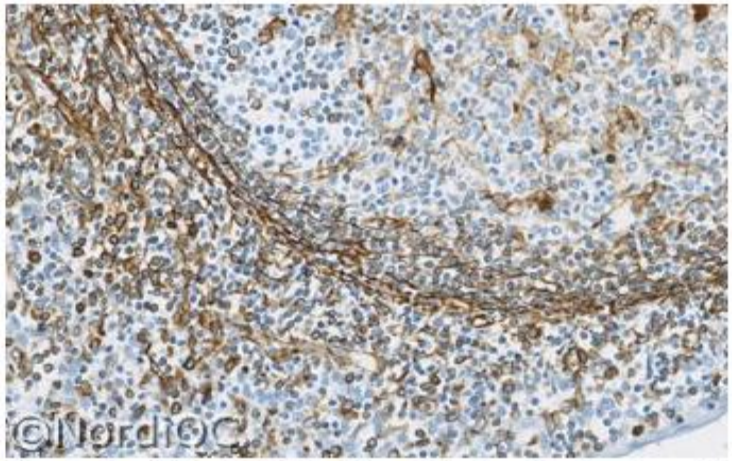


Fig. 4a. Insufficient VIM staining of the tonsil using the mAb clone 3B4 with proteolytic pre-treatment. The germinal centre macrophages and endothelial cells show a moderate staining reaction, whereas the lymphocytes virtually are negative due to excessive proteolysis and digestion of the fragile membranes. Also compare with Fig. 4b - same protocol.

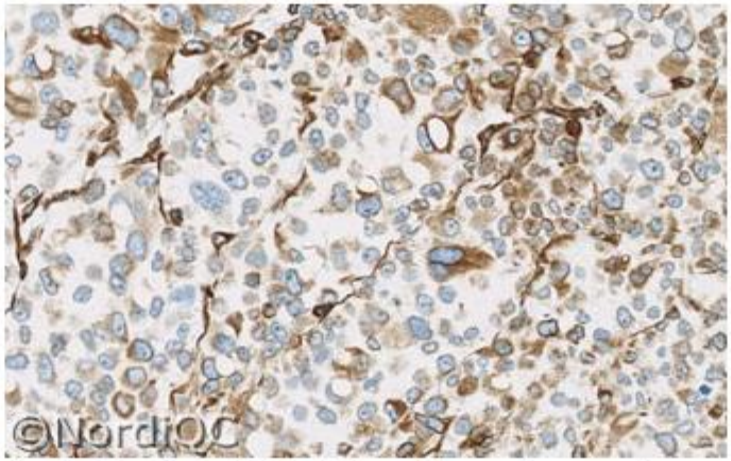
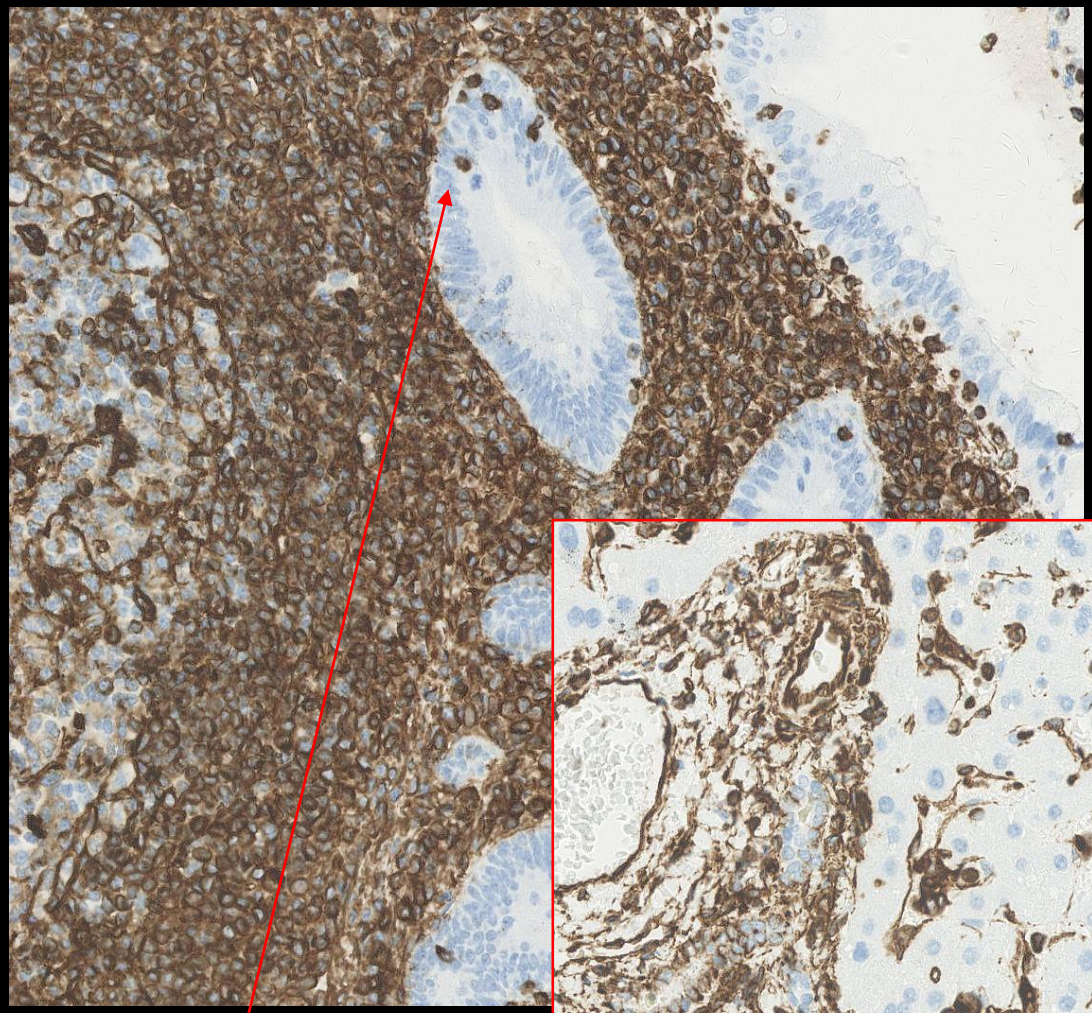


Fig. 4b. Insufficient VIM staining of the melanoma using same protocol as in Fig. 4a - mAb clone 3B4 with proteolytic pre-treatment. The neoplastic cells only show a weak and equivocal staining as the cytoplasmic compartment is digested and only the nuclei are left in the neoplastic cells. Also compare with Fig. 2a - same tissue.

3B4 + prot.

IHC – Protocols and controls for UPT I

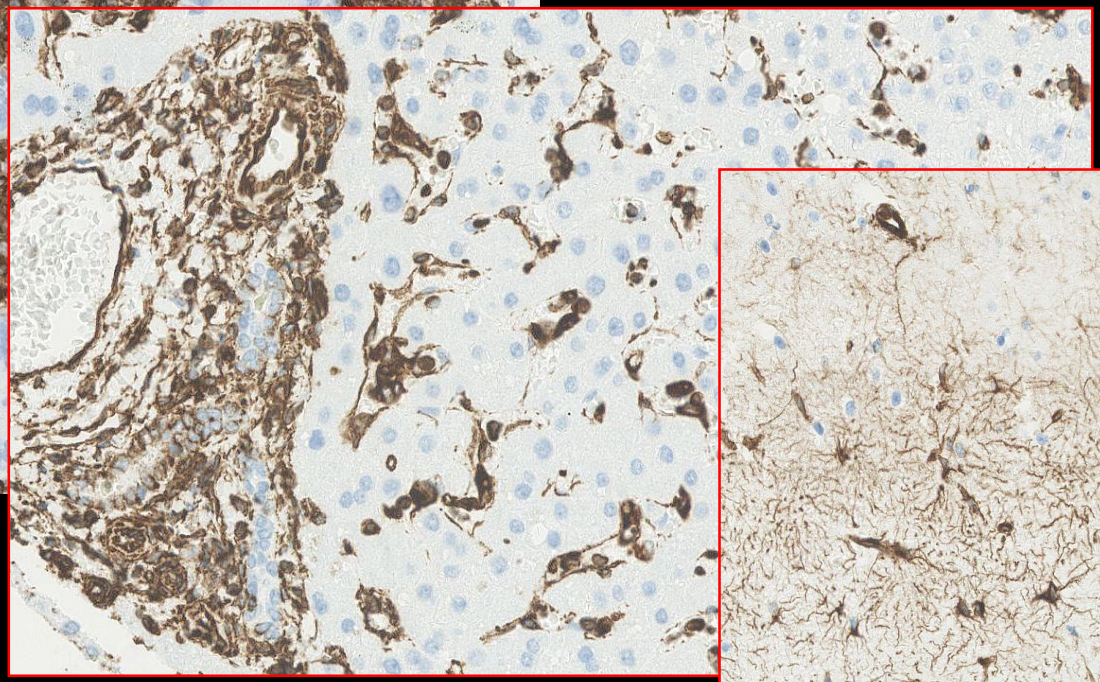


VIM

Appendix

Liver

Brain



ICAPCs:

Isolated lymphocytes & macrophages

IHC – Protocols and controls for UPT I

UPT I: Vimentin

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb 3B4	HIER High	1:150-1.500	2- & 3-step	-	-
mAb V9	HIER	1:100-8.000	2- & 3-step	Dako Ventana	2- & 3-step 2- & 3-step
rmAb SP20	HIER High	1:100-400	2- & 3-step	-	-

IHC – Protocols and controls for UPT I

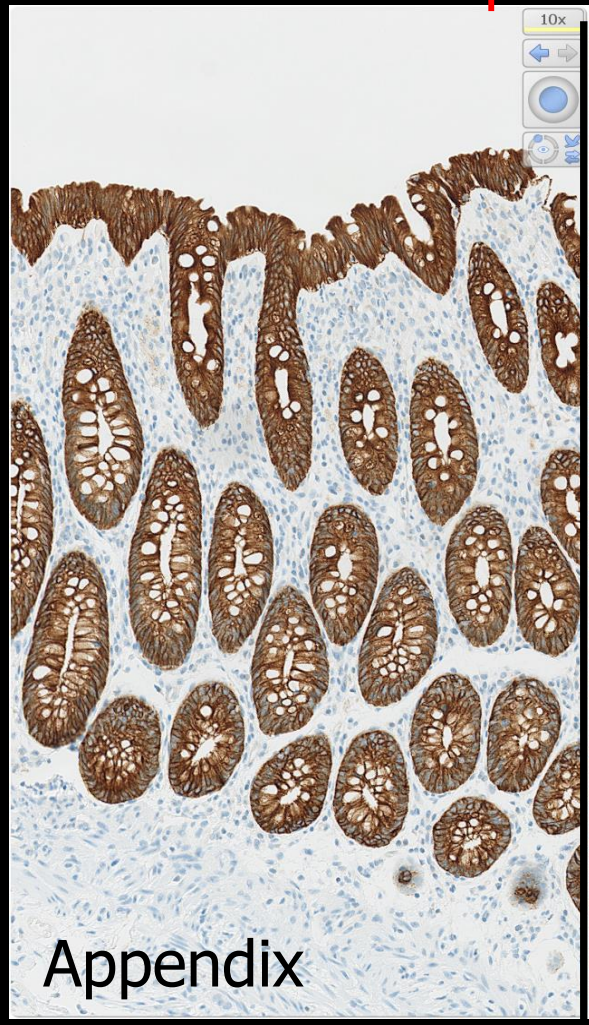
CKs	Recommendable clones (conc.)	Less successful clones (conc.)	RTU ”plug and play” giving optimal result
CK-Low	mAb 5D3 (8,18) mAb B22.1+B23.1(8,18) mAb C51 (18) mAb DC10 (18) mAb TS1 (8) rmAb EP17 (8,18,19) *mAb CAM5.2 (7,8,18,19)*	mAb 35BH11	Dako: mAb DC10 Leica: mAb 5D3 VMS: mAb B22.1+B23.1
CK-High	mAb XM26 (5) mAb LL002 (14) rmAb EP1601Y (5) rmAb SP27 (5) rmAb SP54 (14) *mAb D5/16B4 (5/6)*	mAb 34BH12	VMS: rmAb SP27

IHC – Protocols and controls for UPT I

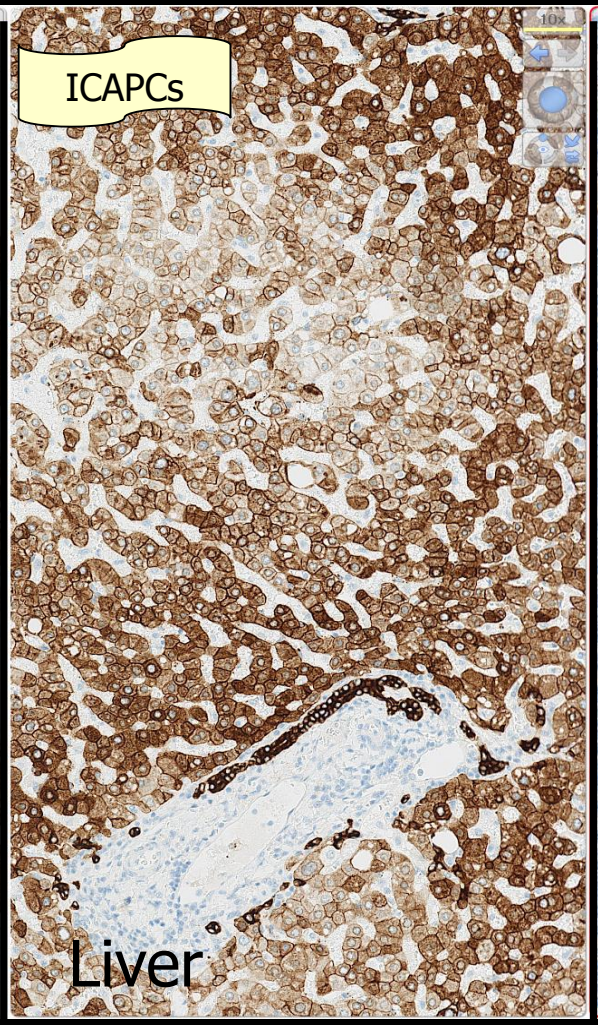
CKs	Positive tissue control HE	Positive tissue control LE	Negative tissue control NE
CK-Low	Liver: Epithelial cells of bile ducts Appendix: Epithelial cells	Liver: Hepatocytes Tonsil: Fibroblastic reticulum cells	Tonsil: Lymphocytes Appendix: Smooth muscle cells
CK-High	Esophagus: Basal squamous epithelial cells	Esophagus: Intermediate squamous epithelial cells	Appendix: Epithelial cells

IHC – Protocols and controls for UPT I

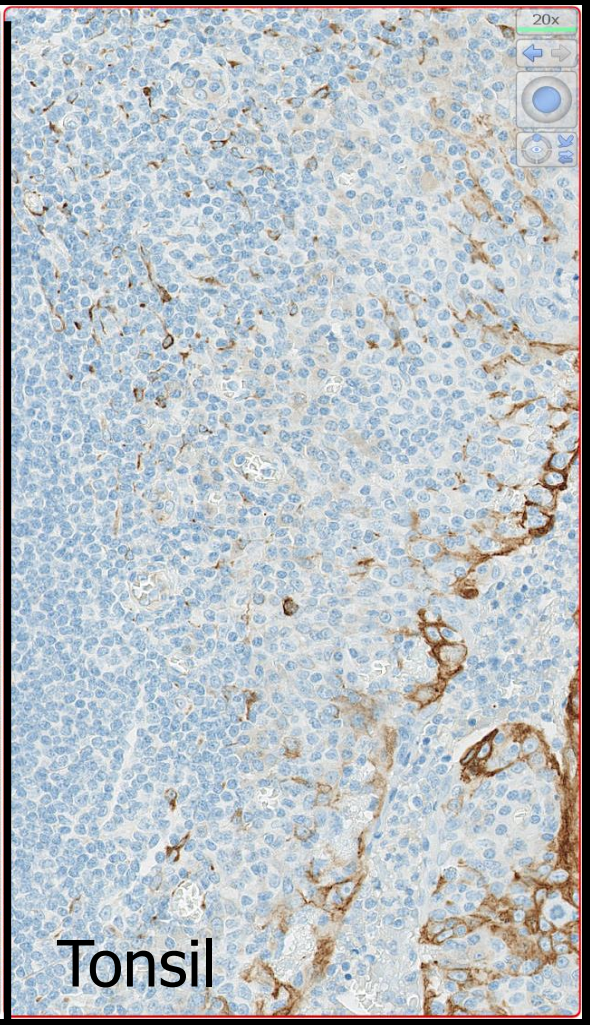
CK-LMW reaction pattern



A moderate to strong distinct cytoplasmic staining reaction in virtually all columnar epithelial cells.



An at least weak to moderate distinct cytoplasmic staining reaction of the vast majority of the hepatocytes (membrane accentuation).



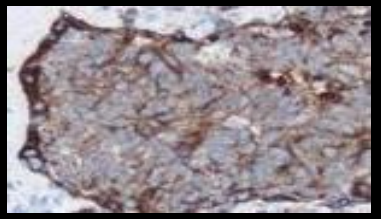
Scattered epithelial cells and fibroblastic reticulum cells can show a weak to moderate staining. No reaction in the vast majority of lymphocytes.

IHC – Protocols and controls for UPT I

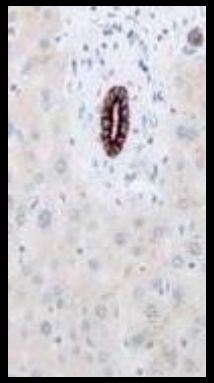
Table 1. Abs and assessment marks for CK-LMW, run 38

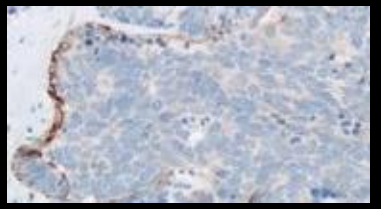
Concentrated Abs	Reactiviti	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **5D3**	CK 8/18	20 4 3 2 2 1	Leica/Novocastra Thermo/Neomarkers Monosan Biocare Biogenex Vector	10	11	9	2	66 %	95 %
mAb clones **B22.1&B23.1**	CK 8/18	1	Cell Marque	0	1	0	0	-	-
mAb clone **BS83**	CK 8/18	1	Nordic Biosite	0	1	0	0	-	-
mAb clone **C51**	CK 18*	4	Invitrogen/Zymed	3	1	0	0	-	-
mAb clone **CAM5.2**	CK 8 (7)	26 2 1	Becton Dickenson Immunologic Zytomed	2	10	13	4	41 %	100 %
mAb clone **DC10**	CK 18	18 9 2 1 1 1	Dako Leica/Novocastra Thermo/Neomarkers Biogenex ID Labs Invitrogen/Zymed	17	14	1	0	97 %	97 %
mAb clone **K8.8+DC10**	CK 8/18	1	Thermo/Neomarkers	0	0	1	0	-	-
mAb clone **TS1**	CK 8	3 2 1	Leica/Novocastra Thermo/Neomarkers Gene Tech	4	1	1	0	83 %	100 %
mAb clone **TS1 + mAb clone DC10**	CK 8/18	1	Homemade cocktail: Thermo/Neomarkers	0	1	0	0	-	-
rmAb clone **EP17**	CK 8	3	Epitomics	3	0	0	0	-	-
rmAb clone **EP1628Y**	CK 8	1	Epitomics	1	0	0	0	-	-
Ready-To-Use Abs									
mAb clone **5D3 PM056**	CK 8/18	1	Biocare	0	1	0	0	-	-
mAb clone **5D3 PA0067**	CK 8/18	6	Leica/Novocastra	5	1	0	0	100 %	100 %
mAb clone **5D3 RTU-5D3**	CK 8/18	2	Leica/Novocastra	2	0	0	0	-	-
mAb clone **35betaH11 760-2637**	CK 8	5	Ventana/Cell Marque	0	0	2	3	-	-
mAb clone **35betaH11 MON-RTU1075**	CK 8	1	Monosan	0	0	1	0	-	-
mAb clones **B22.1&B23.1 760-4344**	CK 8/18	17	Ventana/Cell Marque	15	2	0	0	100 %	100 %
mAb clones **B22.1&B23.1 MAD-001005QD**	CK 8/18	1	Master Diagnostica	1	0	0	0	-	-
mAb clone **CAM5.2 790-4555**	CK 8 (7)	2	Ventana	1	1	0	0	-	-
mAb clone **DC10 IR618**	CK 18	15	Dako	14	1	0	0	100 %	100 %
Total		161		78	46	28	9		
Proportion				48 %	29 %	17 %	6 %	77 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.



Optimal





Poor

Clone !

Retrieval !

Concentration !

IHC – Protocols and controls for UPT I

Table 4: Proportion of sufficient results for CK-LMW in the six NordiQC runs performed

	Run 9 2003	Run 16 2006	Run 20 2007	Run 25 2009	Run 33 2011	Run 38 2013
Participants, n=	54	66	74	99	141	161
Sufficient results	57 %	45 %	67 %	66 %	64 %	77 %

- Use of Abs giving a low sensitivity
- Inappropriate epitope retrieval
- Misleading data-sheets

Table 3. Pass rates for four CK-LMW clones using different epitope retrieval methods

	Pass rate for run 16, 20, 25, 33 & 38							
	Total		HIER		Prot. pre-treatm.		HIER + proteolysis	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb clone CAM 5.2	126	56 (44 %)	41	14 (34 %)	66	39 (59 %)	9	3 (33 %)
mAb clone DC10	159	151 (95 %)	158	149 (95 %)	0	0	2	2 (100 %)
mAb clone 5D3	107	66 (62 %)	80	65 (81 %)	27	3 (11 %)	0	0
mAb clone 35BH11	54	6 (11 %)	32	4 (13 %)	22	2 (11 %)	0	0

IHC – Protocols and controls for UPT I

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Participants, n=	54	66	74	99	141	161
Sufficient results	57 %	45 %	67 %	66 %	64 %	77 %

- Use of Abs giving a low sensitivity
- Inappropriate epitope retrieval
- Misleading data-sheets

CLONE: 5D3

Code	Name	Configuration	Use
PA0067	7ml CK8/18 Bond RTU Primary	Bond ready to use reagent	P(HIER)
5D3-L-CE	1ml NCL-L-5D3	Liquid Concentrated Monoclonal Antibody	P (ENZYME)

Table 3. Pass rates for four CK-LMW clones using different epitope retrieval methods

	Pass rate for run 16, 20, 25, 33 & 38							
	Total		HIER		Prot. pre-treatm.		HIER + proteolysis	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb clone CAM 5.2	126	56 (44 %)	41	14 (34 %)	66	39 (59 %)	9	3 (33 %)
mAb clone DC10	159	151 (95 %)	158	149 (95 %)	0	0	2	2 (100 %)
mAb clone 5D3	107	66 (62 %)	80	65 (81 %)	27	3 (11 %)	0	0
mAb clone 35BH11	54	6 (11 %)	32	4 (13 %)	22	2 (11 %)	0	0

IHC – Protocols and controls for UPT I

Table 2. Optimal results for CK-LMW using concentrated antibodies on the 3 main IHC systems*

Concentrated antibodies	Dako Autostainer Link / Classic		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **5D3**	36 % 4/11**	-	0 % 0/5	-	67 % 2/3	-
mAb clone **DC10**	67 % 2/3	-	64 % 7/11	-	50 % 3/6	-

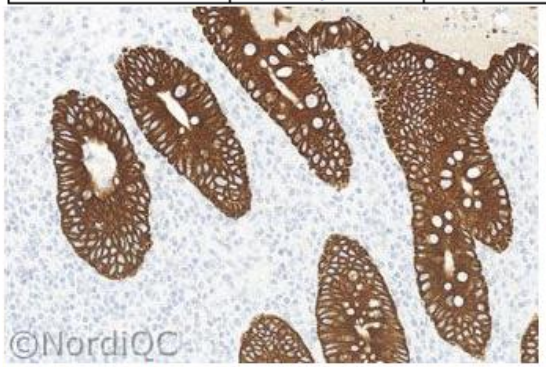


Fig. 1a. Optimal staining for CK-LMW of the appendix using the mAb clone 5D3 for CK 8/18 optimally calibrated, HIER in an alkaline buffer and performed on the Autostainer Link stainer, Dako.
Virtually all the columnar epithelial cells show a strong cytoplasmic staining reaction, while no background staining is seen.
Also compare with Figs. 2a - 3a, same protocol.

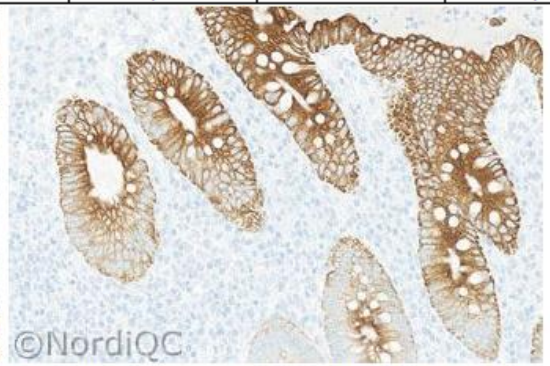


Fig. 1b. Insufficient staining for CK-LMW of the appendix using the mAb clone 5D3 for CK 8/18, HIER in an alkaline buffer and performed on the BenchMark ULTRA stainer, Ventana – same field as in Fig. 1a. The mAb clone 5D3 gave same insufficient staining result by all protocol settings used on the BenchMark stainers.
Only the luminal columnar epithelial cells show a moderate to strong cytoplasmic staining, while virtually no staining is seen in the basal part of the crypts.
Also compare with Figs. 2b - 3b, same protocol.

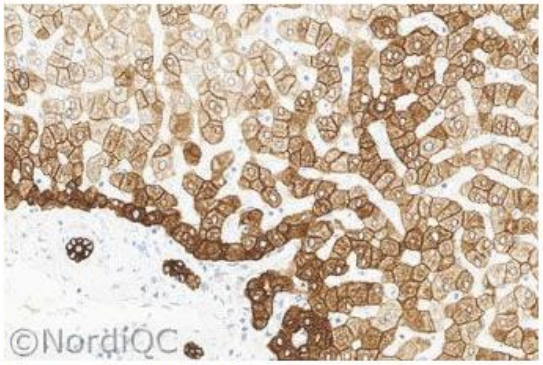


Fig. 2a. Optimal staining for CK-LMW of the liver using the same protocol as in Fig. 1a.
The majority of the hepatocytes show a distinct, moderate staining reaction with a membrane enhancement, while the columnar epithelial cells of the bile ducts show a strong cytoplasmic staining reaction.
Same protocol used in Figs. 1a - 3a.

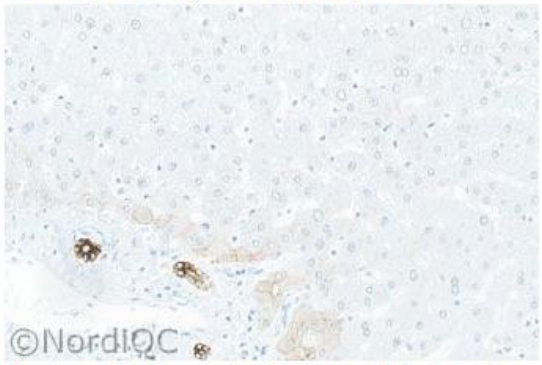
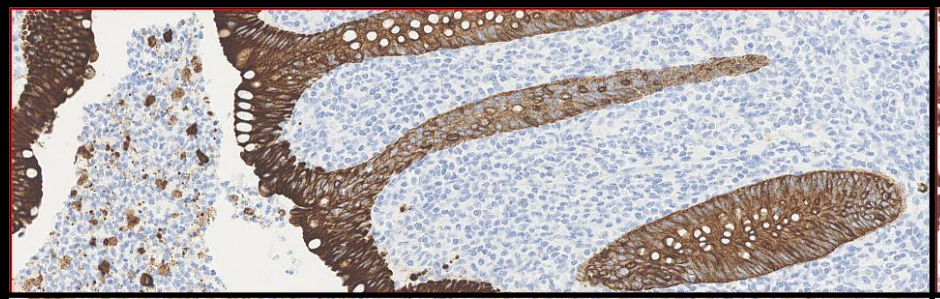
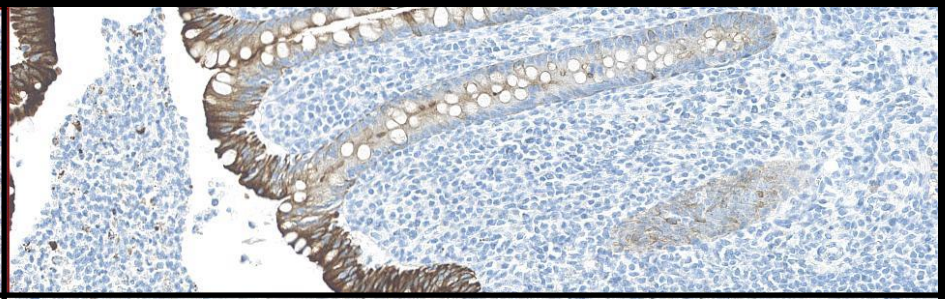
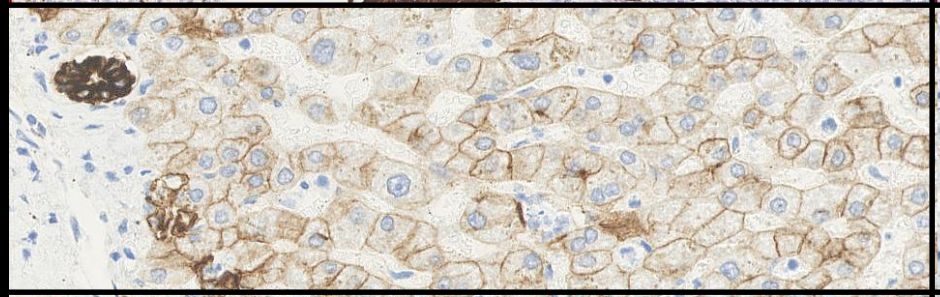
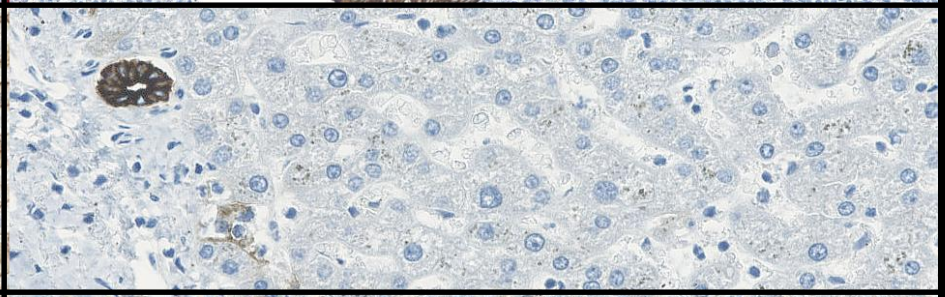
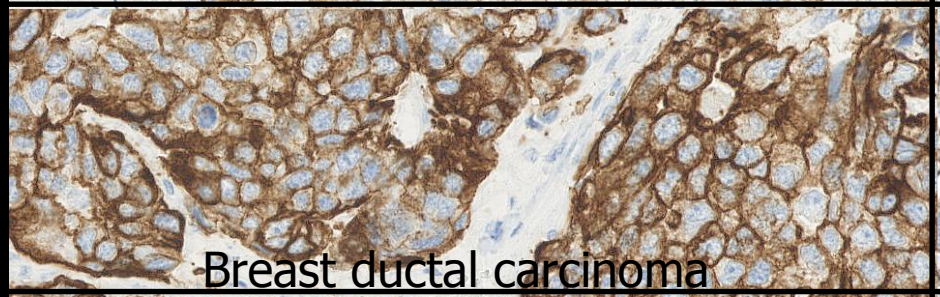


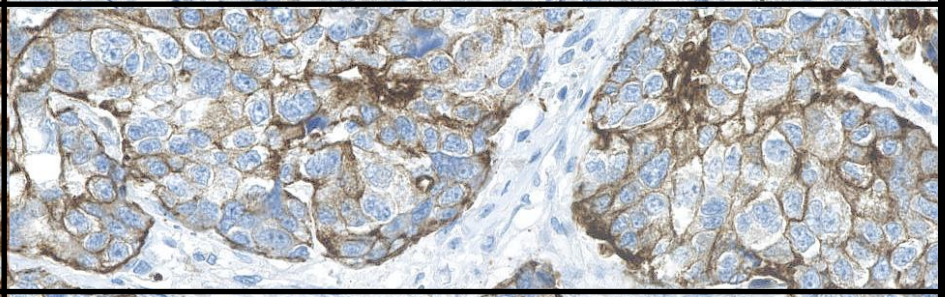
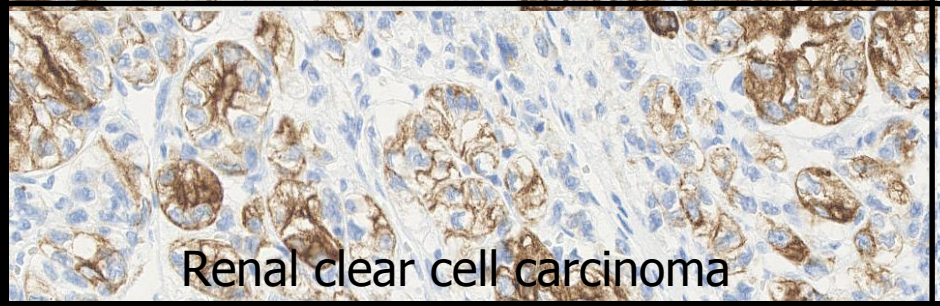
Fig. 2b. Insufficient staining for CK-LMW of the liver using the same protocol as in Fig. 1b - same field as in Fig. 2a.
Only the bile duct epithelial cells are demonstrated, while the hepatocytes are almost negative.
Same protocol used in Figs. 1b - 3b.

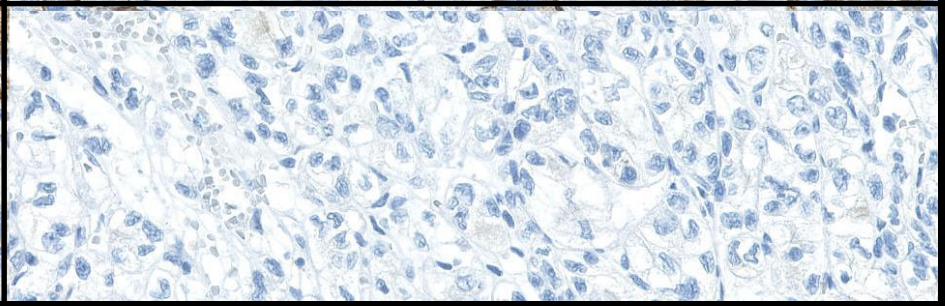
mAb clone 5D3
Less successful on VMS

VMS:
rmAb EP17
mAb DC10
mAb B22.1 + B23.1

IHC – Protocols and controls for UPT I

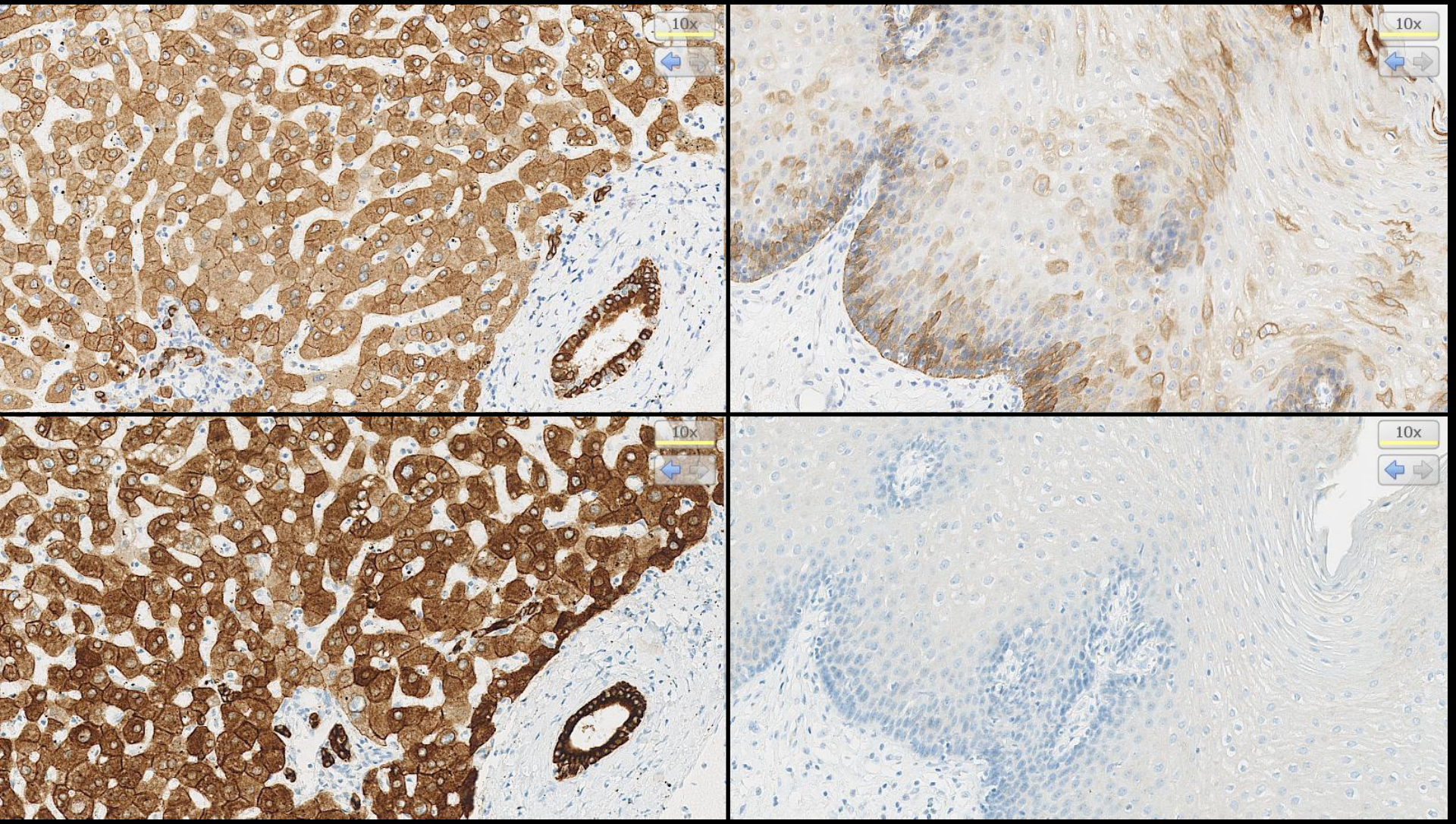


CK LMW – mAb B22.1/B23.1 or DC10

CK LMW – mAb 5D3

VMS Ultra - OptiView

IHC – Protocols and controls for UPT I



Top: CK LMW – rmAb EP17
Bottom: CK LMW – mAb clone DC10

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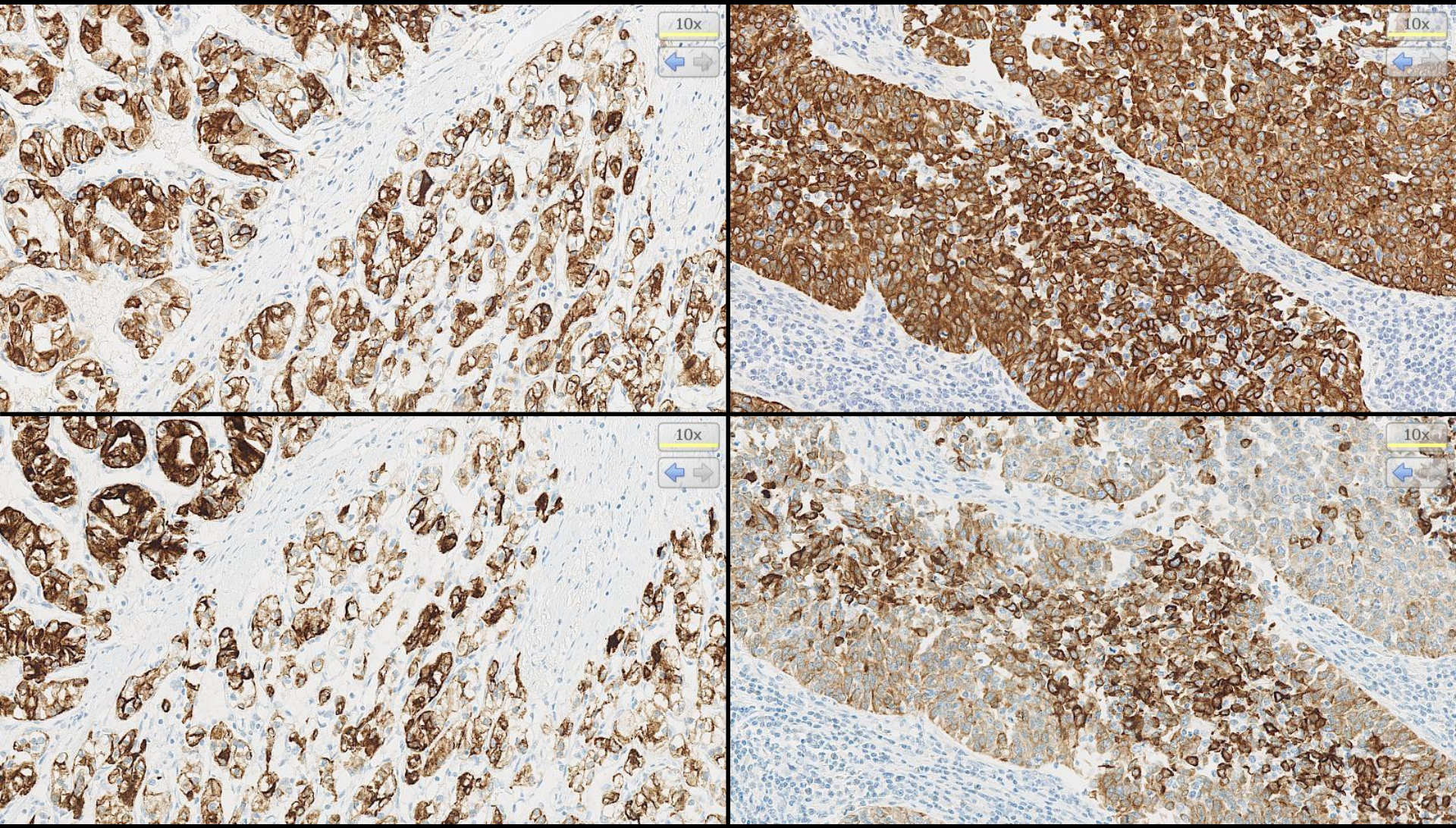

Top: CK LMW – rmAb EP17 – RCC & Lung squamous cell carcinoma
Bottom: CK LMW – mAb clone DC10 - – RCC & Lung squamous cell carcinoma

IHC – Protocols and controls for UPT I

UPT I: CK-LMW
Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb 5D3*	HIER High	1:40-1.400	2- & 3-step	Leica	3-step
mAb B22.1-B23.1	HIER High	1:100-250	3-step	Ventana	2- & 3-step
mAb DC10	HIER High	1:20-1.200	2- & 3-step	Dako	2- & 3-step
mAb CAM 5.2**	Proteolysis	"RTU-BD" 1:100-200CM	3-step	-	-
rmAb EP17	HIER High	1:100	3-step	-	-

*mAb clone 5D3 less successful on VMS stainer platform.
** Becton Dickinson – now mAb clone CAM 5.2 can be applied as concentrate from CM

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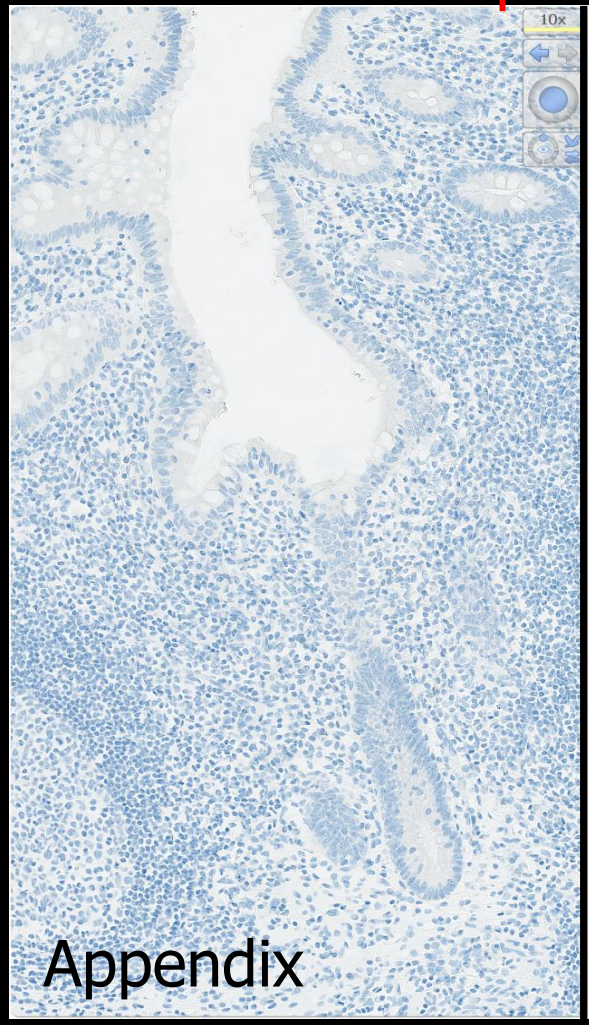
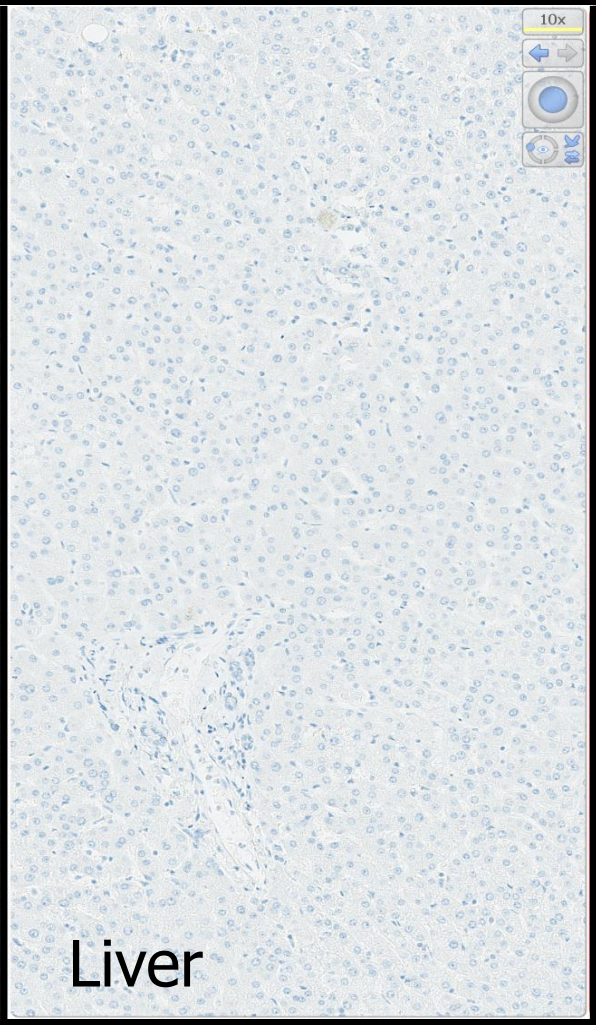
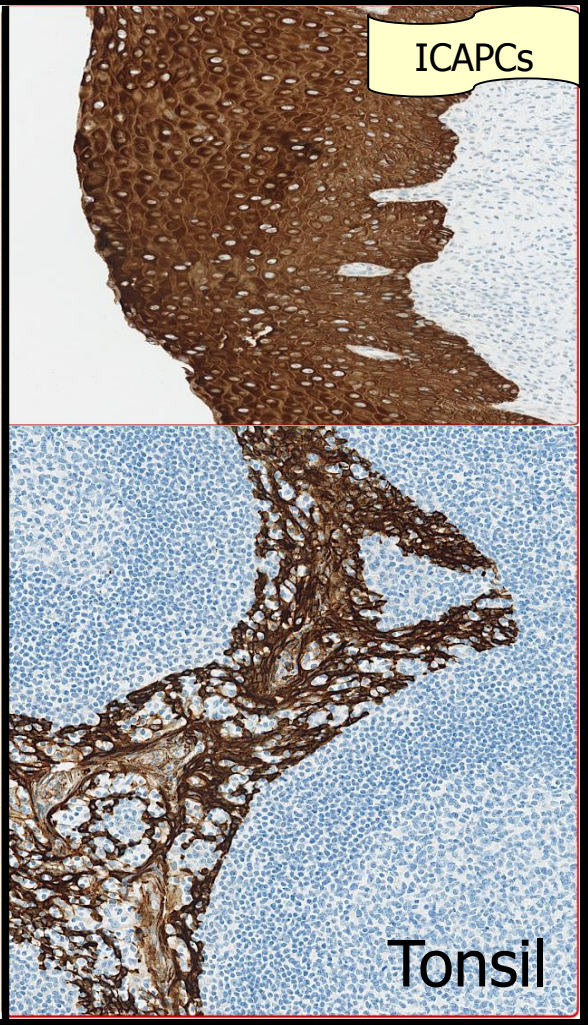
CK-HMW reaction pattern



No staining should be seen.



No staining should be seen.



Virtually all squamous epithelial cells must show a moderate to strong cytoplasmic staining reaction.

IHC – Protocols and controls for UPT I

Table 3: Proportion of sufficient results for CK-HMW in the five NordiQC runs performed

	Run 12 2004	Run 16 2006	Run B6 2008	Run 32 2011	Run 38 2013
Participants, n=	73	87	97	163	207
Sufficient results	77 %	88 %	24 %	23 %	45 %

Table 1. Abs and assessment marks for CK-HMW, run 38

Concentrated Abs	Reactivity	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **34BE12**	CK 1, 5, 10, 14, (19) *	51 2 2 1 1 1 1 1 1	Dako Leica/Novocastra Thermo/Neomarkers Abcam Biocare Bio SB Cell Marque Enzo Gene Tech	0	6	54	1	10 %	0 %
mAb clone **BS42**	Unknown	1	Nordic Biosite	0	0	1	0	-	-
mAb clone **D5/16B4**	CK 5, 6	28 2 1 1	Dako Cell Marque Genemed Zymed	15	13	4	0	88 %	100 %
mAb clone **DE-SQ**	CK 13, 14, 15, 16	1	Thermo/Neomarkers	0	0	1	0	-	-
mAb clone **LL002**	CK 14	6 1 1	Leica/Novocastra AbD Serotec Thermo/Neomarkers	5	1	2	0	75 %	83 %
mAb clone **XM26**	CK 5	23 1	Leica/Novocastra Diagnostic BioSystems	19	5	0	0	100 %	100 %
mAb clone cocktail **XM26+LL002**	CK 5, 14	2 2	Diagnostic BioSystems Zytomed	1	2	1	0	-	-
mAb clone cocktail **Y4A3+XM26+ LL002**	p63, CK 5, 14	1	Zytomed	0	1	0	0	-	-
mAb clone **34BE12** + rmAb clone **EP1601Y**	CK 1, 5, 10, 14, (19) * + CK 5	1	Homemade cocktail: Dako/Cell Marque	0	0	1	0	-	-
mAb clone **XM26** + mAb clone **LL002**	CK 5, 14	1	Homemade cocktail: Leica/Novocastra/ Cell Marque	0	1	0	0	-	-
Ready-To-Use Abs									
mAb clone **34BE12 IR051**	CK 1, 5, 10, 14, (19) *	24	Dako	0	0	24	0	0 %	0 %
mAb clone **D5/16B4 IS/IR780**	CK 5, 6	9	Dako	3	4	2	0	78 %	78 %

mAb clone 34BE12 gives an aberrant staining with an unidentified CK-LMW subtype complicating the use as a reliable marker for CK-HMW

mAb clone XM26 or D5/16B4

Conc & RTU

Alternatively:
CK5: rmAb EP1601Y

CK14: rmAb SP53 & mAb LL002

IHC – Protocols and controls for UPT I

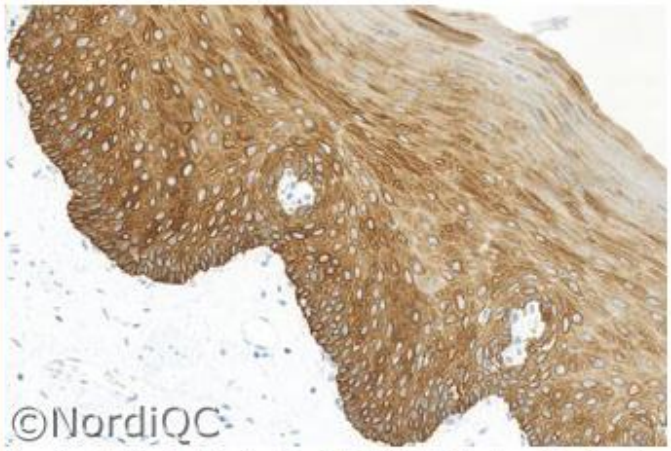


Fig. 1a. Optimal staining for CK-HMW of the tonsil using the mAb clone D5/16B4 against CK5/6 optimally calibrated and with HIER in an alkaline buffer.
Virtually all the squamous epithelial cells show a distinct, moderate to strong cytoplasmic staining, while no background staining is seen.

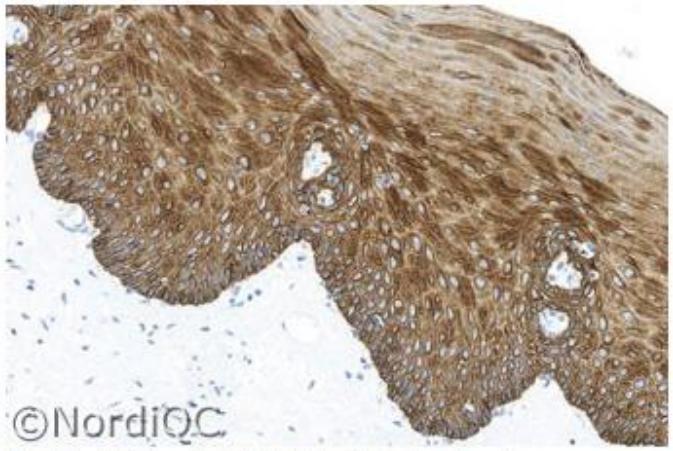


Fig. 1b. Staining for CK-HMW of the tonsil using an insufficient protocol based on the mAb clone 34BE12 against CK-HMW with HIER in an alkaline buffer, same field as in Fig. 1a.
Virtually all the squamous epithelial cells show a distinct, moderate to strong cytoplasmic staining, while no background staining is seen. However, compare with Fig. 3b, same protocol.

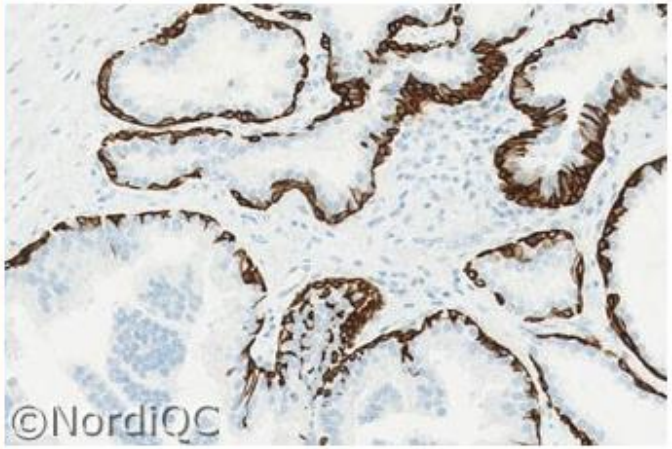


Fig. 2a. Optimal staining for CK-HMW of the prostate hyperplasia/PIN lesion using same protocol as in Fig. 1a. Virtually all the basal cells show a strong cytoplasmic staining. No background staining is seen.

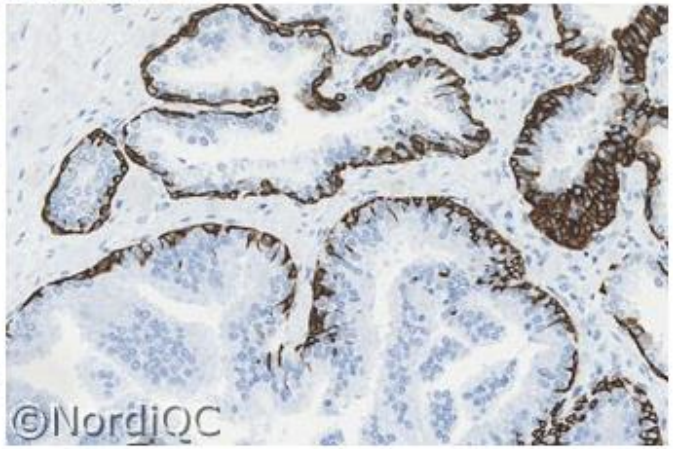


Fig. 2b. Staining for CK-HMW of the prostate hyperplasia/PIN lesion using same insufficient protocol as in Fig. 1b, same field as in Fig. 2a. Virtually all the basal cells show a strong cytoplasmic staining. No background staining is seen, same field as in Fig. 2a. However, compare with Fig. 3b., same protocol.

IHC – Protocols and controls for UPT I

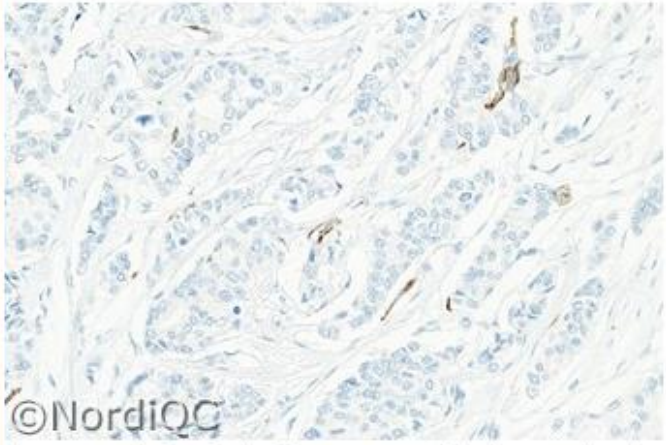


Fig. 3a. Optimal staining for CK-HMW of the breast ductal carcinoma using same protocol as in Figs. 1a. & 2a.
The neoplastic cells expressing CK-LMW are negative, while the remnants of entrapped myoepithelial cells expressing the CK-HMW subtypes CK5 & CK14 show a moderate cytoplasmic staining.

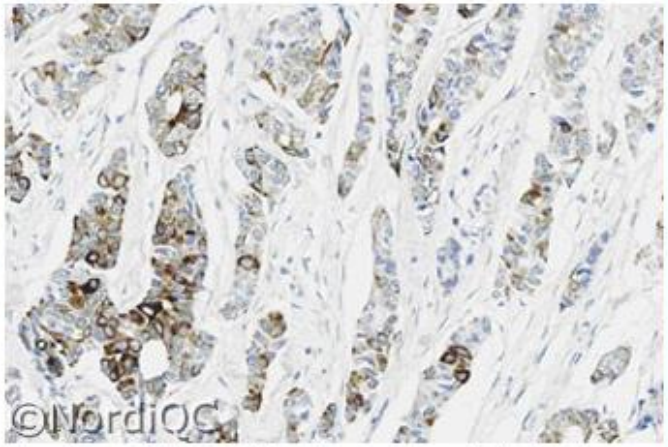


Fig. 3b. Insufficient staining for CK-HMW of the breast ductal carcinoma using same protocol as in Figs. 1b. & 2b, same field as in Fig. 3a. A moderate to strong aberrant cytoplasmic staining is seen in the majority of the neoplastic cells. This false positive cross reaction with an unidentified subtype of CK-LMW was typically seen, when the mAb clone 34BE12 was used with HIER.

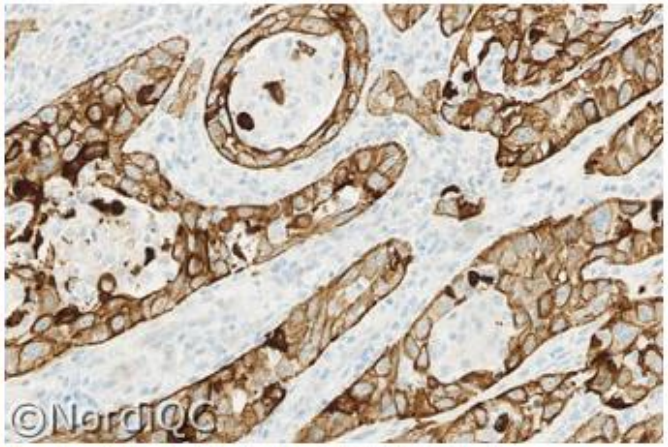


Fig. 4a. Optimal staining for CK-HMW of the lung squamous cell carcinoma using same protocol as in Figs. 1a. - 3a.
Virtually all the neoplastic cells expressing CK-HMW show a moderate to strong cytoplasmic staining.

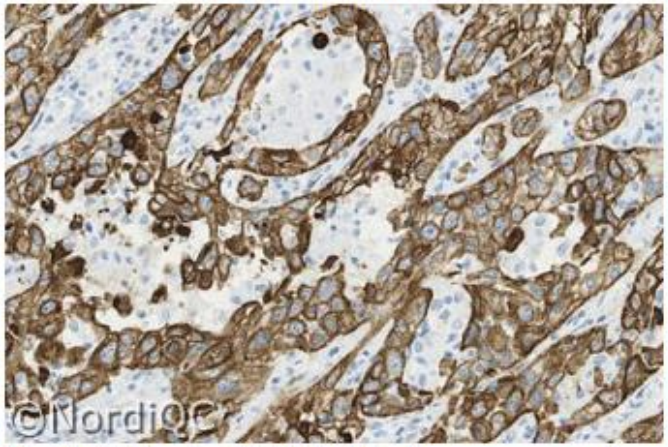


Fig. 4b. Staining for CK-HMW of the lung squamous cell carcinoma using same insufficient protocol as in Figs. 1b – 3b, same field as in Fig. 4a.
Virtually all the neoplastic cells expressing CK-HMW show a moderate to strong cytoplasmic staining. However, as the epithelial cells of the breast ductal carcinoma in Fig. 3b showed same staining characteristics, the staining for CK-HMW is not reliable.

IHC – Protocols and

Tonsil/esophagus & liver:

HE: Basal squamous epithelial cells of tonsil/esophagus

LE: Intermediate and superficial squamous epithelial cells of tonsil/esophagus

NE: Columnar epithelial cells of the bile ducts in the liver

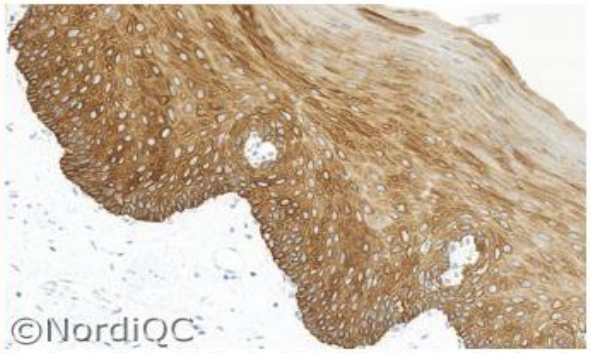


Fig. 1a. Optimal staining for CK-HMW of the tonsil using the mAb clone D5/16B4 against CK5/6 optimally calibrated and with HIER in an alkaline buffer.
Virtually all the squamous epithelial cells show a distinct, moderate to strong cytoplasmic staining, while no background staining is seen.

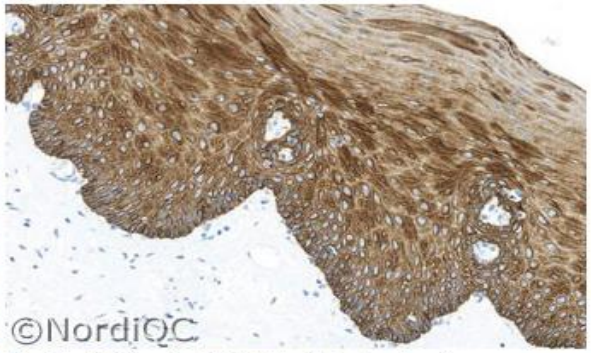


Fig. 1b. Staining for CK-HMW of the tonsil using an insufficient protocol based on the mAb clone 34BE12 against CK-HMW with HIER in an alkaline buffer, same field as in Fig. 1a.
Virtually all the squamous epithelial cells show a distinct, moderate to strong cytoplasmic staining, while no background staining is seen. However, compare with Fig. 3b, same protocol.

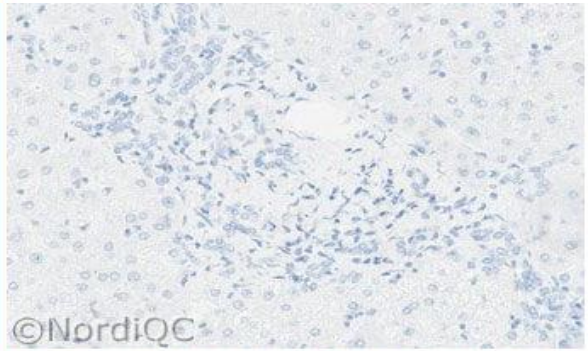


Fig. 4a. Optimal staining for CK-HMW of liver using same protocol as in Figs. 1a. - 3a.
No staining is seen in neither the liver cells nor the epithelial cells of the bile ducts.

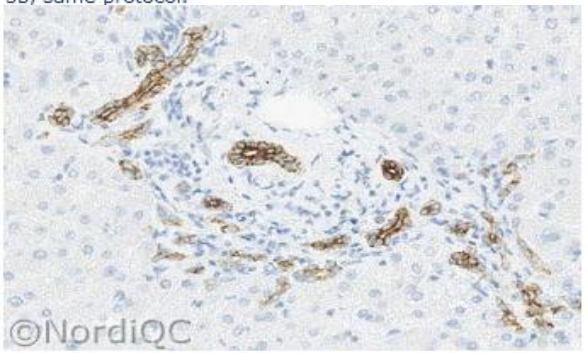
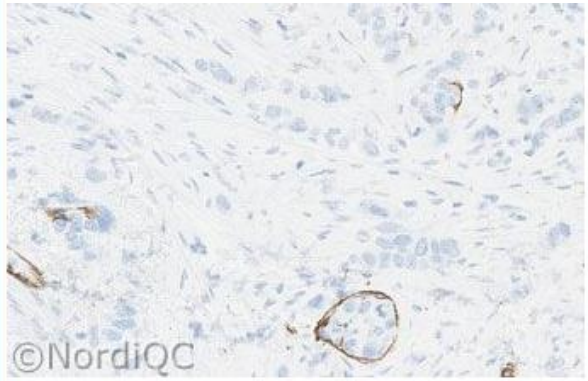
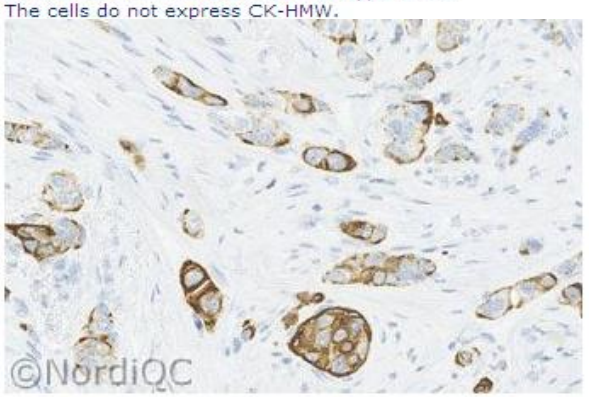


Fig. 4b. Insufficient false positive staining for CK-HMW of the liver using the mAb clone 34BE12 with HIER in an alkaline buffer – same field as in Fig. 4a.
A moderate to strong and aberrant cytoplasmic staining reaction is seen in the epithelial cells of the bile ducts.
The aberrant positive staining reaction most likely is caused by a cross-reaction of the mAb clone 34BE12 with a denatured form of the CK-LMW subtype CK19.
The cells do not express CK-HMW.

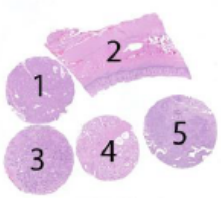




IHC – Protocols and controls for UPT I

Assessment Run 46 2016
CK5

Material
The slide to be stained for CK5 comprised:



1: Lung squamous cell carcinoma 2: Esophagus 3: Lung adenocarcinoma
4: Prostate hyperplasia 5: Lung squamous cell carcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CK5 staining as optimal included:

- A moderate to strong and distinct cytoplasmic staining reaction of all squamous epithelial cells in esophagus throughout all the cell layers.
- A strong and distinct cytoplasmic staining reaction of the majority of basal cells in the hyperplastic prostate glands.
- A moderate to strong cytoplasmic staining reaction of virtually all neoplastic cells in the lung squamous cell carcinoma, tissue core no. 1.
- An at least weak to moderate cytoplasmic staining reaction of the majority of neoplastic cells in the lung squamous cell carcinoma, tissue core no. 5.
- No staining of neoplastic cells in the lung adenocarcinoma.

Participation

Number of laboratories registered for CK5, run 46	281
Number of laboratories returning slides	266 (95%)

Results
266 laboratories participated in this assessment. 181 (68%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful CK5 antibodies
- Too low concentration of the primary Ab
- Insufficient HIER - too short efficient HIER time

Performance history
This was the second NordiQC assessment of CK5. The pass rate in this run was improved compared to the previous run from 2004 as shown in table 2.

Table 2. **Proportion of sufficient results for CK5 in the two NordiQC runs performed**

	Run 12 2004	Run 46 2016
Participants, n=	74	266
Sufficient results	47%	68%

IHC – Protocols and controls for UPT I

Table 1. **Antibodies and assessment marks for CK5, run 46**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **10C11E6**	1	Immunologic	0	1	0	0	-	-
mAb clone **D5/16 B4**	74 1 6 3 1 2 1	Dako/Agilent Invitrogen Cell Marque Zytomed Thermo Scientific Biocare Immunologic	21	34	28	5	63%	66%
mAb **BS42**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **XM26**	49 2 1	Leica/Novocastra Zytomed Sanbio	25	15	11	1	77%	79%
mAb clone **XM26/LL002**	1	Zytomed	0	1	0	0	-	-
rmAb clone **EP1601Y**	8 1	Cell Marque Biocare	6	3	0	0	100%	100%
rmAb clone **SP27**	3	Immunologic	3	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **D5/16 B4 1072**	1	Monosan	0	1	0	0	-	-
mAb clone **D5/16 B4 BMS017**	1	Zytomed	0	1	0	0	-	-
mAb clone **D5/16 B4 IR/IS780**	36	Dako/Agilent	1	8	22	5	25%	67%
mAb **D5/16 B4 GA780**	11	Dako/Agilent	1	8	2	0	82%	82%
mAb clone **D5/16 B4 790-4554**	38	Ventana/Roche/Cell Marque	15	15	5	3	79%	85%
mAb clone **XM26 PA0468**	2	Leica/Novocastra	0	2	0	0	-	-
mAb clone **XM26 PM234**	1	Biocare	0	1	0	0	-	-
rmAb clone **EP1601Y/LL002 760-4939**	1	Ventana/Cell Marque	1	0	0	0	-	-
rmAb clone **EP1601Y/LL002 905H-8**	3	Cell Marque	1	1	1	0	-	-
rmAb clone **EP1601Y 305R-18**	2	Cell Marque	0	1	1	0	-	-
rmAb clone **EP24/EP67 MAD-000651QD**	2	Master Diagnostica	2	0	0	0	-	-
rmAb clone **SP27 760-4935**	12	Ventana /Cell Marque	11	0	1	0	92%	92%
rmAb clone **SP27 RMA-0612**	1	Maixin	0	0	1	0	-	-
Total	266		88	92	72	14	-	
Proportion			33%	35%	27%	5%	68%	

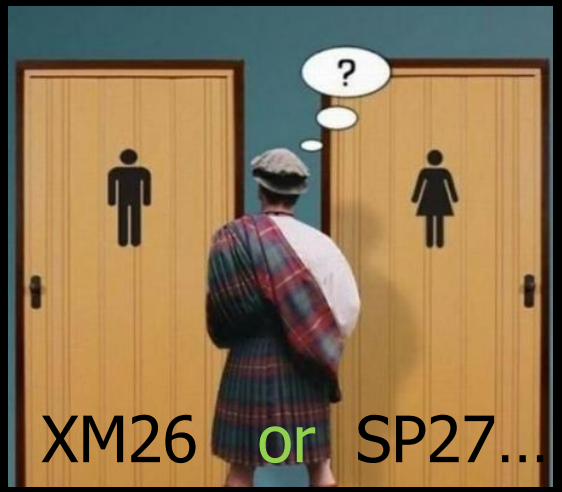
1)Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Clone

HIER buffer

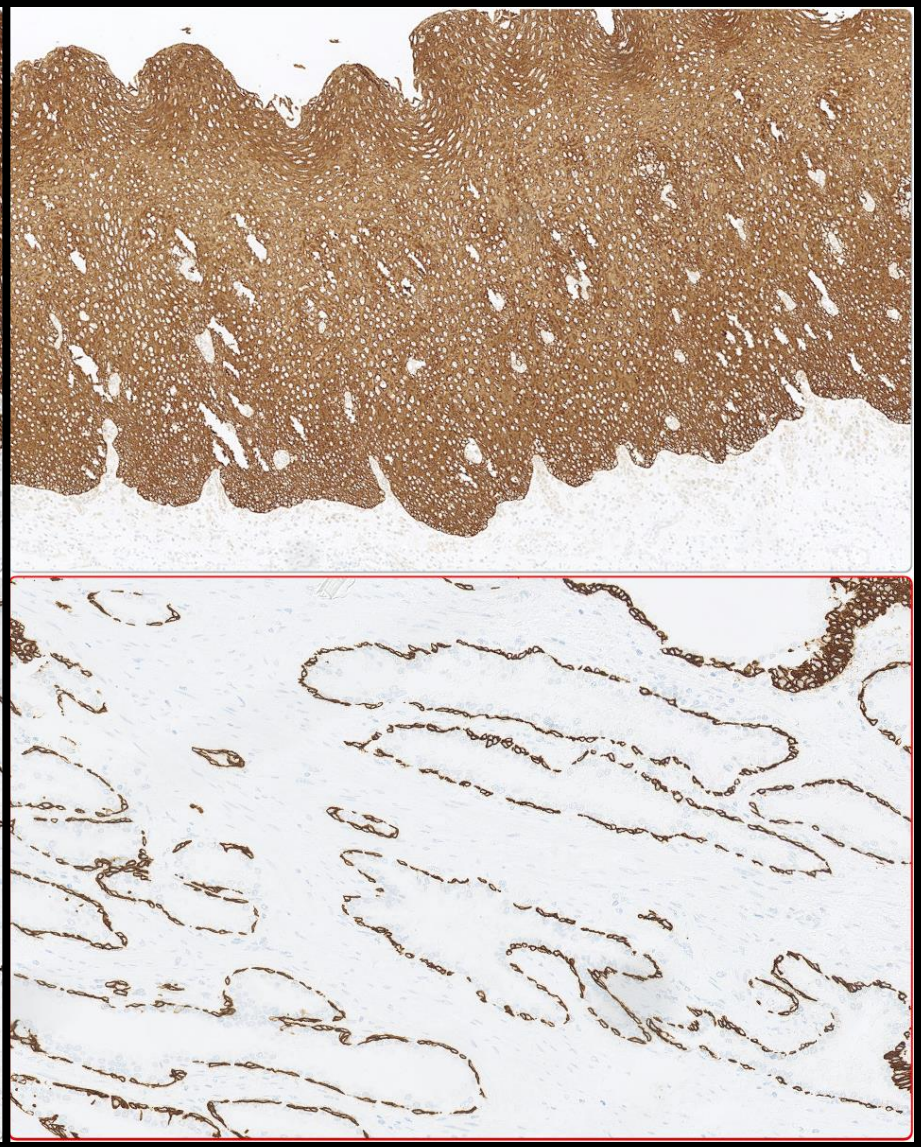
Detection kit

High pH + 3-step



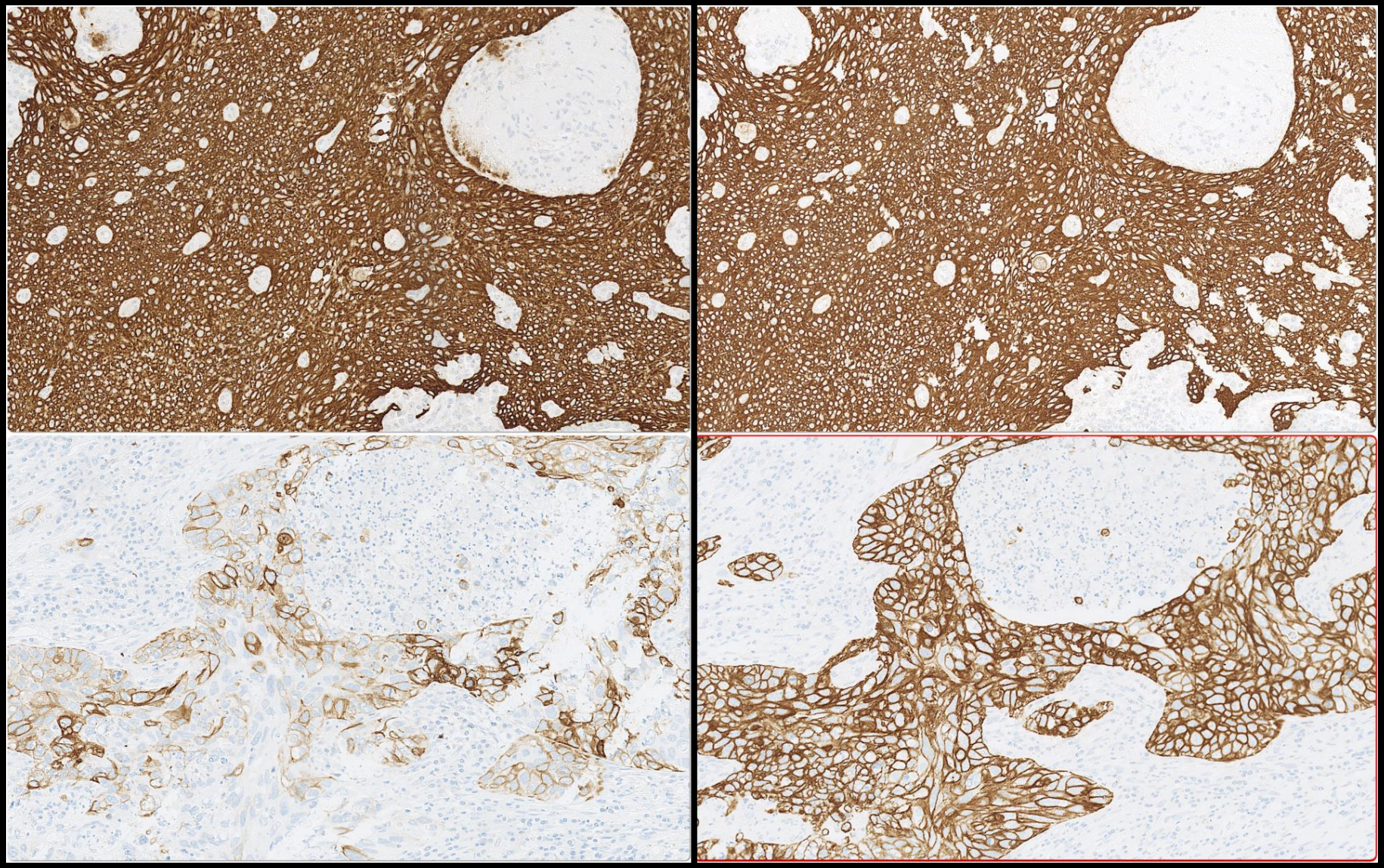
IHC – Protocols and controls for UPT I

mAb XM26



rmAb SP27

IHC – Protocols and controls for UPT I

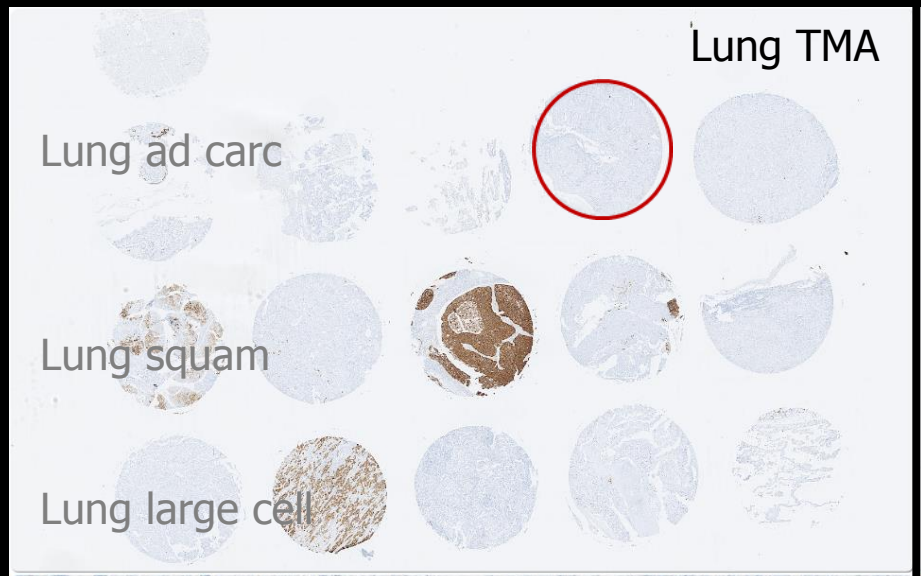


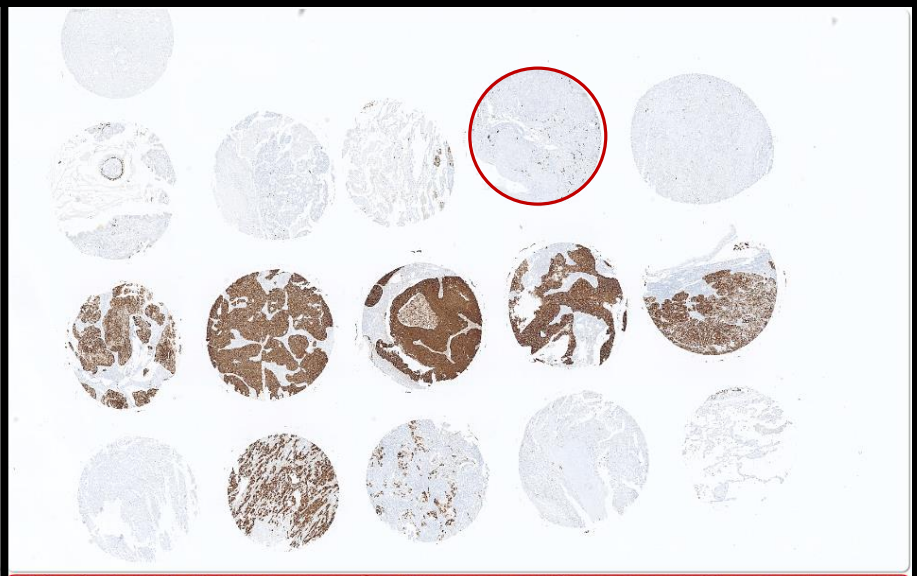
mAb XM26

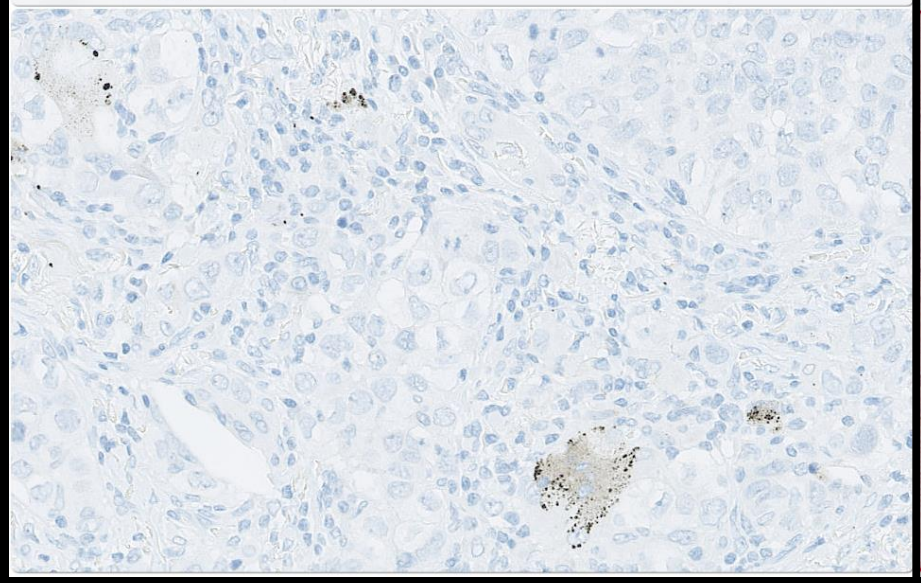
Lung squam cell carc.

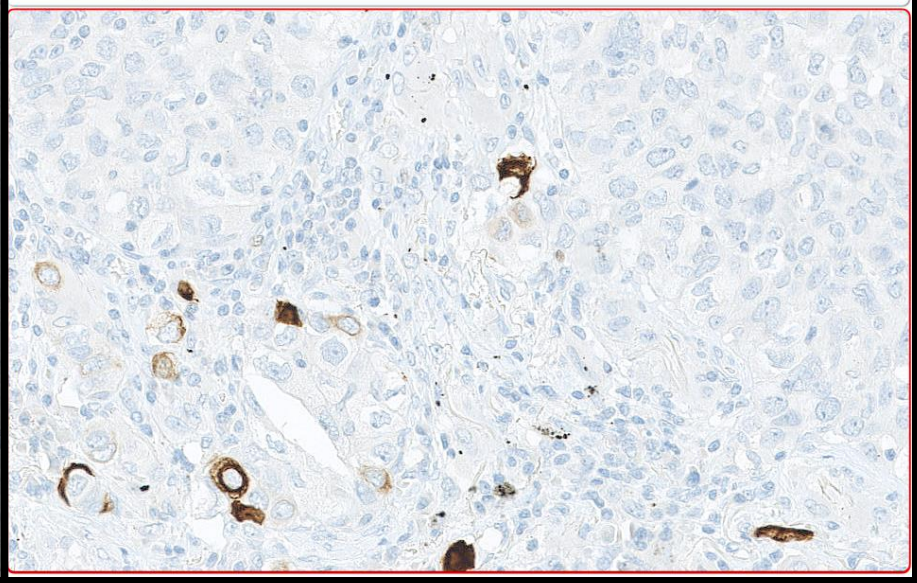
rmAb SP27

IHC – Protocols and controls for UPT I







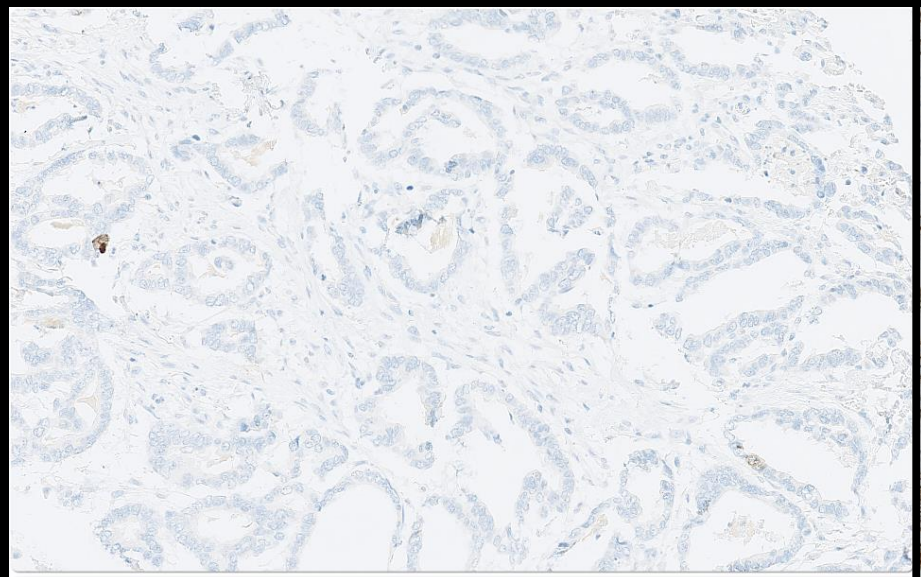


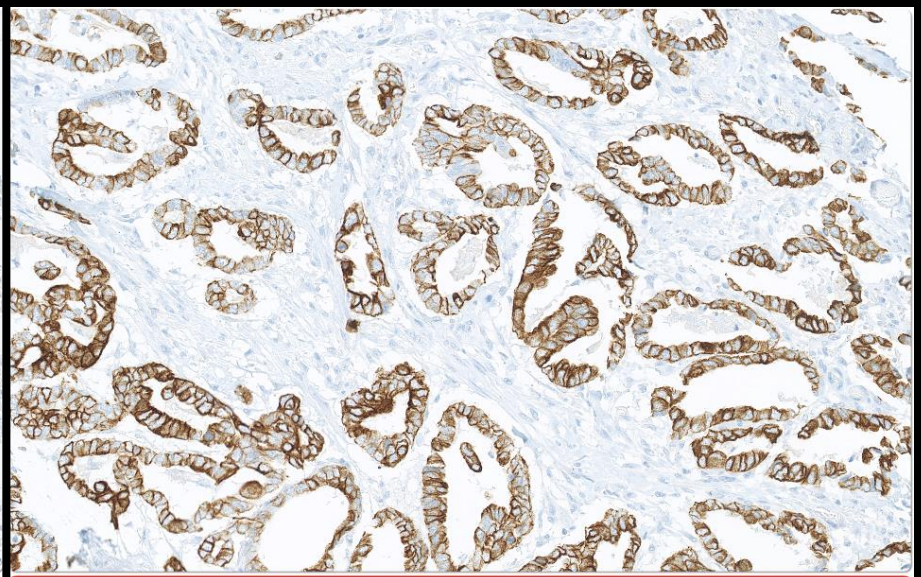
mAb XM26

Lung carc.

rmAb SP27

IHC – Protocols and controls for UPT I





mAb XM26

TMA Neoplasia
Gastic ad. carc.

rmAb SP27

IHC – Protocols and controls for UPT I

UPT I: CK-HMW
Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb XM26 (5)	HIER High	1:20-200	3-step	Leica	3-step
mAb D5/16B (5&6)	HIER High	1:20-200	3-step	Ventana	3-step
rmAb EP1601Y (5)	HIER High	1:50-200	3-step	-	-
rmAb SP27 (5)	HIER High	1:50-250	3-step	Ventana	2- & 3-step
mAb LL002 (14)	HIER High	1:10-200	2-& 3-step	Ventana	3-step
rmAb SP53 (14)	HIER High	1:40-80	3-step	Ventana	3-step

IHC – Protocols and controls for UPT I

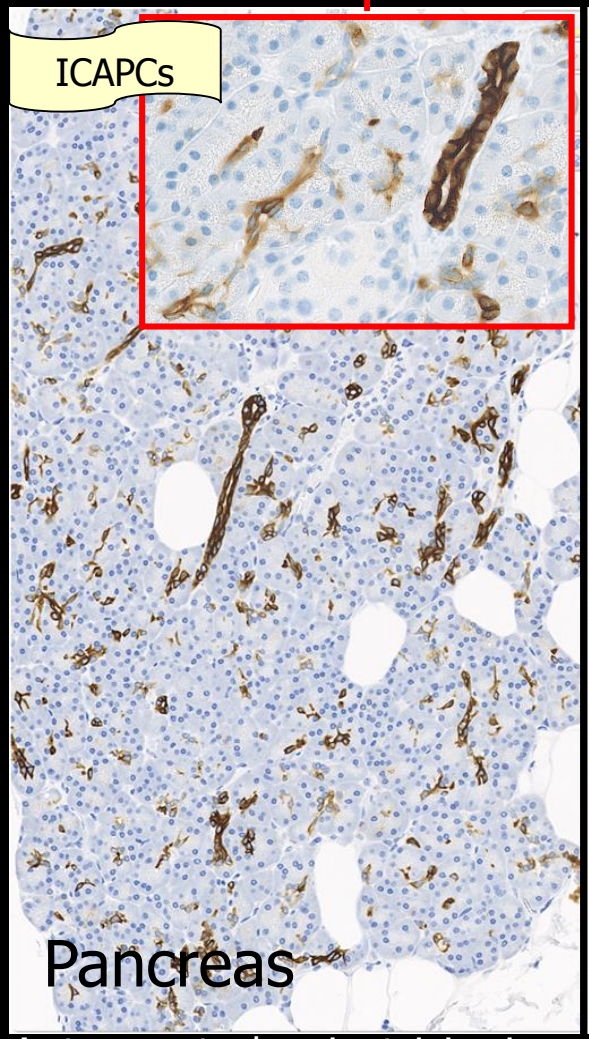
CKs	Recommendable clones (conc.)	Less successful clones (conc.)	RTU ”plug and play” giving optimal result
CK 7	mAb OV-TL 12/30 mAb RN7 rmAb SP52		Dako: mAb OV-TL 12/30 Leica: mAb RN7 VMS: mAb SP52
CK 19	mAb A53-B/A2.26 mAb B170 mAb BA17	mAb Rck108	VMS: mAb A53-B/A2.26
CK 20	mAb BS101 mAb Ks20.8 rmAb E19-1 rmAb SP33	mAb PW31	Dako: mAb Ks20.8 Leica: mAb Ks20.8 VMS: rmAb SP33

IHC – Protocols and controls for UPT I

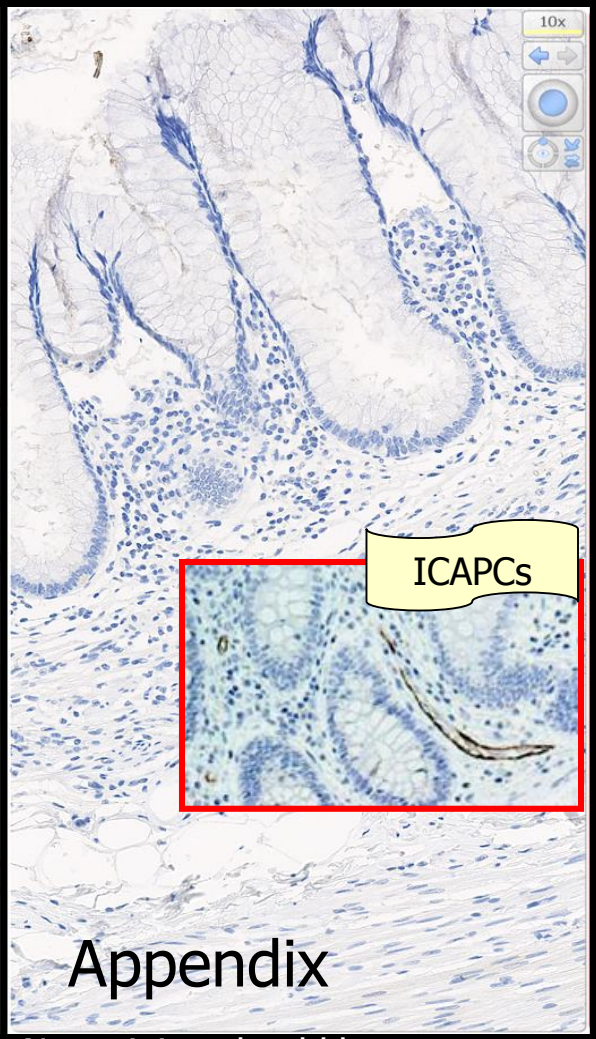
CKs	Positive tissue control HE	Positive tissue control LE	Negative tissue control NE
CK 7	Pancreas: Epithelial cells of large ducts Tonsil: Squamous epithelial cells	Pancreas: Epithelial cells of intercalating ducts Appendix: Endothelial cells	Appendix: Vast majority of epithelial cells Tonsil: Lymphocytes
CK 19	Appendix: Virtually all epithelial cells.	Tonsil / Esophagus: Basal squamous epithelial cells	Tonsil: Lymphocytes Appendix: Endothelial cells
CK 20	Appendix: Luminal epithelial cells	Appendix: Epithelial cells, basal crypts	Tonsil: Squamous epithelial cells

IHC – Protocols and controls for UPT I

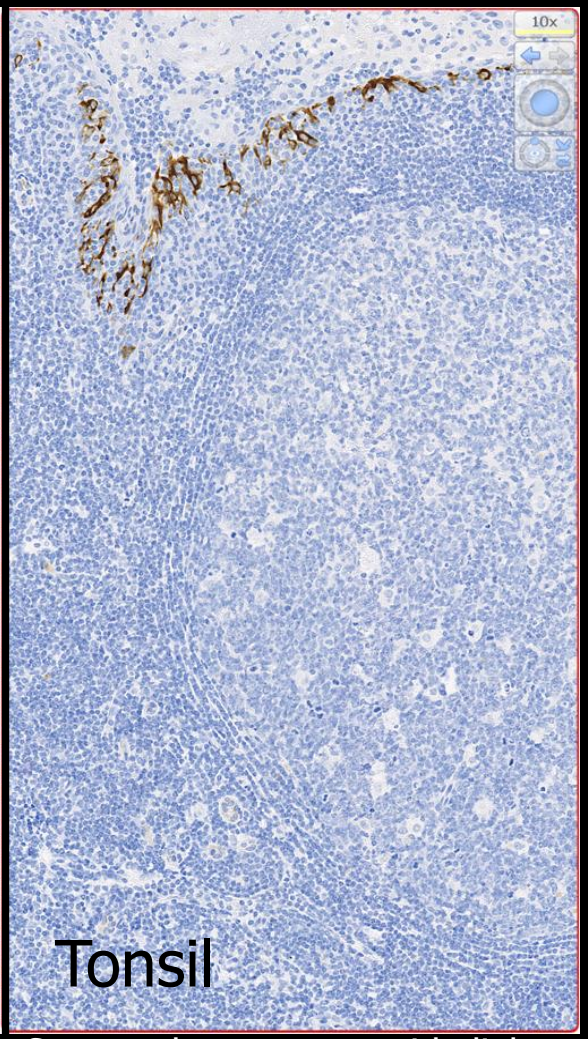
CK7 reaction pattern



A strong cytoplasmic staining in virtually all epithelial cells of the large pancreatic ducts & weak to moderate cytoplasmic staining in cells of intercalating ducts.



No staining should be seen. Endothelial cells can be demonstrated.



Scattered squamous epithelial cells can show a weak to strong cytoplasmic staining reaction.

IHC – Protocols and controls for UPT I

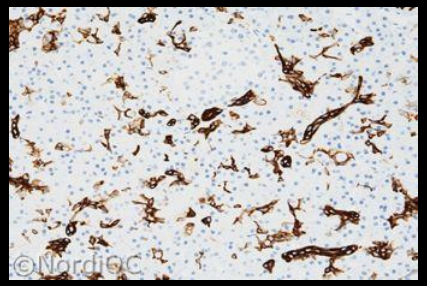
Table 1. **Antibodies and assessment marks for CK7, run 40**

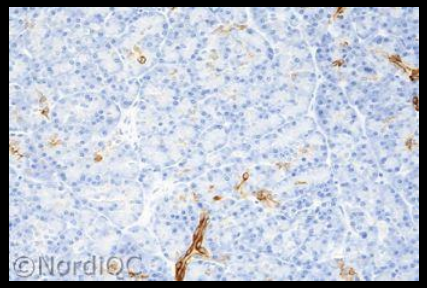
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **OV-TL 12/30**	97 14 14 4 3 2 2 2 1 1	Dako Leica/Novocastra BioGenex Thermo S/ NeoMarkers Monosan Biocare Cell Marque Genemed ZytoMed Nordic Biosite	40	66	32	2	76%	94%
mAb clone **RN7**	3	Leica/Novocastra	1	2	-	-	-	-
rmAb clone **EPR1619Y**	1	Abcam	-	-	1	-	-	-
mAb clone **K72.7**	1	Thermo S/ NeoMarkers	-	1	-	-	-	-
Ready-To-Use antibodies:								
mAb clone **OV-TL 12/30, IR619**	41	Dako	36	5	0	0	100%	100%
mAb clone **OV-TL 12/30, MAD-001004QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **OV-TL 12/30, 307M-98**	1	Cell Marque	1	0	0	0	-	-
mAb clone **OV-TL 12/30, MON-RTU1074**	1	Monosan	1	0	0	0	-	-
mAb clone **OV-TL 12/30, PDM 097**	1	Diagnostic Biosystem	0	1	0	0	-	-
mAb clone **OV-TL 12/30, E061**	1	Linaris	0	1	0	0	-	-
rmAb clone **SP52, 790-4462**	45	Ventana	26	18	1	0	98%	98%
mAb clone **RN7, PA0942**	7	Leica/Novocastra	2	4	1	0	86%	100%
rmAb clone **BC1, PRM 339**	1	Biocare	0	0	1	0	-	-
Clone **unknown ZM-0071**	1	Zhongshan	1	0	0	0	-	-
Total	246		109	99	36	2	-	
Proportion			44%	40%	15%	1%	84%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

HIER > proteolysis

OV-TL	Pass	Optim.
HIER	89%	34%
Proteol.	32%	11%





Calibration of titre

RTU > to in-house

IHC – Protocols and controls for UPT I

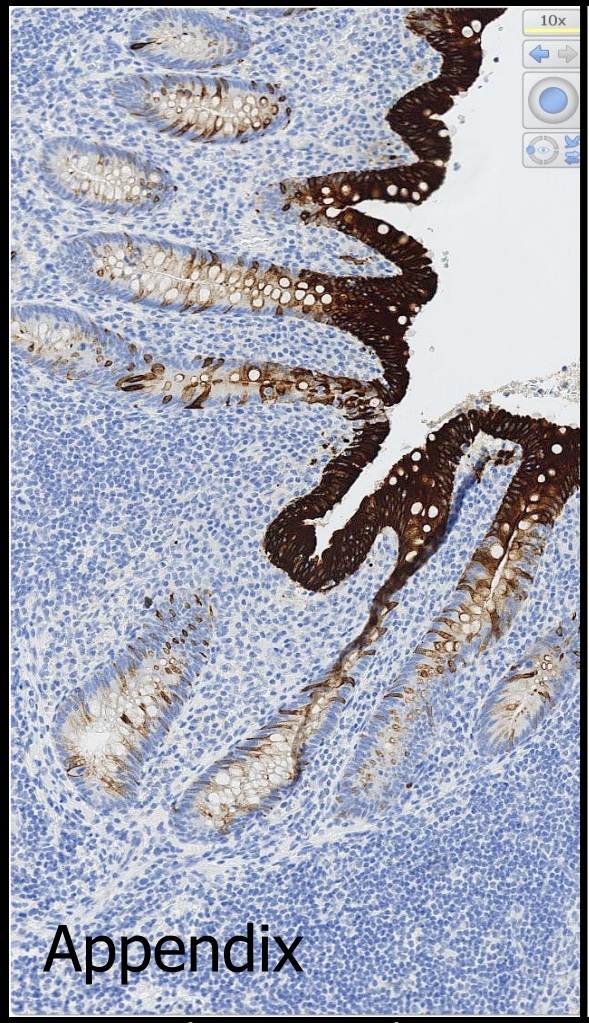
UPT I: CK7

Basic protocol settings for an optimal staining result (NQC)

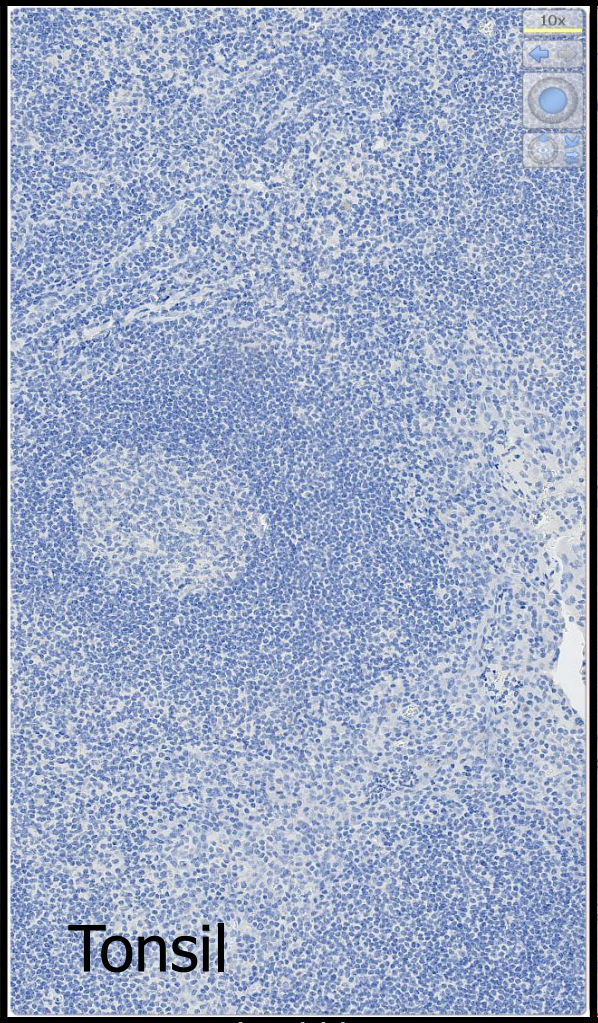
	Retrieval	Titre	Detection	RTU	Detection
mAb OV-TL 12/30	HIER	1:30-300	2- & 3-step	Dako	2- & 3-step
mAb RN7	HIER High	-		Leica	3-step
rmAb SP52	HIER High	-	-	Ventana	2- & 3-step

IHC – Protocols and controls for UPT I

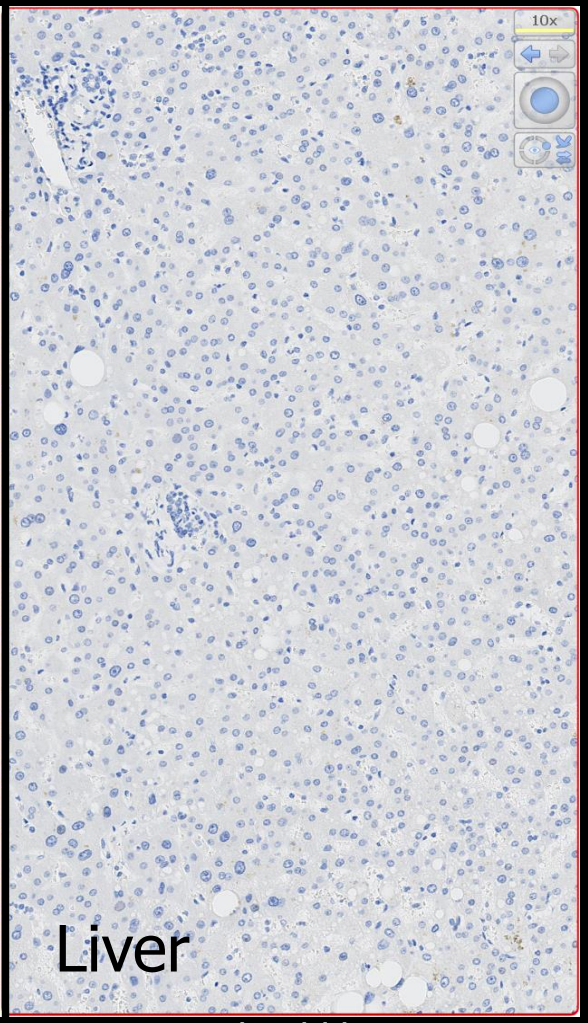
CK20 reaction pattern



A strong, distinct cytoplasmic staining reaction of all the surface epithelial cells and at least a weak to moderate staining reaction in most crypt cells.



No staining should be seen.



No staining should be seen.

IHC – Protocols and controls for UPT I

Table 1. Abs and assessment marks for CK20, run 35

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Ks20.8**	92 10 2 2 2 1 1 1 1 1	Dako Leica/Novocastra Cell Marque Eurodiagnostics Thermo/NeoMarkers Biocare DBS Europroxima Master Diagnostica Progen	56	42	12	3	87 %	91 %
mAb clone **PW31**	1	Leica/Novocastra	0	0	1	0	-	-
rmAb clone **EP23**	1	Epitomics	0	1	0	0	-	-
pAb **E16444**	7	Spring Bioscience	4	3	0	0	100 %	100 %
Unknown	1	Unknown	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **Ks20.8 IR/IS777**	25	Dako	13	12	0	0	100 %	100 %
mAb clone **Ks20.8 PM062**	1	Biocare	0	0	1	0	-	-
mAb clone **Ks20.8 320M-17**	1	Cell Marque	0	1	0	0	-	-
mAb clone **Ks20.8 RTU-CK20**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **Ks20.8 E062**	1	Linaris	1	0	0	0	-	-
mAb clone **Ks20.8 mon-rtu1083**	1	Monosan	0	0	1	0	-	-
mAb clone **Ks20.8 ZM-0075**	1	Zhongshan	0	1	0	0	-	-
mAb clone **PW31 PA0918**	4	Leica/Novocastra	0	0	4	0	-	-
rmAb clone **SP33 790-4431**	37	Ventana	20	10	6	1	81 %	100 %
Total	195		94	72	25	4	-	
Proportion			48 %	37 %	13 %	2 %	85 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

Table 2: Pass rate for laboratories using mAb clone Ks20.8 with HIER and enzymatic pre-treatment

	HIER	Enzymatic pre-treatment
Sufficient[1]	76/100 (76 %)	5/26 (19 %)

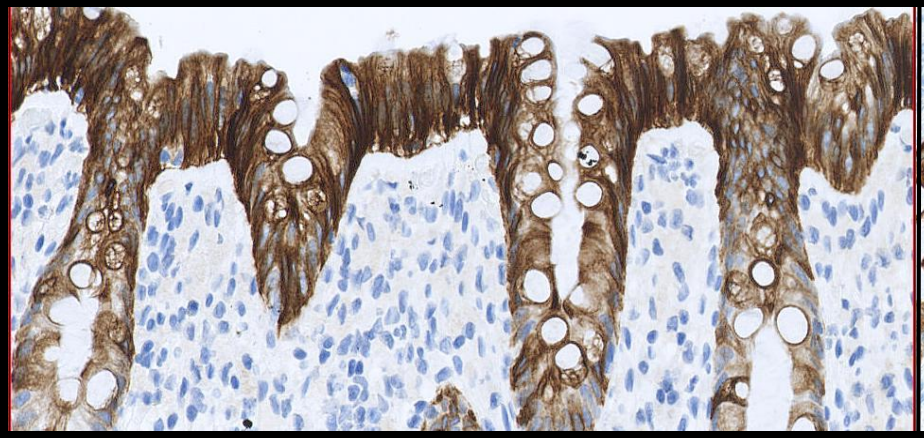
1) Proportion of sufficient stains (optimal or good),

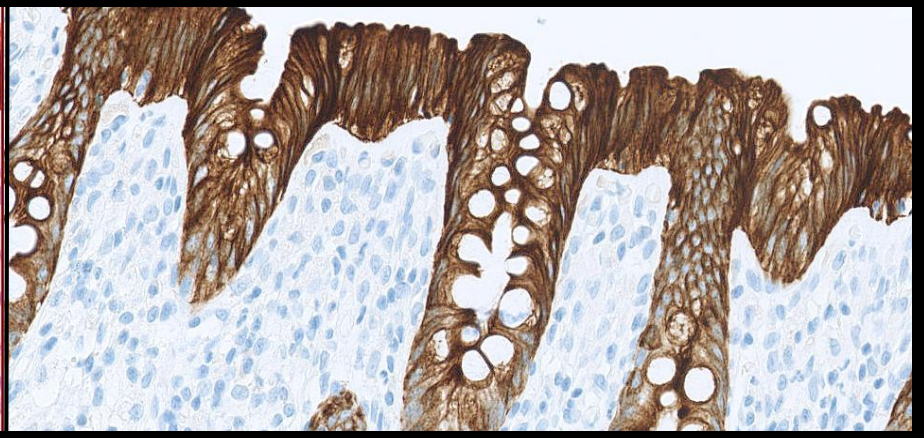
Insuff.:

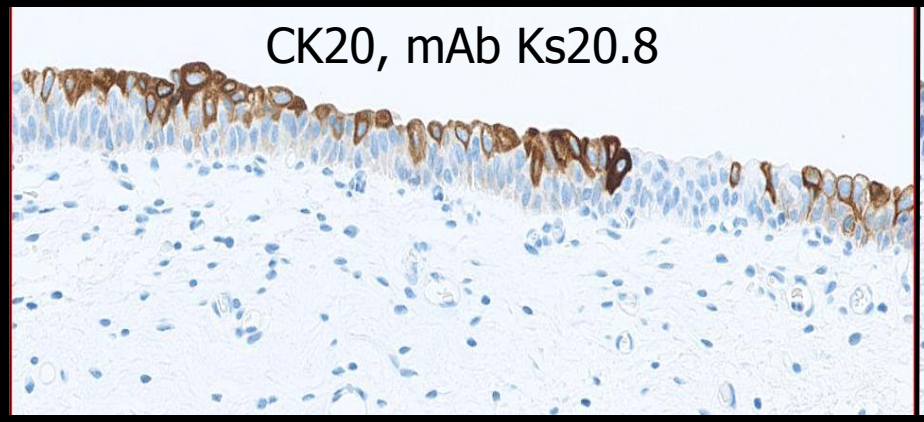
mAb clone Ks20.8 by proteolysis and/or too low sensitivity

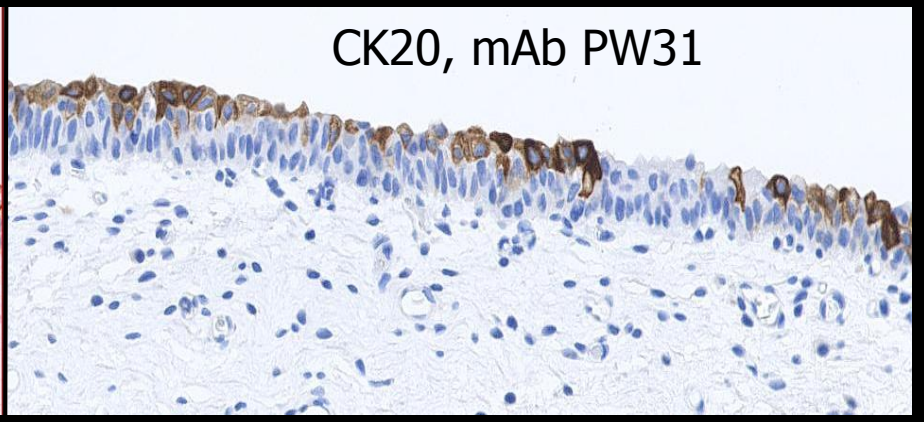
mAb clone PW31 less succesful (5/5 protocols ins.)

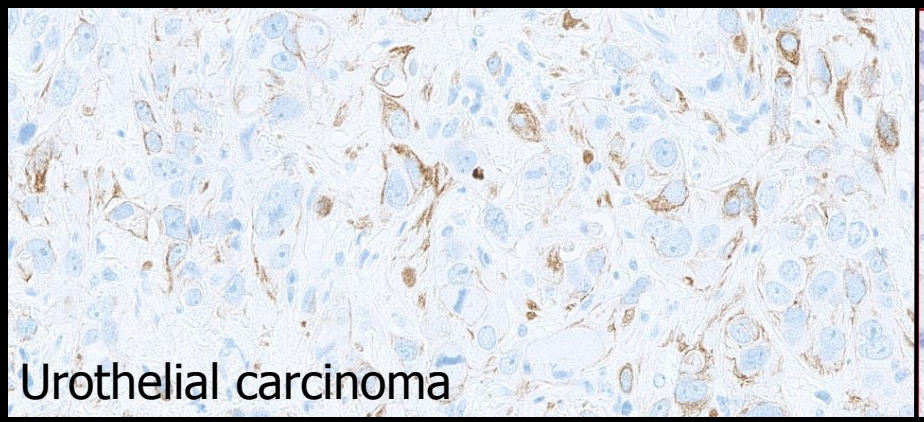
IHC – Protocols and controls for UPT I

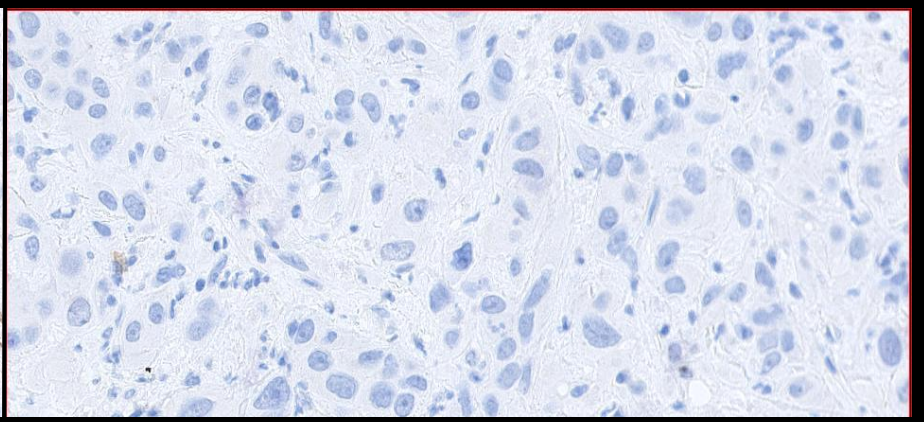












IHC – Protocols and controls for UPT I

UPT I: CK20

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb Ks20.8	HIER High	1:25-400	2- & 3-step	Dako Leica	2- & 3-step 3-step
rmAb E19-1	HIER	1:50-200	2- & 3-step	-	-
rmAb SP33	HIER High	-	-	Ventana	2- & 3-step

IHC – Protocols and controls for UPT I

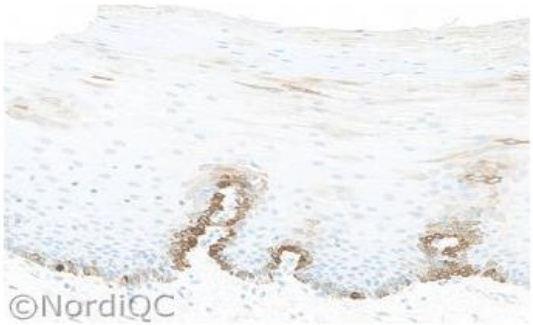


Fig. 1a. Optimal CK19 staining of the esophagus using the mAb clone A53-B/A2.26 in an optimally calibrated protocol with HIER in an alkaline buffer. The majority of the basal squamous epithelial cells show a weak to moderate cytoplasmic staining reaction, while a weak staining reaction is seen in scattered intermediate epithelial cells.

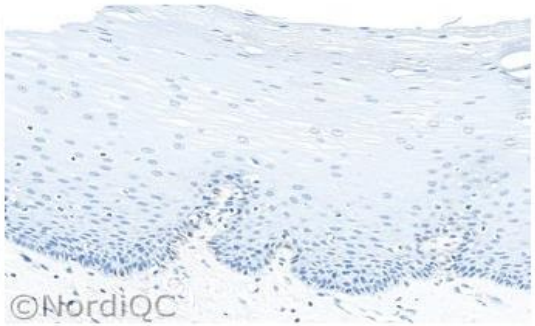


Fig. 1b. Insufficient CK staining of the esophagus using the mAb clone A53-B/A2.26 with a protocol giving a too low sensitivity – too low concentration of the primary Ab., same field as in Fig. 1a. No staining reaction is seen in the squamous epithelial cells – also compare with Figs. 2b - 4b same protocol.

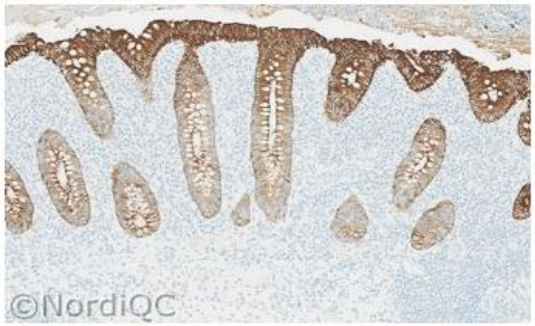


Fig. 2a. Optimal CK19 staining of the appendix using the same protocol as in Fig. 1a. The surface columnar epithelial cells show a strong cytoplasmic staining reaction, while the columnar epithelial cells in the basal parts of the crypts show a weak to moderate staining reaction.

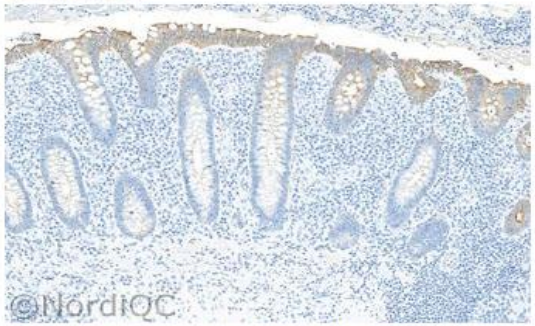


Fig. 2b. Insufficient CK19 staining of the appendix using same protocol as in Fig. 1b., same field as in Fig. 2a. Only the surface columnar epithelial cells show a moderate cytoplasmic staining reaction, while virtually no staining reaction is seen in the basal part of the crypts – also compare with Figs. 3b - 4b same protocol.

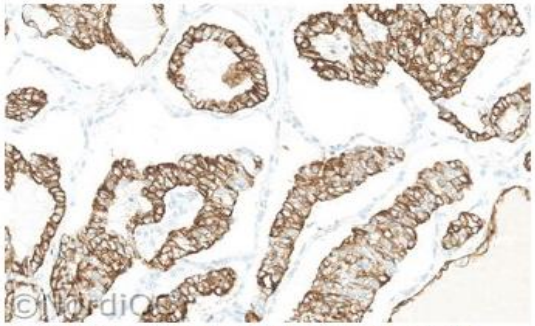


Fig. 3a. Optimal CK19 staining of the papillary thyroid carcinoma using same protocol as in Figs. 1a - 2a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic staining reaction. No background reaction is seen.

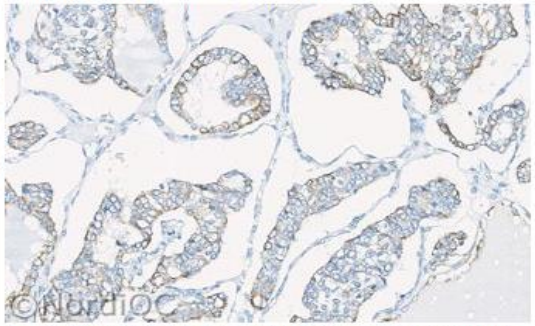


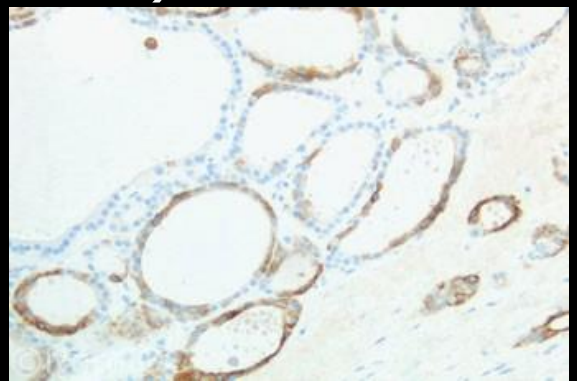
Fig. 3b. Insufficient CK19 staining of the papillary thyroid carcinoma using same protocol as in Figs. 1b & 2b., same field as in Fig. 3a. Only scattered neoplastic cells show a weak to moderate staining reaction.

CK19:

Tonsil / esophagus:
Basal squam. cells

Appendix:
Virtually all epithelial cells

(Normal thyroid:
Scattered epithelial cells)



IHC – Protocols and controls for UPT I

Table 1. Abs and assessment marks for CK19, run 34

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **A53-B/A2.26**	6 6 1 1 1	Cell Marque Thermo/NeoMarkers DBS IDLabs Zytomed	4	5	5	1	60 %	86 %
mAb clone **BA17**	3 1	Thermo/NeoMarkers Master Diagnostica	3	1	0	0	-	-
mAb clone **b170**	7	Leica/Novocastra	5	2	0	0	100 %	100 %
mAb clone **K19.2**	1	Thermo/NeoMarkers*	0	0	0	1	-	-
mAb clone **Ks19.1**	4	Biocare	2	2	0	0	-	-
mAb **RCK108**	61 3 1 1 1 1	Dako Biogenex Abcam Eurodiagnostica EuroProxima Thermo/NeoMarkers	7	16	24	21	34 %	57 %
rmAb **EP72**	1	Epitomics	1	0	0	0	-	-
pAb **RB-9021**	1	Thermo/NeoMarkers	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone **A53-B/A2.26 760-4281**	17	Ventana/Cell Marque	6	6	3	2	71 %	90 %
mAb clone **A53-B/A2.26 319M-17**	1	Cell Marque	0	1	0	0	-	-
mAb clone **A53-B/A2.26 ZM-0074**	1	Zhongshan	0	1	0	0	-	-
mAb clone **b170 PA0799**	3	Leica	0	0	1	2	-	-
mAb clone **Ks19.1 PM242**	1	Biocare	0	1	0	0	-	-
mAb clone **RCK108 IS/IR615**	22	Dako	2	3	12	5	23 %	50 %
mAb clone **RCK108 MS-1902-R7**	1	Thermo/NeoMarkers	0	0	1	0	-	-
Total	147		30	38	46	33	-	
Proportion			22 %	26 %	31 %	23 %	46 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.
* Product has been discontinued by the vendor

Insuff.:

mAb clone RCK108 by proteolysis and/or too low sensitivity

Other abs, too low conc. and/or proteolysis…

14/17 using proteolysis were ins. none optimal…

IHC – Protocols and controls for UPT I

Table 2: Proportion of sufficient results for CK19 in the two NordiQC runs performed

	Run 29 2010	Run 34 2012
Participants, n=	109	147
Sufficient results	69 %	46 %

- Use of Abs giving a low sensitivity
- Inappropriate epitope retrieval
- Misleading data-sheets

IHC – Protocols and controls for UPT I

Specimen preparation

Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin.

Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is required. For heat-induced epitope retrieval, optimal results are obtained with Dako Target Retrieval Solution, pH 9.0, Code S2368, or Dako Target Retrieval Solution, Code S1700. The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure.

Frozen sections and cell preparations: The antibody can be used for labelling frozen sections (6).

Staining procedure

Dilution: Monoclonal Mouse Anti-Human Cytokeratin 19, Code M0888, may be used at a dilution range of 1:50-1:100 when applied on formalin-fixed, paraffin-embedded sections of human breast carcinoma and using 5 minutes proteolytic epitope retrieval with Dako Proteinase K, Code S3020, and 30 minutes incubation at room temperature with the primary antibody. Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended negative control is Dako Mouse IgG1, Code X0931, diluted to the same mouse IgG concentration as the primary antibody. Unless the stability of the diluted antibody and negative control has been established in the actual staining procedure, it is recommended to dilute these reagents immediately before use, or dilute in Dako Antibody Diluent, Code S0809. Positive and negative controls should be run simultaneously with patient specimen.

Visualization: Dako LSAB™+/HRP kit, Code K0679, and Dako EnVision™+/HRP kits, Codes K4004 and K4006, are recommended for formalin-fixed, paraffin-embedded sections. Follow the procedure enclosed with the selected visualization kit.

Automation: The antibody is well-suited for immunohistochemical staining using automated platforms, such as the Dako Autostainer.

mAb clone RCK108 used by > 60% of the labs

- Use of Abs giving a low sensitivity
- Inappropriate epitope retrieval
- Misleading data-sheets

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CLONE: B170

Code	Name	Configuration	Use	Datasheet	MSDS	Qty
PA0799	7ml Cytokeratin19 BondRTU Prim	Bond ready to use reagent	P (ENZYME)			1
CK19-S	0.1ml NCL-CK19	Lyophilised Concentrated Monoclonal Antibody	F, P (ENZYME)			1
CK19	1ml NCL-CK19	Lyophilised Concentrated Monoclonal Antibody	F, P (ENZYME)			1

Recommendations on Use	Immunohistochemistry: Typical working dilution 1:100–1:150. Trypsin digestion of paraffin sections is recommended. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Not evaluated.
Positive Controls	Immunohistochemistry: Skin. Western Blotting: Not evaluated.
Staining Pattern	Cytoplasmic.

IHC – Protocols and controls for UPT I

UPT I: CK19

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb A53-B/A2.26	HIER High	1:25-200	3-step	Ventana	2- & 3-step
mAb b170	HIER High	1:50-200	3-step	-	-
mAb BA17	HIER	1:50-200	3-step	-	-
mAb RCK108	*HIER High*	*1:25-100*	*3-step*	*Dako*	*3-step*

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