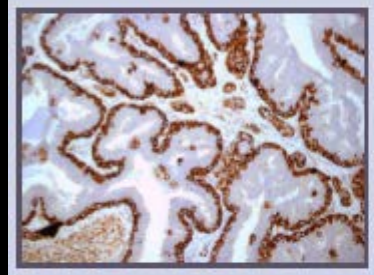

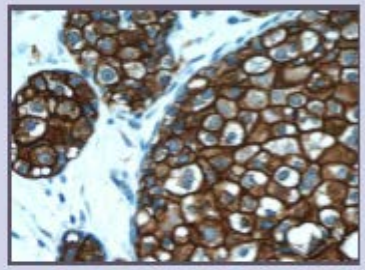

NordiQC

TATA MEDICAL CENTER

ISIMm
International Society for IHC and Molecular Morphology

International Symposium on Immunohistochemistry

January 4th - 7th, 2018

QA of IHC in Gynaecological, GI and Liver pathology

Søren Nielsen
Global Pathology Manager
Agilent Technologies

(Former Scheme Manager, NordiQC)

IHC – Protocols and controls – GYN, GI, Liver

NordiQC

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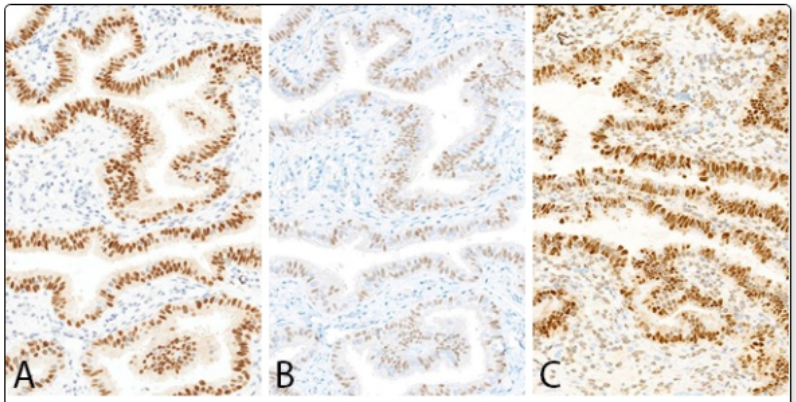

Serial sections of Fallopian tube stained for PAX8 in three laboratories. Lab A gets an optimal result, lab B a too weak staining with false negative reaction in ciliated cells, and lab C a too strong staining with false positive reaction in stromal cells. See the details in the PAX8 assessment, run 51.

Results - module 51, B24, H12, C2

15-Dec-2017

Individual results for the runs 51, B24 and H12 are now available (after logging in). Results from C2 will be available on the 14th January 2018. Click to see an overview of the results.

All news

Events

QuIP/NordiQC Workshop in Applied Immunohistochemistry
13–15 Jun 2018: Brugge, Belgium

NordiQC Workshop in Diagnostic Immunohistochemistry
18–20 Sep 2018: Aalborg, DK

Important dates

Run 52, H13, C3, B25
Slide circulation
9 Jan 2018
Slide return deadline
13 Feb 2018
Publication of results
20 Apr 2018

Questions

Check out our FAQ (Frequently asked questions) or contact us

Google Custom S Search

Collaborators

BIOCARE MEDICAL · CELL MARQUE a SIGMA-ALDRICH company · Dako · Diagnostic BioSystems · horizon · ISIMm · Leica BIOSYSTEMS · Roche · ThermoFisher SCIENTIFIC · VISIOPHARM

IHC – Protocols and controls – GYN, GI, Liver

NordiQC

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- News
- Contact
- New participant
- PD-L1
- Accompanying letters
- Questions (FAQ)

Serial sections of Falll … in three laboratories.
Lab A gets an optima… …ining with false negative
reaction in ciliated ce… …aining with false positive
reaction in stromal ce… …X8 assessment, run 51.

Re… …, H12, C2

15-Dec-2017

Individual results for t… …are now available (after logging in). Results from C2 w… …January 2018. Click to see an overview of the results.

All news

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IHC – Protocols and controls – GYN, GI, Liver

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IHC –

NordiQC

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NordiQC

Register new user

In order to apply for participation in NordiQC, please fill in the form below.
NordiQC will review your information and accept or decline your application within a few days.

Laboratory address

Hospital *	
Department *	
Address 1	
Address 2	
Zip and City *	
Country *	

** = required*

Contacts addresses

Contact 1 *	
Email *	
Contact 2 *	
Email *	
Contact 3	
Email	
Contact 4	
Email	

Modules

General Module	○ YES	○ NO
Breast Cancer Module	○ YES	○ NO
HER2-ISH Module	○ YES	○ NO
Companion Diagnostic Module	○ YES	○ NO

Comments and security

Comments

*To prevent robot-registration, you have to enter a security code **without** letters.*
*For example, security code: **a6h8k3**, you enter: **683***

Security code: d6e7c6

Invoices and payments

Invoice

Laboratories and sponsors are invoiced in the beginning of each year. Participation fees are based on modules, not individual tests. There is no tax on health benefits in Denmark, so invoice amounts are tax exempt. Invoices are sent to the email addresses specified in the user profile, but also available in the user control center.

Deadline for payment is indicated in the invoice. Payment is indicated in DKK and EUR. However, any convertible currency is accepted. Fee is non-refundable. If the amount is not paid by deadline, an electronic reminder will be sent to all the e-mail addresses indicated. Laboratories neither paying the fee invoiced nor responding to a reminder will have their participation closed until the account is settled.

NordiQC is a not-for-profit organization. Rates indicated in the table below only cover running cost. For this reason, discounts are not available. Apart from new participants, laboratories can only participate on an annual basis paying the fee indicated above (fees are not reduced if a laboratory does not stock an antibody or omits to submit protocols or return slides).

Questions

Check out our FAQ (Frequently asked questions) or contact us

Google Custom S Search

Annual subscription rates (2017)

Module	Runs	Tests	DKK	EUR
General	3	17	7,500	1,008
Breast cancer	2	4	3,600	483
HER2 ISH	2	2	1,700	228
All modules above (discount)	7	23	11,600	1,559
Companion (PD-L1)	2	2	3,200	428

Annual subscription rates (2018)

Module	Runs	Tests	DKK	EUR
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All modules above (discount)	7	23	11,600	1,559
Companion (PD-L1)	2	2	3,200	428

New labs enrolled during the year (2018)

General (after 3rd January)	2	11	5,500	739
General (after 24th March)	1	5	3,500	470
Breast (after 3rd January)	1	2	2,500	336
HER-2 ISH (after 3rd January)	1	1	1,200	161
Companion (after 3rd January)	1	1	2,000	268

Sponsorships

NordiQC assessment scheme 2018

Module	Winter	Spring	Autum
General	**Run 52** CR MSH6 SYP TdT VIM	**Run 53** BSAP CGA ECAD OCT3/4 PMS2	**Run 54** CD8 CEA CK-PAN GATA3 Podop
Breast	**Run B25** ER HER2 IHC		**Run B26** ER HER2 IHC PR
HER2	**Run H13** HER2 ISH		**Run H14** HER2 ISH
Companion	**Run C3** PD-L1		**Run C4** PD-L1

Dates	Winter	Spring	Autum
Protocol submission opens	1 Dec 2017	12 Feb	1 Aug
Protocol submission closes	3 Jan	13 Mar	4 Sep
Shipping of slides	9 Jan	21 Mar	12 Sep
Deadline for slide return	13 Feb	1 May	11 Oct
Assessment General	6 Mar - 8 Mar	23 May - 25 May	24 Oct - 26 Oct
Assessment Breast	15 Mar - 16 Mar		8 Nov - 9 Nov
Assessment HER2	23 Mar		16 Nov
Assessment Companion	5 Apr		20 Nov
Publication of results	20 Apr	9 Jul	7 Dec

IHC – Protocols and controls – GYN, GI, Liver

- CDX2 — GI
- CEA
- Cadherin 17
- SMAD4

- PAX8 — Fem. Gen.
- CA125
- WT1

- Arginase — Liver
- Glypican 3
- Glutamin synthease
- Hepatocellular antigen

- MLH1 — GI + Fem. Gen
- MSH2
- MSH6
- PMS2

IHC – Protocols and controls – GYN, GI, Liver

	Recommendable clones (conc.)*	Less successful clones (conc.)	RTU ”plug and play”** giving optimal result
CEA	mAb II-7 mAb CEA31 mAb COL1	mAb 12-140-10 mAb PARLAM4 mAb TF3H8-1	Leica: mAb II-7 VMS: mAb CEA31
CDX2	mAb DAK-CDX2 rmAb EPR2764Y	mAb AMT28 mAb CDX2-88	Dako: mAb DAK-CDX2 VMS: rmAb EPR2764Y
CAD17	rmAb SP183***		
SMAD4	mAb BC8 rmAb EP618Y***		

* Potential to provide optimal result by a laboratory developed test (LDT)

** Using the protocol settings as recommended by the vendor – incubation, retrieval, detection kit.

*** Aalborg University Hospital data

IHC – Protocols and controls – GYN, GI, Liver

	Positive tissue control HE*	Positive tissue control LE**	Negative tissue control NE***
CEA	Appendix: Brushborder of columnar epith. cells	Appendix: Cytoplasm. compartm. of col. epith. cells.	Liver: Hepatocytes
CDX2	Appendix: Columnar epithelial cells	Pancreas: Epith. cells of intercalating ducts	Liver: Hepatocytes
CAD17	Appendix: Columnar epithelial cells	Pancreas: Scatered col. epith. cells of large ducts	Liver: Hepatocytes
SMAD4	Tonsil: Dispersed squam. epith. cells + plasma cells	Tonsil: Lymphocytes	Tumour: Neoplasia with loss of SMAD4

* HE = High expression

** LE = Low expression

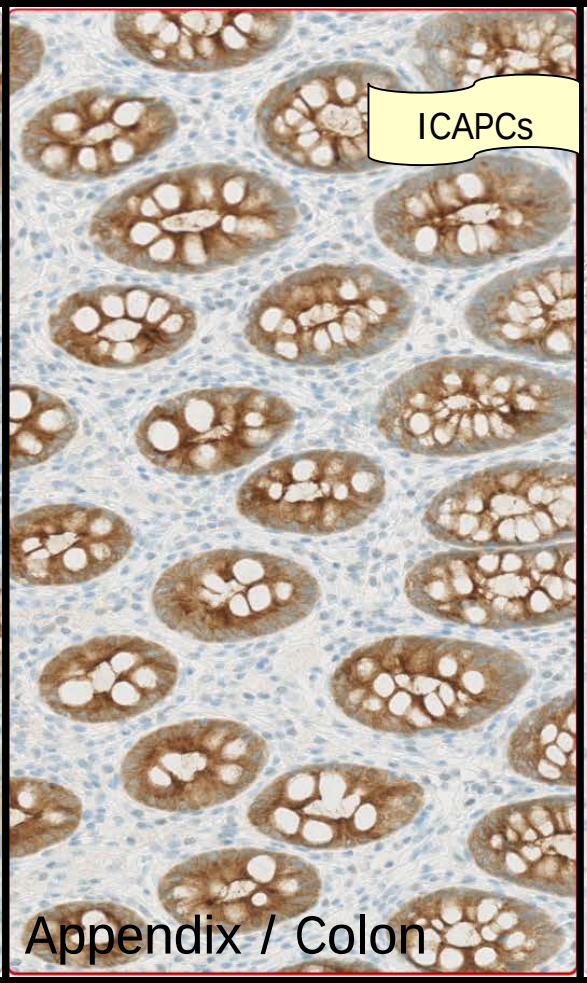
*** NE = No expression

IHC – Protocols and controls – GYN, GI, Liver

CEA reaction pattern

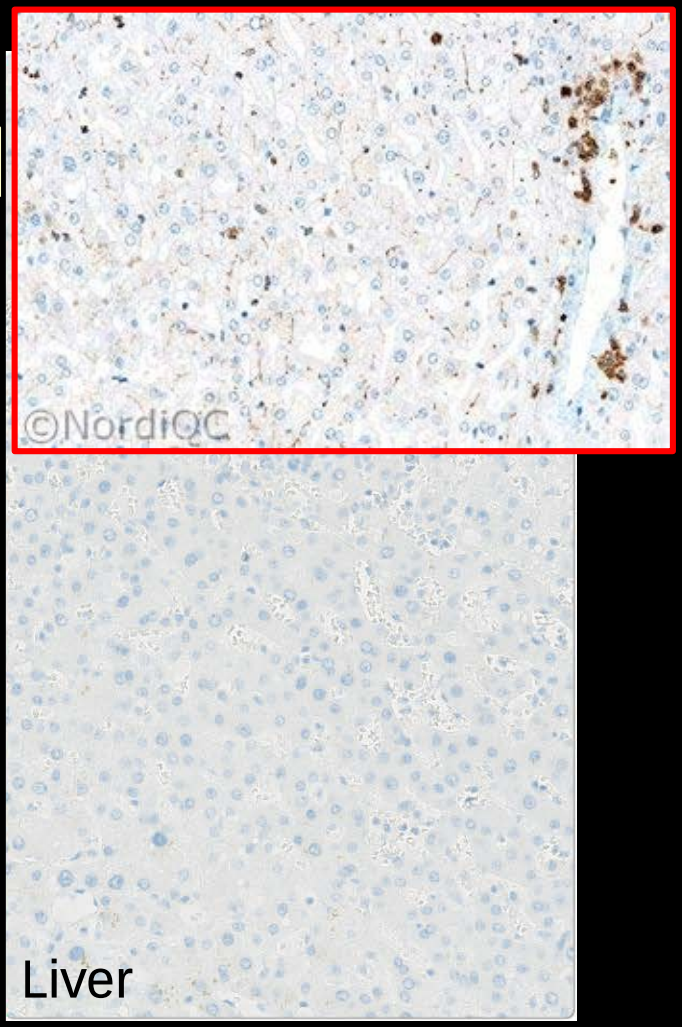
Appendix / Colon

A strong accentuation of the brushborder / glycocalyx of the luminal epithelial cells.



Appendix / Colon

An at least weak to moderate and distinct cytoplasmic staining reaction of the vast majority of epithelial cells.



Liver

No staining reaction.

No staining of bile canaliculi, leucocytes and Kupffer cells (NCA, BGP)

IHC – Protocols and controls – GYN, GI, Liver

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

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **12-140-10**	6	Leica/Novocastra	0	0	1	5	-	-
mAb clone **CEA31**	1	Cell Marque	1	0	0	0	-	-
mAb **COL-1**	9 4 4 3 1	Thermo/Neomarkers Biocare Invitrogen/Zymed Immunologic Zytomed	13	4	4	0	81 %	100 %
mAb **II-7**	89	Dako	12	42	33	2	61 %	93 %
mAb **PARLAM 4**	1	BioScience Products AG	0	0	1	0	-	-
rmAb **EP216**	1	Epitomics	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **B01-94-11-M AM009**	1	Biogenex	0	0	1	0	-	-
mAb clone **CEA31 760-4594**	12	Ventana/Cell Marque	9	2	1	0	92 %	92 %
mAb clone **CEA31 236M**	1	Cell Marque	1	0	0	0	-	-
mAb clone **CEA31 ZM-0062**	1	Zhongshan	0	0	1	0	-	-
mAb clone **COL-1 PM058**	1	Biocare	0	1	0	0	-	-
mAb clone **II-7 IR/IS622**	33	Dako	1	19	13	0	61 %	80 %
rmAb clone **II-7 N1586**	2	Dako	0	2	0	0	-	-
mAb clone **II-7 PA0004**	4	Leica	1	3	0	0	-	-
mAb clone **TF3H8-1 760-2507**	16	Ventana	0	0	0	16	0 %	0 %
Total	190		38	74	55	23	-	
Proportion			20 %	39 %	29 %	12 %	59 %	

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

COL-1 & CEA31

Optimal on all platforms

II-7 less successful especially on VMS and Omnis

Clone !

HIER
Titre

IHC – Protocols and controls – GYN, GI, Liver

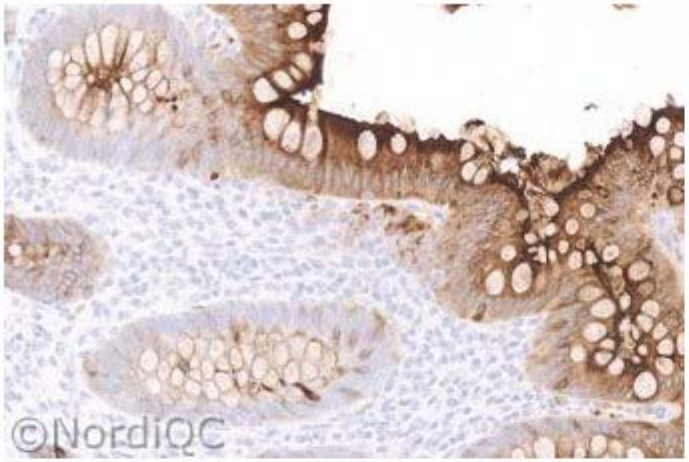


Fig. 1a. Optimal CEA staining of the appendix using the mAb clone CEA31 optimally calibrated and with HIER. A weak to moderate staining reaction is seen in the vast majority of the luminal epithelial cells of the appendix, whereas the glycocalyx show an intense staining reaction. Also compare with Figs. 2a – 4a, same protocol.

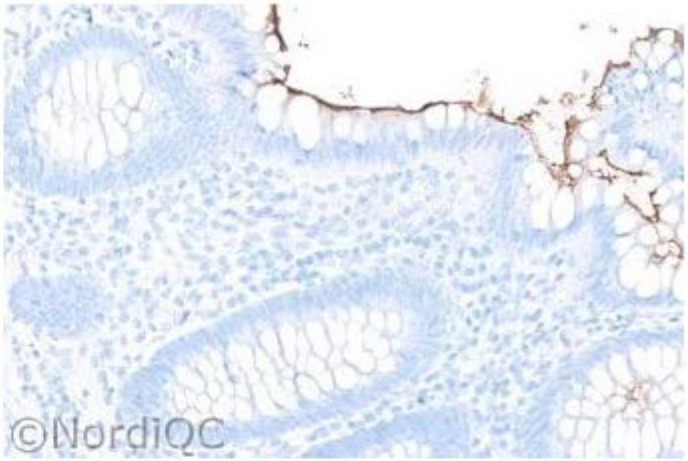


Fig. 1b. Insufficient CEA staining of the appendix using the mAb clone II-7 with a less successful protocol – insufficient HIER and too diluted Ab. Only the glycocalyx is distinctively demonstrated, while the cytoplasmic compartment of the epithelial cells is unstained - same field as in Fig. 1a. Also compare with Figs. 2b & 3b, same protocol.

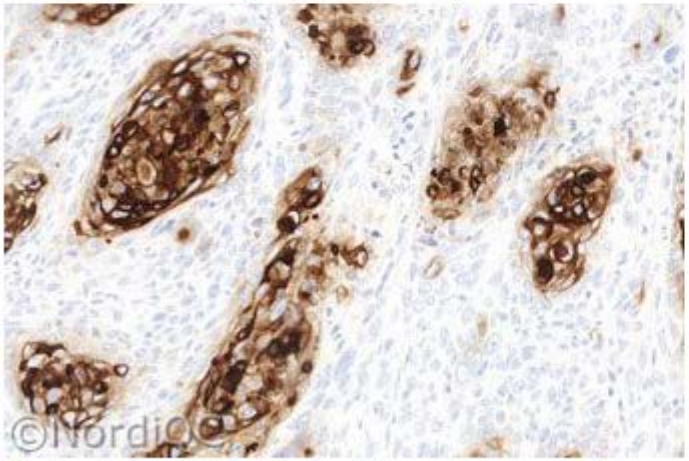


Fig. 2a. Optimal CEA staining of the colon adenocarcinoma, tissue core no. 4 using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining reaction. No background staining is seen.

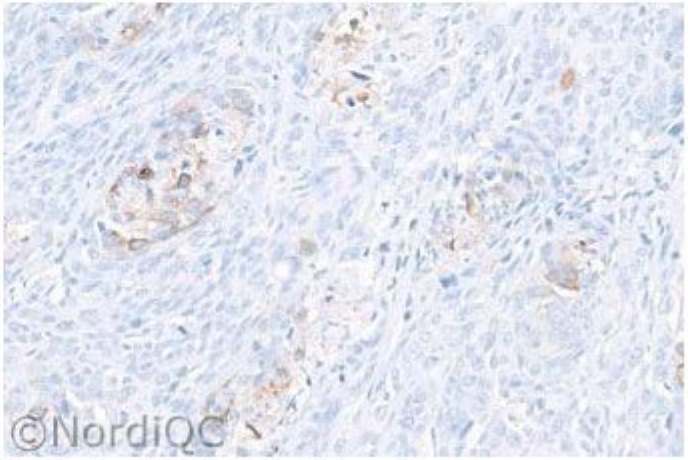
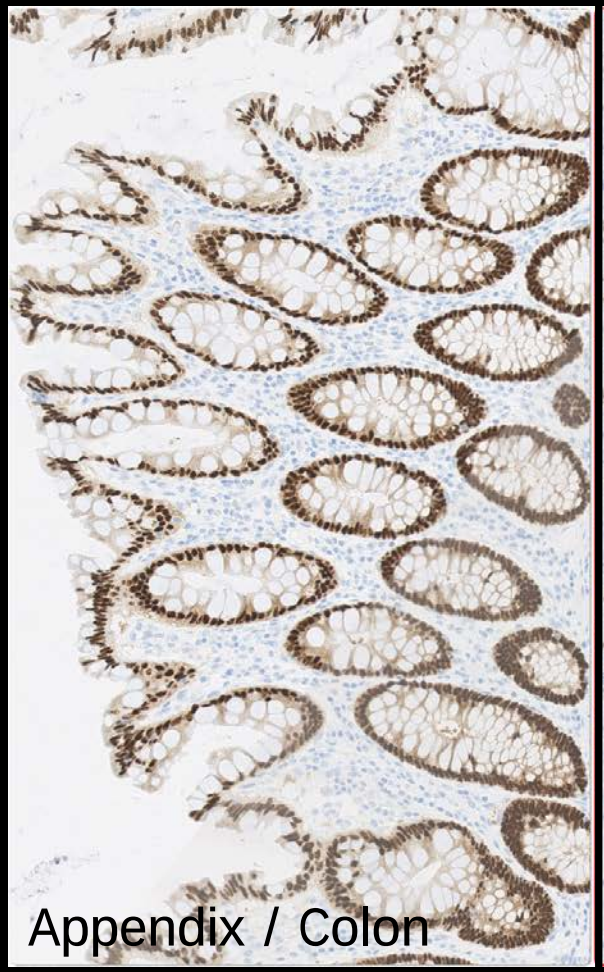


Fig. 2b. Insufficient CEA staining of the colon adenocarcinoma, tissue core no. 4 using same protocol as in Fig. 1b. – same field as in Fig. 2a. The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 2a.

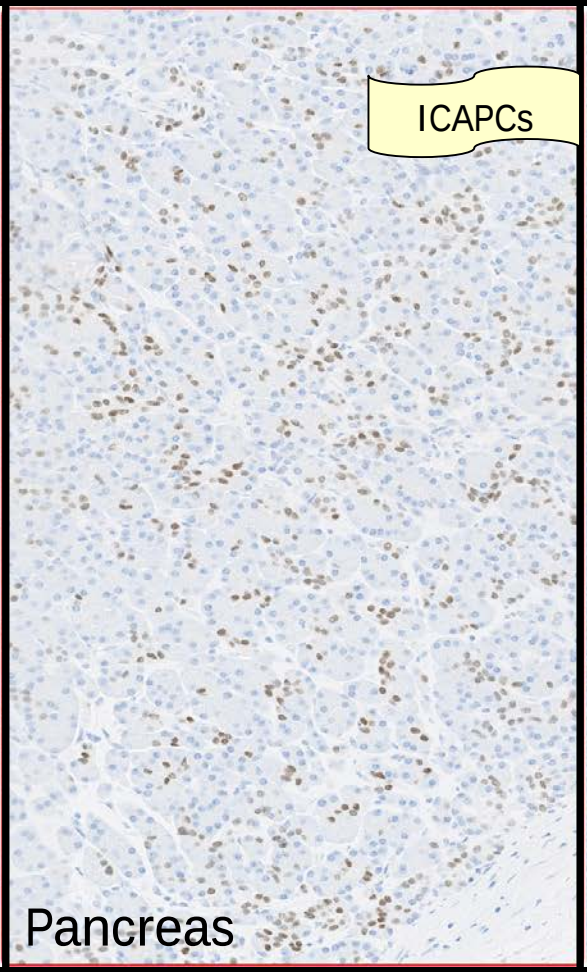
IHC – Protocols and controls – GYN, GI, Liver

CDX2 reaction pattern



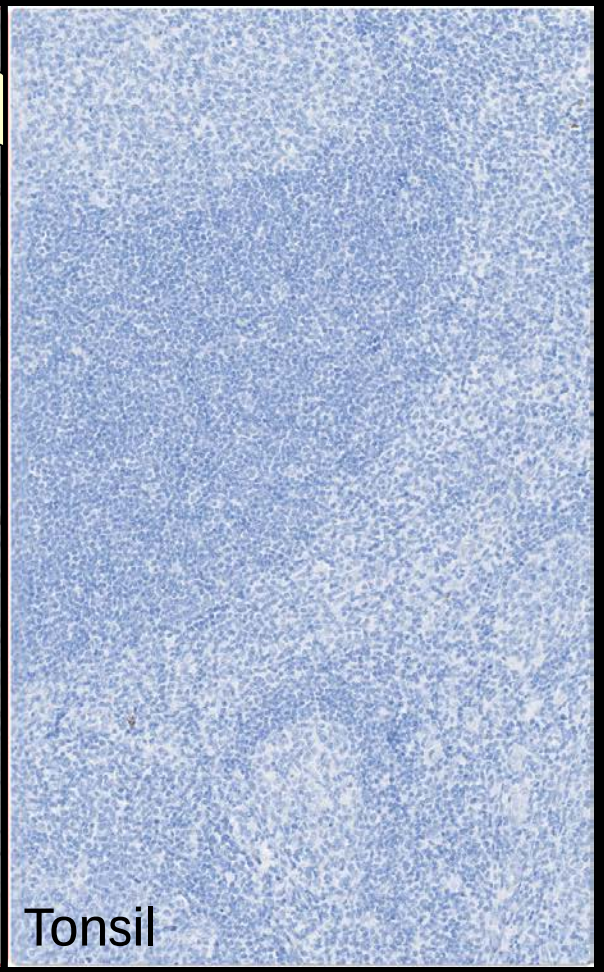
Appendix / Colon

A strong nuclear staining reaction of virtually all epithelial cells. A weak to moderate cytoplasmic staining reaction can be expected.



Pancreas

An at least weak to moderate and distinct nuclear staining reaction of the vast majority of epithelial cells of intercalating ducts.



Tonsil

No staining reaction. *Few lymphocytes may show a faint nuclear staining reaction.*

IHC – Protocols and controls – GYN, GI, Liver

Table 1. **Antibodies and assessment marks for CDX2, run 48**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **AMT28**	2	Leica/Novocastra	0	0	0	2	-	-
mAb clone **CDX2-88**	2 2	Biocare Biogenex	0	0	1	3	-	-
mAb clone **DAK-CDX2**	31	Agilent/Dako	6	9	7	9	48%	57%
rmAb clone **EPR2764Y**	31 5 4 4 2 2 1 1 1 1	Cell Marque Thermo/Neomarkers Immunologic Zytomed Monosan Zeta Corporation A.Menarini Abcam Nordic Biosite Thermo/Pierce	28	14	7	3	81%	81%
Ready-To-Use antibodies								
mAb clone **BC39 API3184**	1	Biocare	0	0	0	1	-	-
mAb clone **CDX2-88 PM226**	1	Biocare	0	1	0	0	-	-
mAb clone **CDX2-88 AM392**	1	Biogenex	0	1	0	0	-	-
mAb **DAK-CDX2 IR080/IS080**	34	Agilent/Dako	18	10	5	1	82%	93%
mAb **DAK-CDX2 GA080**	26	Agilent/Dako	16	4	3	3	77%	100%
rmAb clone **EP25 RMPD059**	1	Diagnostic Biosystems	0	0	1	0	-	-
rmAb clone **EP25 PA0375**	7	Leica/Novocastra	4	3	0	0	100%	100%
rmAb clone **EP25 MAD-000645QD**	3	Master Diagnostica	0	3	0	0	-	-
rmAb clone **EPR2764Y RMA-0631**	1	Maixin	1	0	0	0	-	-
mAb clone **EPR2764Y RM-2116-R7**	1	Thermo/Neomarkers	0	0	1	0	-	-
rmAb clone **EPR2764Y 760-4380/ 235R***	103	Ventana/Cell Marque	81	15	5	2	93%	96%
Total	268		154	60	30	24	-	
Proportion			58%	22%	11%	9%	80%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
* Products merged due to imprecise antibody selection at the NordiQC homepage for protocol submission.

AMT28 and CDX2-88 lower pass rate – used by a significantly lower proportion – 3% in this run vs 70% in run 27 2009.

DAK-CDX2 & EPR2764Y

RTU superior

Control tissue !

IHC – Protocols and controls – GYN, GI, Liver

Table 2: **Optimal results for CDX2 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 **CDX2-88**	50 % 1/2**	-	0 % 0/8	-	50 % 1/2	-
mAb clone **DAK-CDX2**	67 % 6/9	-	0 % 0/7	-	-	-
rmAb clone **EPR2764Y**	60 % 3/5	100 % 1/1	63 % 10/16	-	80 % 4/5	0 % 0/2

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms. ** Number of optimal results/number of laboratories using this buffer.

Dako conc. and RTU format of mAb clone DAK-CDX2 will show an inferior pass-rate on a VMS platform

Frequently Dako RTU format was applied on VMS.....!

Table 2. **Proportion of sufficient results for CDX2 in the five NordiQC runs performed**

	Run 22 2008	Run 27 2009	Run 33 2011	Run 38 2013	Run 48 2016
Participants, n=	56	93	148	200	268
Sufficient results	64%	46%	51%	73%	80%

< use of mAb clones CDX2-88 and AMT28

IHC – Protocols and controls – GYN, GI, Liver

- Inappropriate RTU settings
  - use of modified protocol settings of otherwise successful RTU product

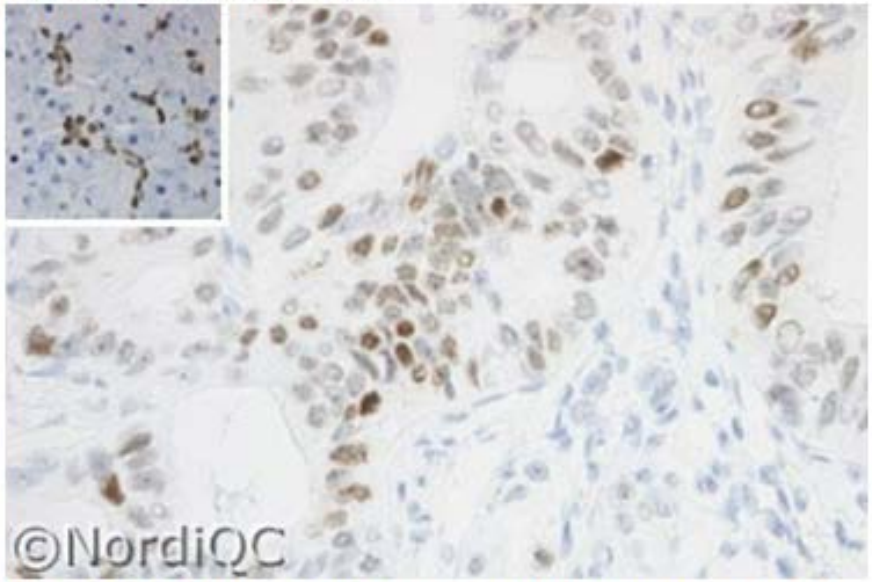


Fig. 4a. Pancreas adenocarcinoma and normal pancreas (insert) showing optimal staining for CDX2 with clone the mAb clone DAK-CDX2 in a Ready-To-Use format and performed at the Autostainer platform. A weak to moderate staining is seen in the majority of the ductal epithelial cells of the pancreas and in the majority of the tumour cells in the pancreas adenocarcinoma.

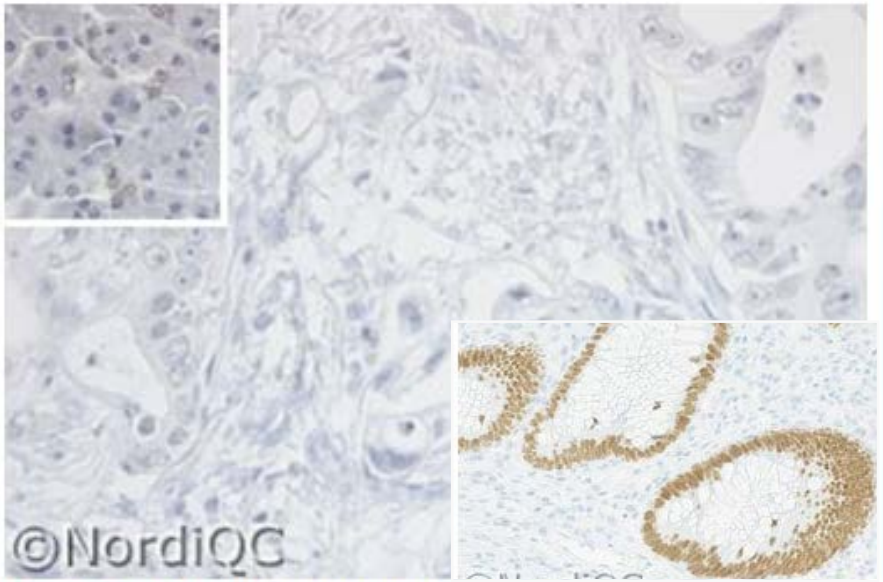
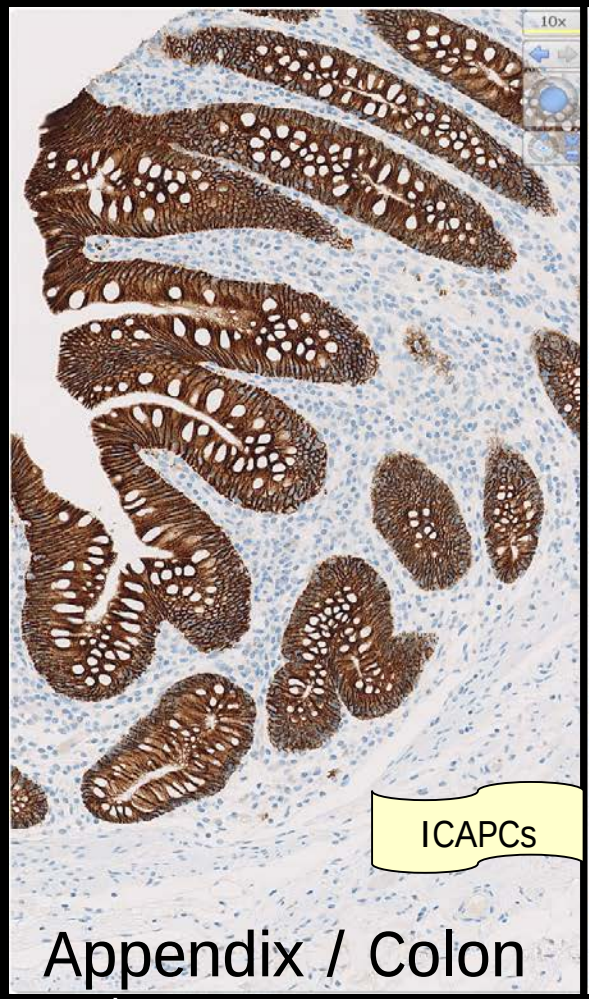


Fig. 4b. Pancreas adenocarcinoma and normal pancreas (insert) showing an insufficient staining for CDX2. Same Ready-To-Use product of the mAb clone DAK-CDX2 as in Fig. 4a was used, but performed at the Ventana Benchmark platform. Only a faint staining in very few ductal epithelial cells is seen and the tumour cells are negative. The mAb clone DAK-CDX2 was found to have an suboptimal performance on the Ventana Benchmark platform.

Dilution of RTU format, use of RTU format ”out of system” !!!!

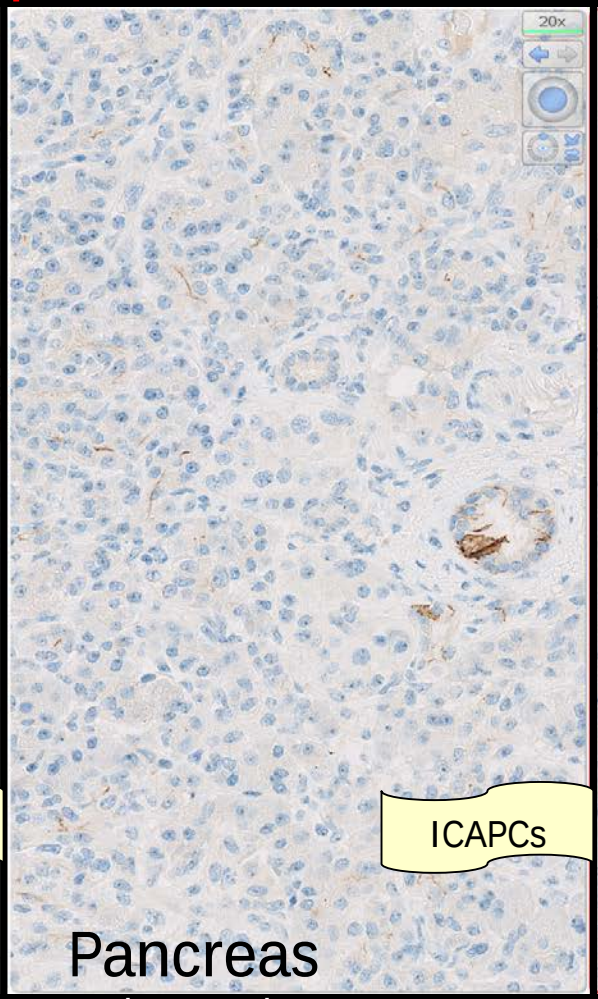
IHC – Protocols and controls – GYN, GI, Liver

Cadherin 17 reaction pattern



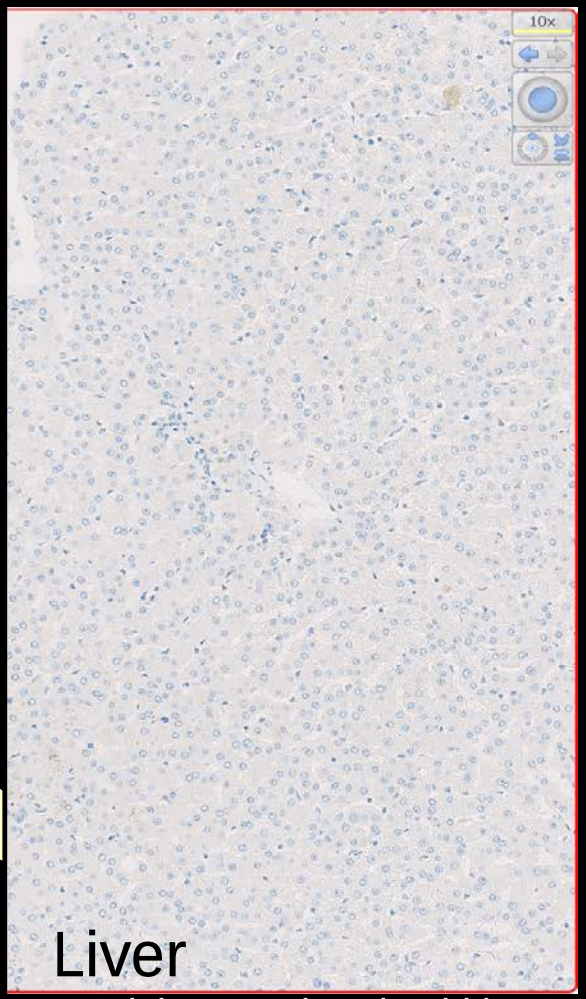
Appendix / Colon

A moderate to strong membranous staining of virtually all columnar epithelial cells – both luminal and crypt base.
.



Pancreas

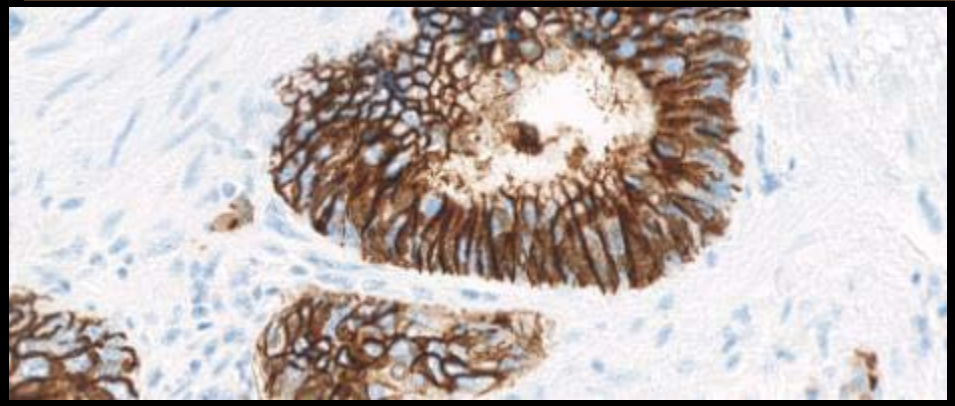
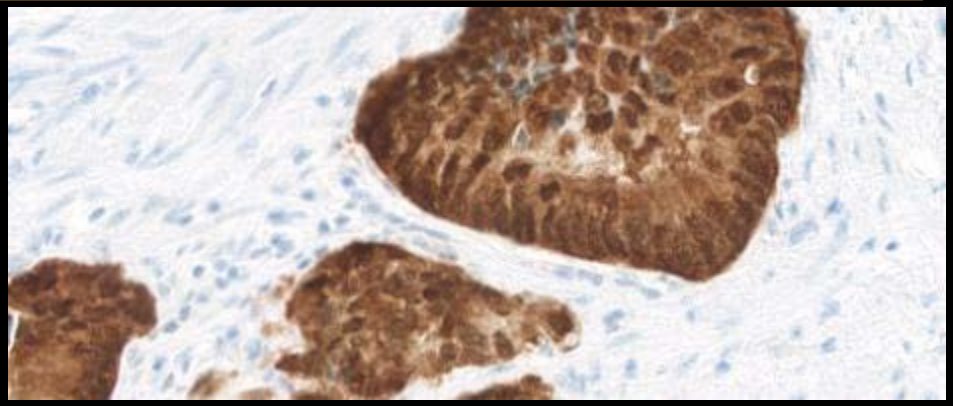
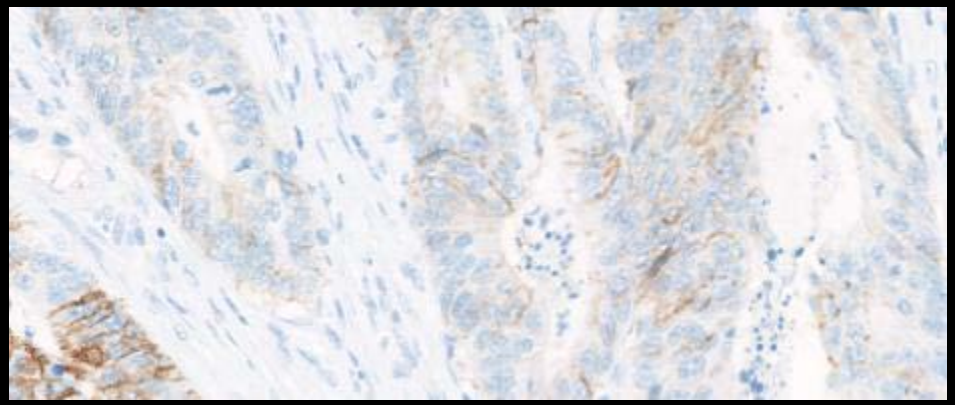
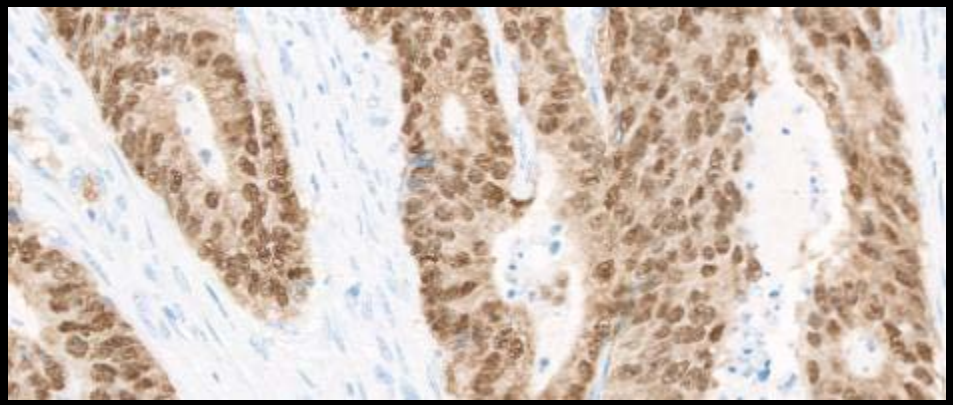
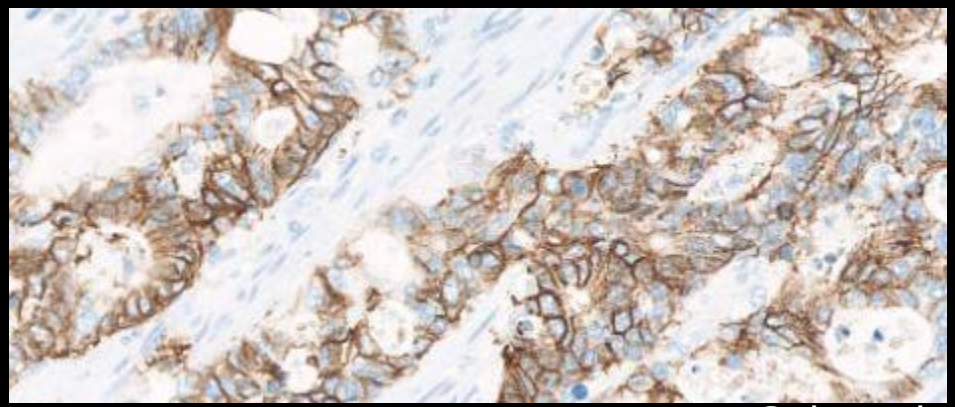
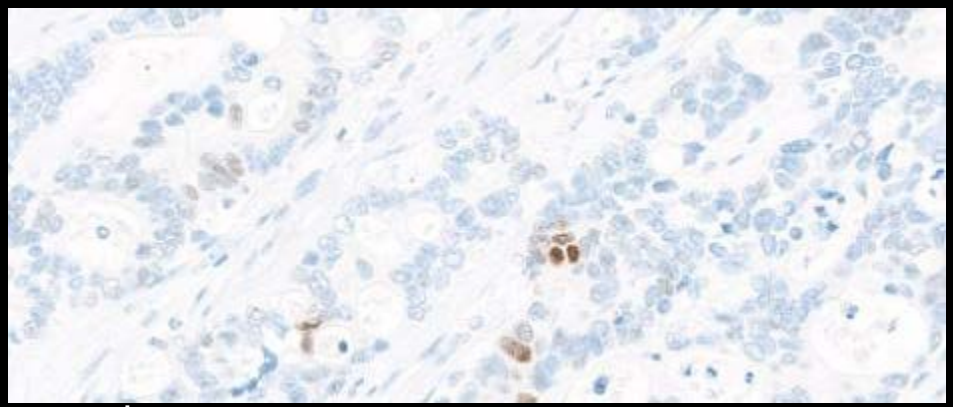
A weak to moderate membranous staining of dispersed columnar epithelial cells of large ducts and exocrine acini.



Liver

No staining reaction should be seen.

IHC – Protocols and controls – GYN, GI, Liver

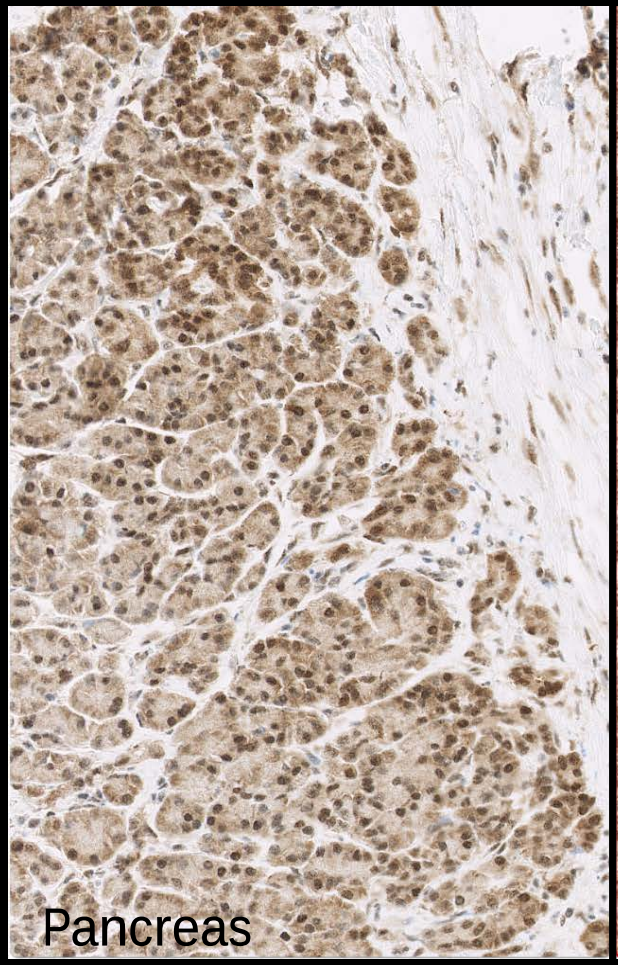
Colon adenocarcinomas

Cadherin 17, rmAb SP183

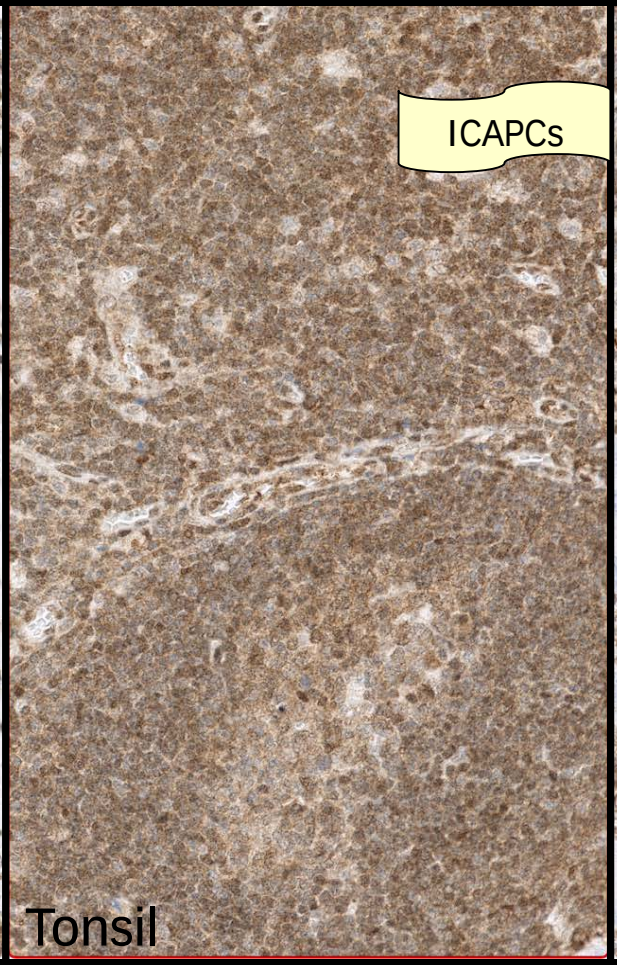
CDX2, rmAb EPR2764Y

IHC – Protocols and controls – GYN, GI, Liver

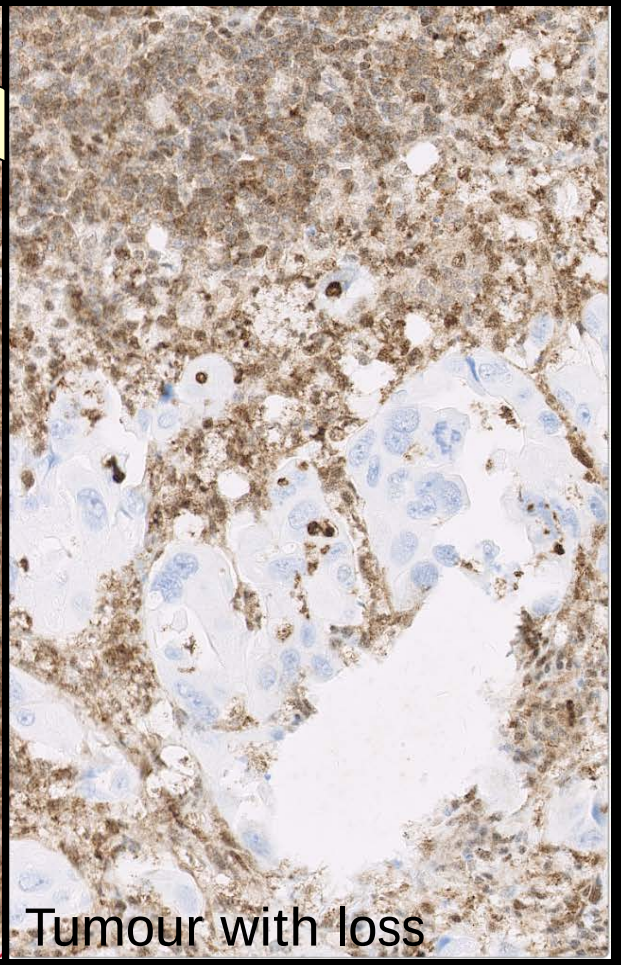
SMAD4 reaction pattern



A moderate to strong nuclear staining reaction of the majority of all cells. A weak to moderate cytoplasmic staining reaction can be expected.



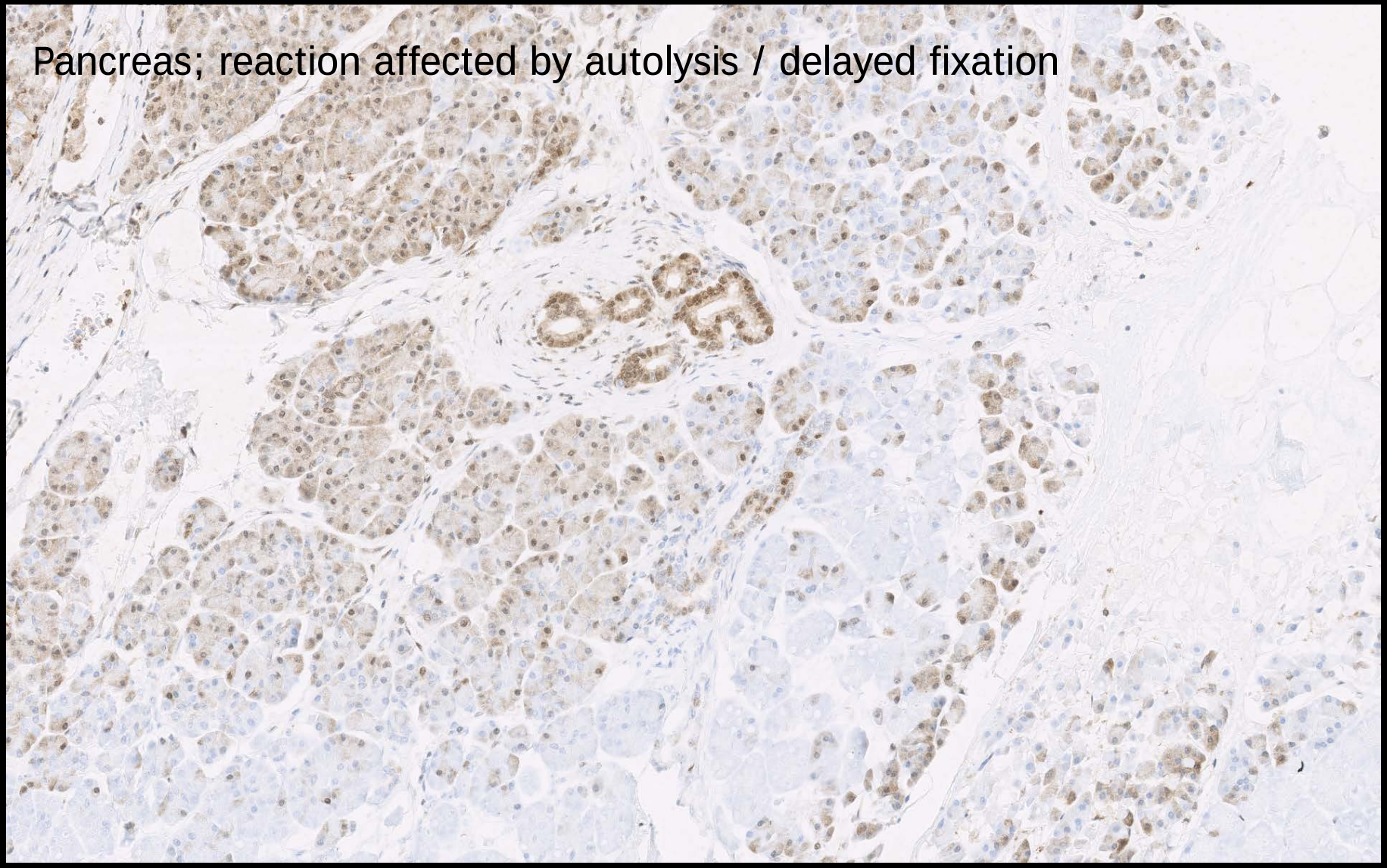
A moderate to strong nuclear staining reaction of the majority of all cells. A weak to moderate cytoplasmic staining reaction can be expected.



No nuclear staining reaction in neoplastic cells. Stromal cells serving as internal positive control.

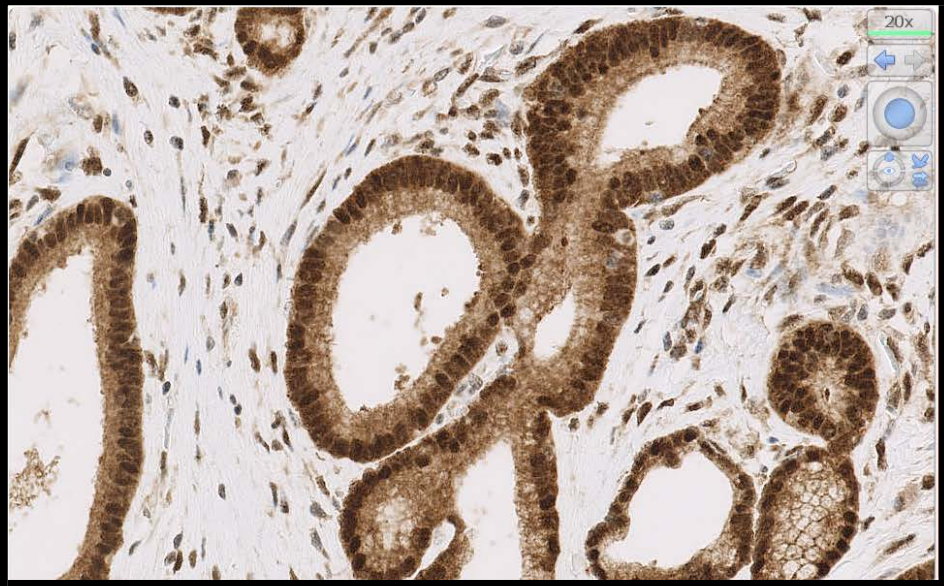
IHC – Protocols and controls – GYN, GI, Liver

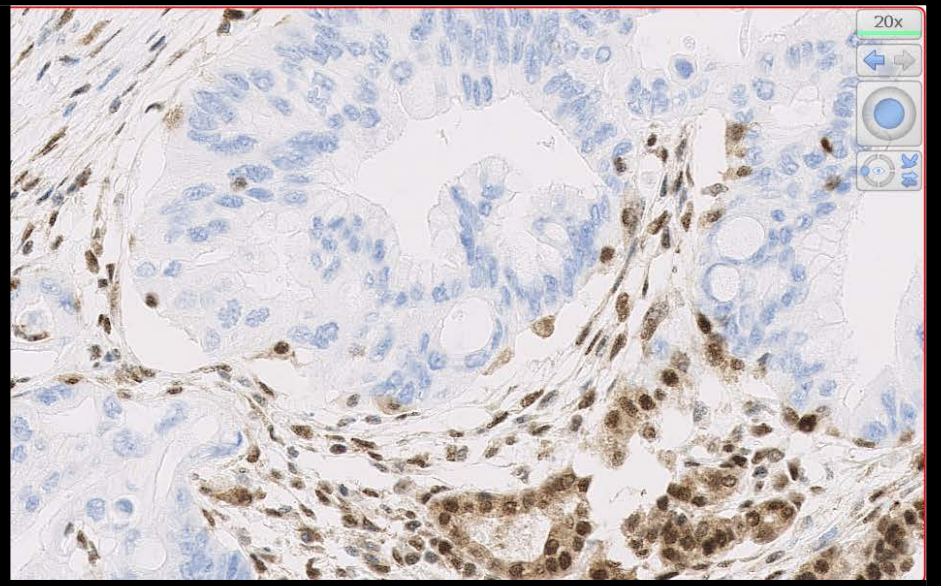
SMAD4 reaction pattern

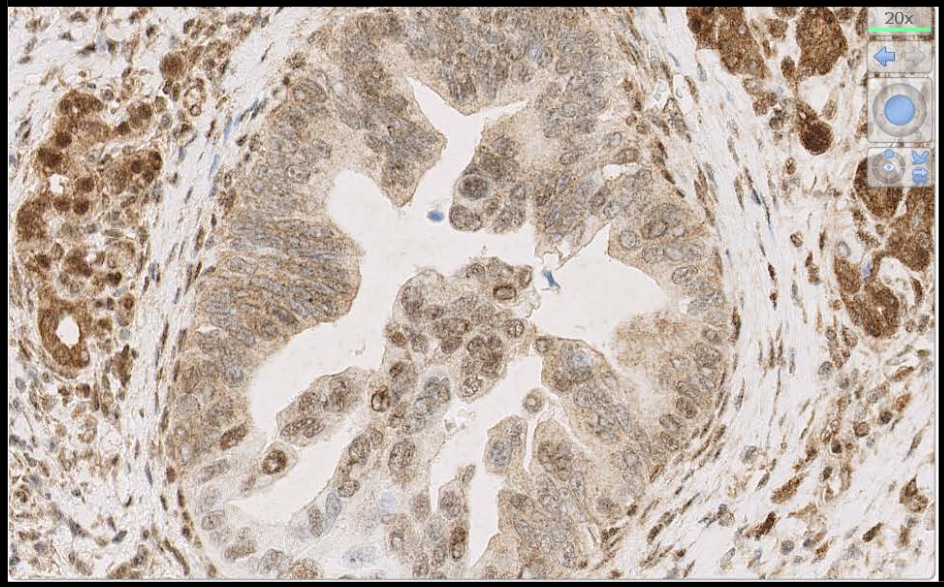


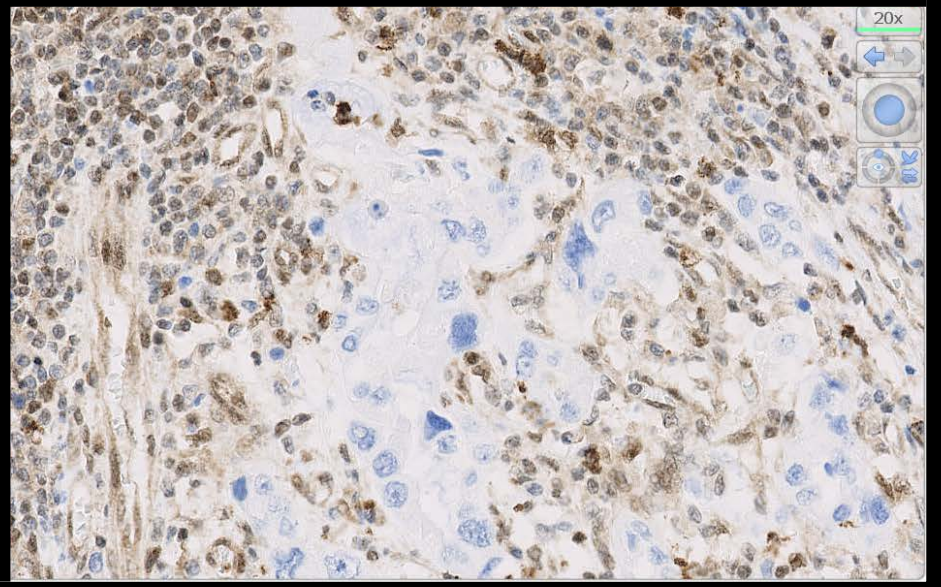
IHC – Protocols and controls – GYN, GI, Liver

IHC – Protocols and controls – GYN, GI, Liver





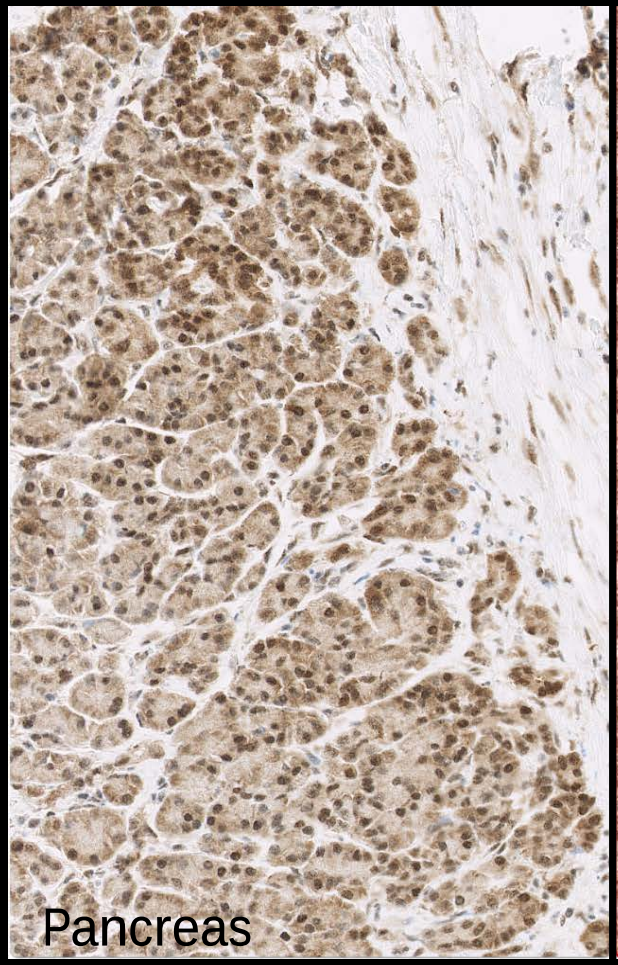




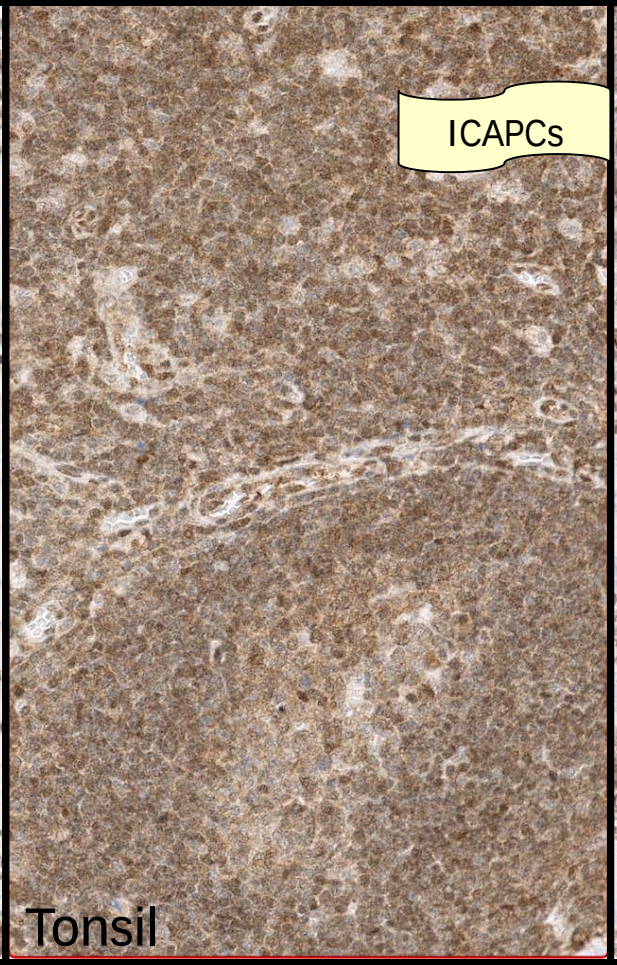
SMAD4 staining: mAb BC8, HIER TE pH 9, FLEX+

IHC – Protocols and controls – GYN, GI, Liver

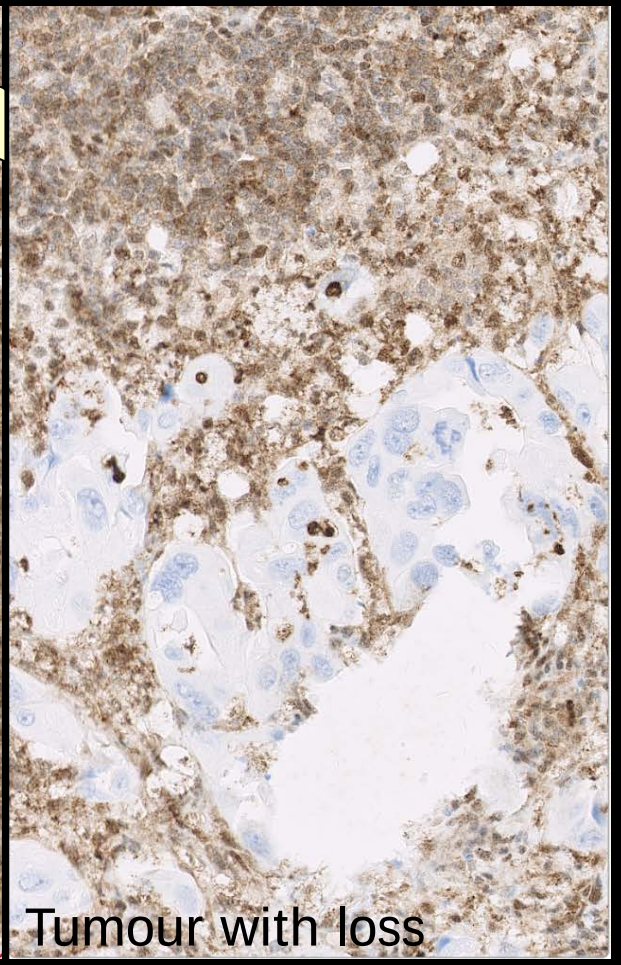
SMAD4 reaction pattern



A moderate to strong nuclear staining reaction of the majority of all cells. A weak to moderate cytoplasmic staining reaction can be expected.



A moderate to strong nuclear staining reaction of the majority of all cells. A weak to moderate cytoplasmic staining reaction can be expected.



No nuclear staining reaction in neoplastic cells. Stromal cells serving as internal positive control.

IHC – Protocols and controls – GYN, GI, Liver

UPT II: CDX2

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb DAK-CDX2*	HIER high pH	1:10-30	3-step	Dako	2- & 3-step
rmAb EPR2764Y	HIER high pH	1:50-100	3-step	Ventana	2- & 3-step

UPT II: CEA

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb II-7*	HIER high pH	1:25-200	3-step	Dako, Leica	3-step
mAb CEA31	HIER high pH	1:100-400	2- & 3-step	Ventana	2- & 3-step
mAb COL-1	HIER high pH	1:100-500	2- & 3-step		

* Inferior performance on VMS stainer platform

IHC – Protocols and controls – GYN, GI, Liver

UPT II: SMAD4

Basic protocol settings for an optimal staining result (Internal data)

	Retrieval	Titre	Detection	RTU	Detection
mAb BC8*	HIER high	1:200-400	3-step	-	-
rmAb EP618Y	HIER high	1:800-1.500	3-step	-	-

UPT II: Cadherin 17

	Retrieval	Titre	Detection	RTU	Detection
rmAb SP183	HIER high pH	1:50-100	3-step	-	-

* Inferior performance on Dako OMNIS and VMS stainer platform

IHC – Protocols and controls – GYN, GI, Liver

	Recommendable clones (conc.)	Less successful clones (conc.)	RTU ”plug and play” giving optimal result
PAX8	mAb BC12* mAb ILQ-50 mAb MRQ-50*, ** rmAb ZR-1** rmAb EP298 (rmAb EP331) pAb 10336-1-AP	pAb 363	
CA125	mAb M11 rmAb OV185:2		Dako: mAb M11
WT1	mAb 6F-H2 mAb WT49		Dako: mAb 6F-H2 Leica: mAb WT49

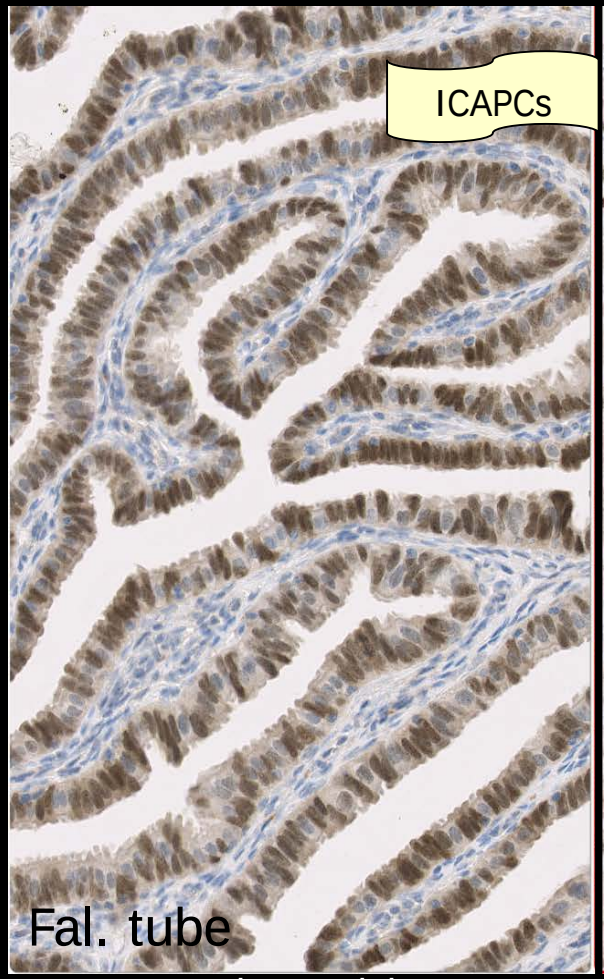
* Inferior performance on VMS & Leica stainer platforms
** Inferior performance on Dako Omnis stainer platform
** Lot-to-lot variations

IHC – Protocols and controls – GYN, GI, Liver

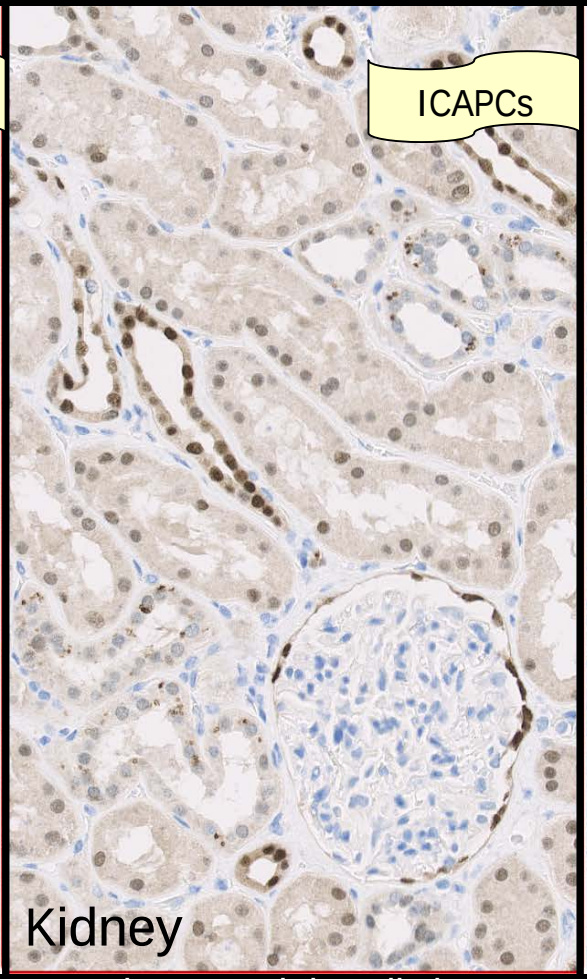
	Positive tissue control HE	Positive tissue control LE	Negative tissue control NE
PAX8	Fallopian tube: Secretory epithelial cells. Kidney: Epithelial cells of collecting ducts and lining Bowman capsules.	Fallopian tube: Ciliated epithelial cells. Kidney: Epithelial cells of proximale tubules.	Appendix: Epithelial cells
CA125	*Fallopian tube: Brushborder of columnar epith. cells.*	*Fallopian tube: Brushborder of columnar epith. cells.*	Appendix: Columnar epithelial cells
WT1	Fallopian tube: Columnar epithelial cells	Kidney: Parietal epithelial cells and podocytes	Tonsil: Lymphocytes, endothelial cells (nuclei)

IHC – Protocols and controls – GYN, GI, Liver

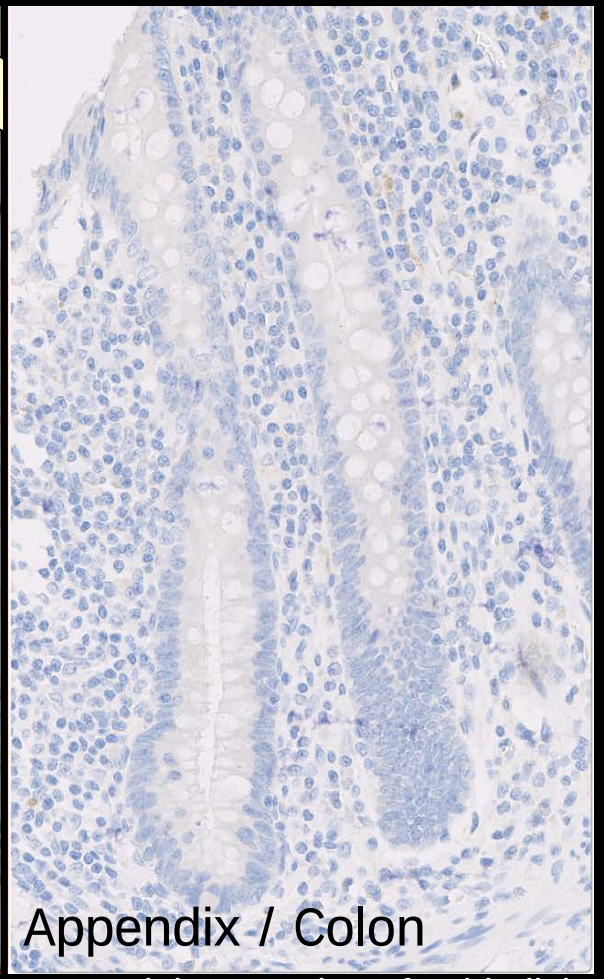
PAX8 reaction pattern



A strong nuclear staining reaction of virtually all secretory epithelial cells. A weak to moderate nusclear staining reaction of the majority of ciliated cells.



An at least weak but distinct nuclear staining of the majority of epithelial cells of proximal tubules. Moderate to strong nuclear staining of epithelial cells of distale tubules and Bowman.



No staining reaction of epithelial cells.

IHC – Protocols and controls – GYN, GI, Liver

Table 1. Antibodies and assessment marks for PAX8, run 42

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **MRQ-50**	33	Cell Marque	19	8	6	0	82%	81%
mAb clone **BC12**	7	BioCare	1	3	1	2	57%	-
mAb clone **ILQ-150**	1	Immunologic	1	0	0	0	-	-
mAb clone **PAX8R1**	1	Abcam	0	1	0	0	-	-
rmAb clone **ZR-1**	1 1 1	Abcam Zeta Zhongshan	2	0	0	1	-	-
pAb, **363A**	11	Cell Marque	0	4	7	0	36%	-
pAb, **10336-1-AP**	11	Protein Tech	5	5	0	1	91%	100%
pAb, **CP379**	4	Biocare	1	2	1	0	-	-
pAb, **RBK047**	2	Zytomed Systems	0	1	1	0	-	-
pAb, **HPA030062**	1	Atlas Antibodies	0	0	0	1	-	-
pAb, **ILP3633-C05**	1	Immunologic	0	1	0	0	-	-
pAb, **ABE671**	1	Millipore	0	0	1	0	-	-
pAb, **NBP1-32440**	1	Novus	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **MRQ-50 760-4618**	36	Ventana/Cell Marque	2	20	12	2	61%	73%
mAb clone **MRQ-50 MAD-000550QD**	3	Master Diagnostica	3	0	0	0	-	-
mAb clone **MRQ-50 363M**	2	Cell Marque	1	1	0	0	-	-
mAb clone **BC12 API438**	3	BioCare	3	0	0	0	-	-
mAb clone **BC12 PDM 180**	1	Diagnostic Biosystems	0	0	0	1	-	-
mAb clone **ILQ-150 ILM4403**	1	Immunologic	1	0	0	0	-	-
Total	125		41	47	29	8	-	
Proportion			33%	38%	23%	6%	71%	

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

Choose clone depending on platform...

Use 3-step system

IHC – Protocols and controls – GYN, GI, Liver

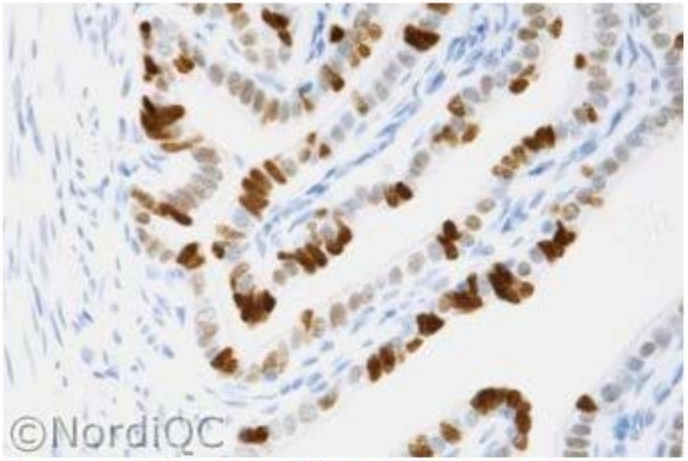


Fig. 1a. Optimal PAX8 staining of the Fallopian tube using the mAb clone MRQ-50 as a concentrate, HIER in TRS pH 6.1 and a 3-step polymer based detection system. Virtually all the ciliated epithelial cells show a distinct, weak to moderate nuclear staining reaction, while the secretory epithelial cells are strongly labelled.

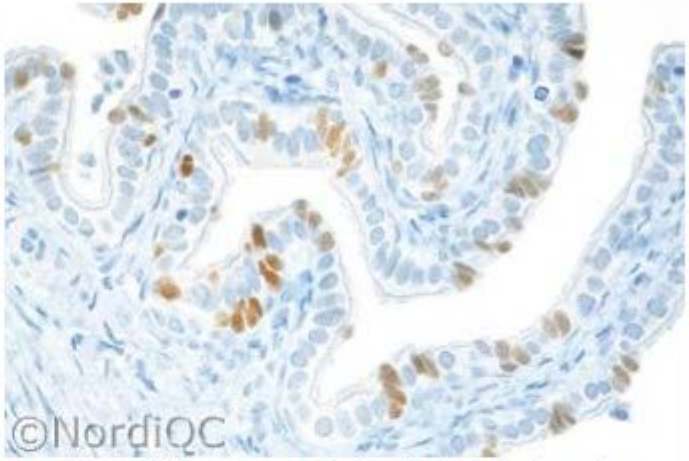


Fig. 1b. Insufficient PAX8 staining of the Fallopian tube using the mAb clone MRQ-50 as a concentrate with a protocol giving a too low sensitivity (a too low concentration of the primary Ab and a 2-step multimer based detection system) - same field as in Fig. 1a. The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result obtained in Fig. 1a. Also compare with Fig. 2b, same protocol.

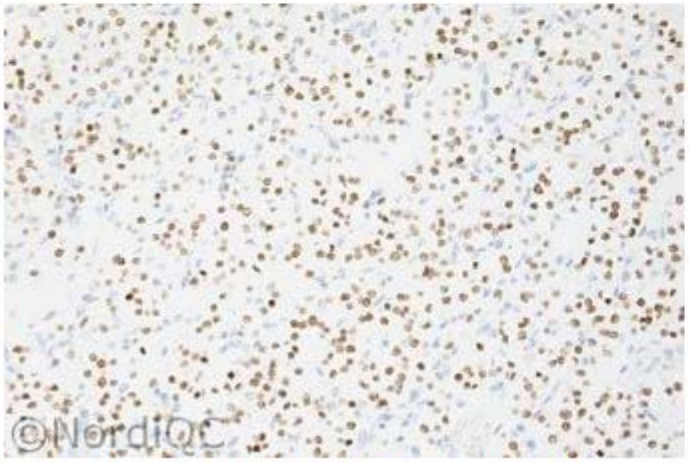


Fig. 2a. Optimal PAX8 staining of the renal clear cell carcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a moderate to strong nuclear staining reaction.

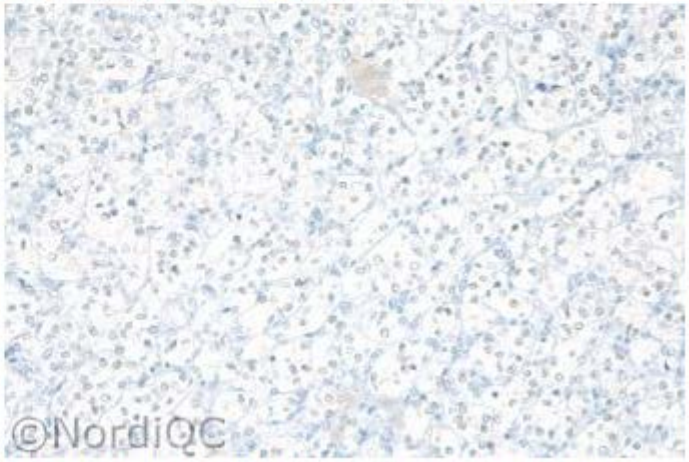


Fig. 2b. Insufficient PAX8 staining of the renal clear cell carcinoma using same protocol as in Fig. 1b - same field as in Fig. 2a. Only scattered neoplastic cells show an equivocal staining reaction.

Dako AS48:

EP298, ZR-1, BC12, MRQ-50 or Prot. Tech

VMS, Dako OMNIS:

EP298, ZR1 or Prot.Tech

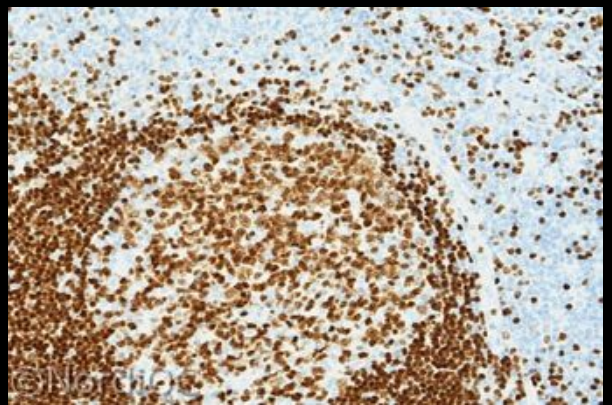
IHC – Protocols and controls – GYN, GI, Liver

MODERN PATHOLOGY (2011), 1–6

N-terminal PAX8 polyclonal antibody shows cross-reactivity with N-terminal region of PAX5 and is responsible for reports of PAX8 positivity in malignant lymphomas

Lucas Moretti[1], L Jeffrey Medeiros[1], Kranthi Kunkalla[1], Michelle D Williams[2], Rajesh R Singh[1] and Francisco Vega[1]

[1]*Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA and* [2]*Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA*



Tonsil stained for PAX8 =

Same pattern as for PAX5

IHC – Protocols and controls – GYN, GI, Liver

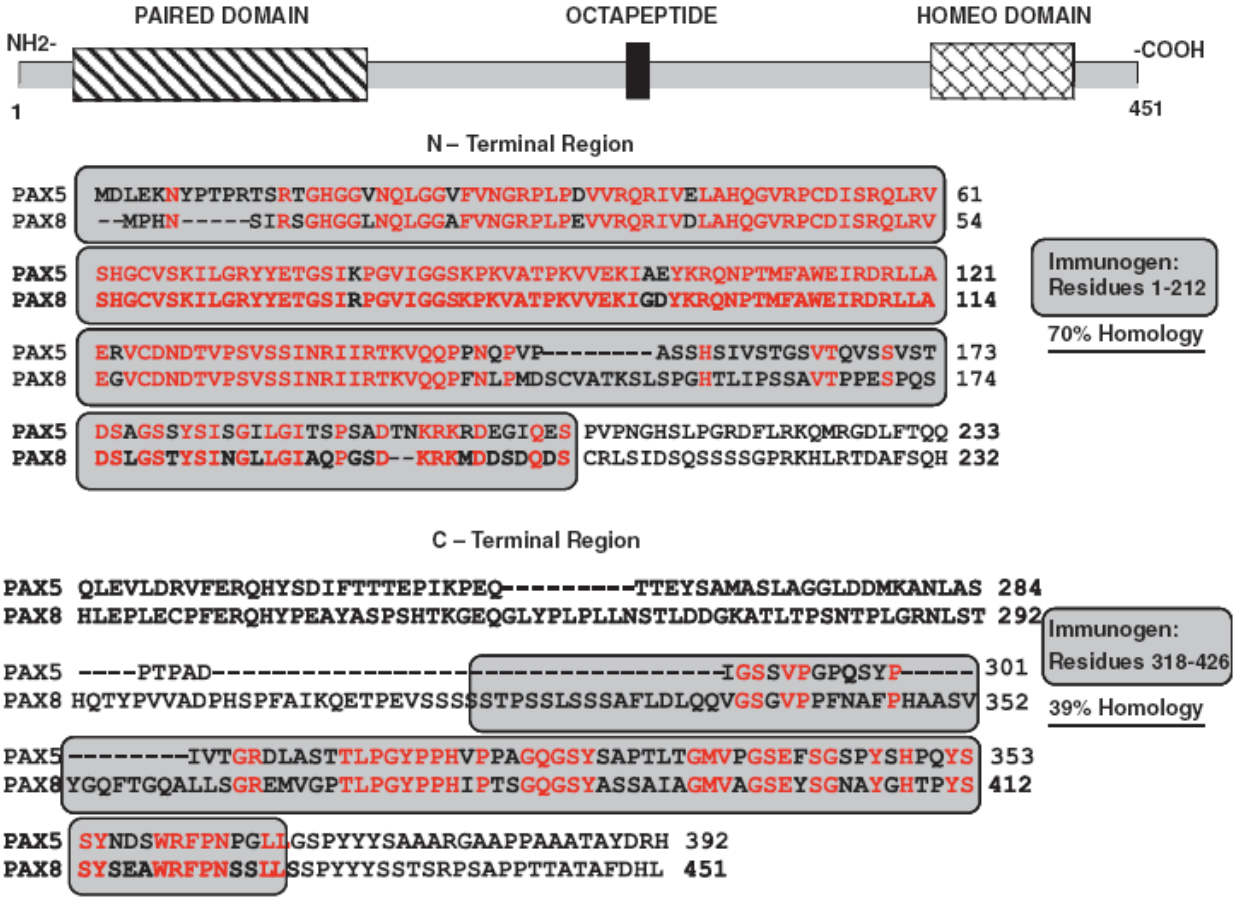
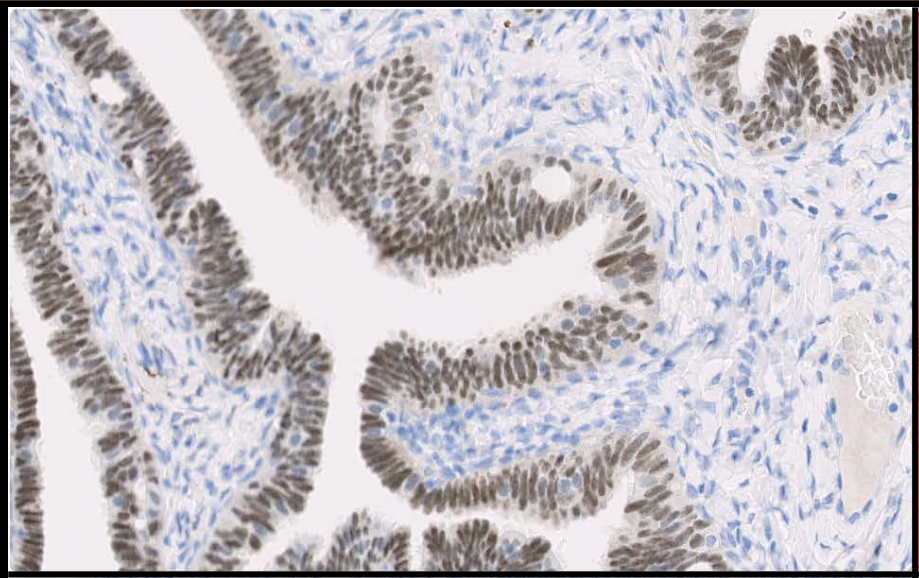
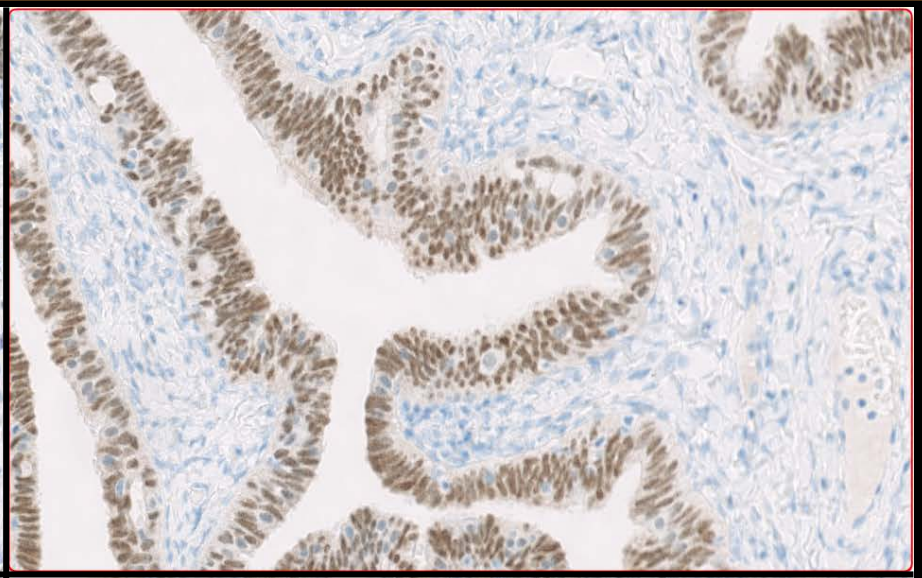
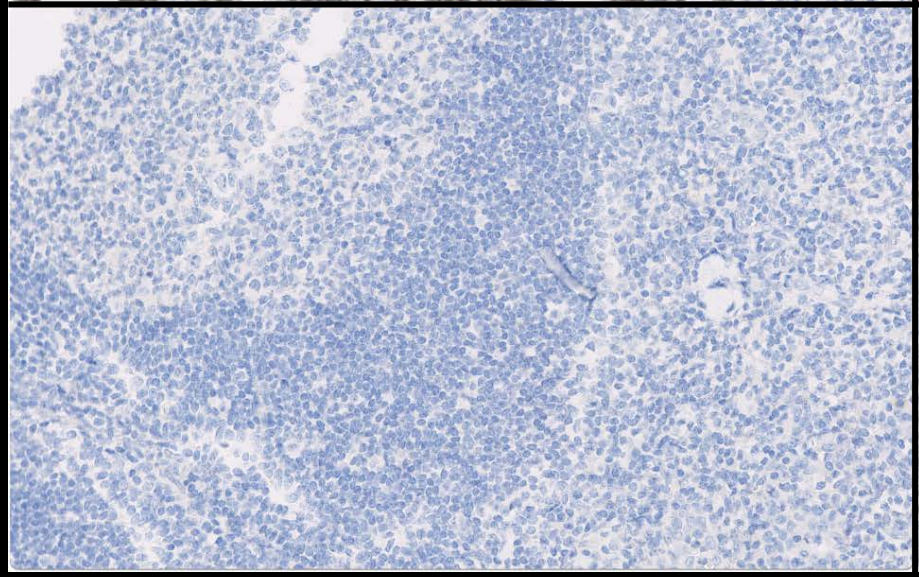


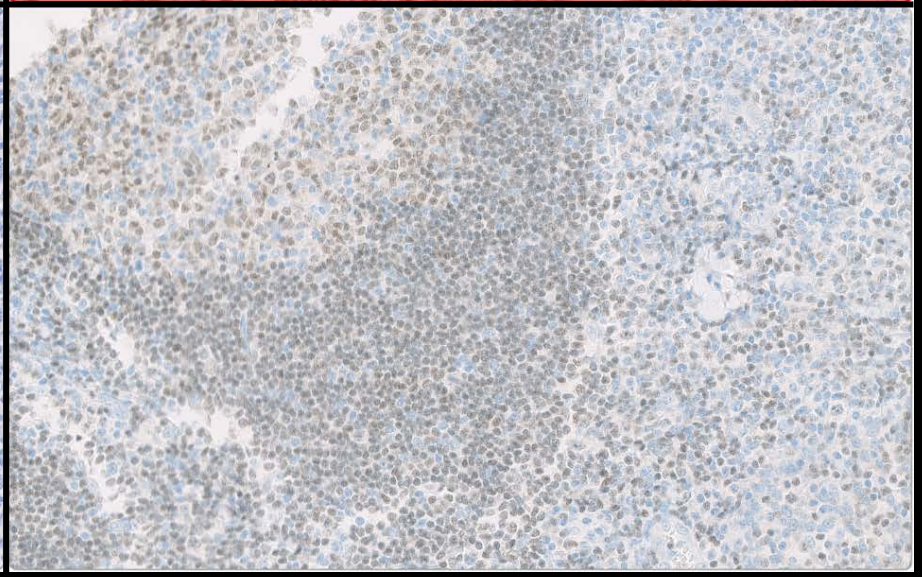
Figure 1 Schematic representation of PAX8 protein, and human PAX5 and PAX8 protein sequence comparison. The region in gray cover the sequence of the PAX8 antibodies (against N-terminal region top and C-terminal region bottom) and their homology with the sequences of PAX-5, N-terminal region (top) and C-terminal region (bottom).

IHC – Protocols and controls – GYN, GI, Liver









mAb clone BC12 (C-term.)
rmAb clone EP298 (C-term)

mAb clone MRQ-50 (N-term.)

IHC – Protocols and controls – GYN, GI, Liver

PAX8 antibodies towards N-terminal (most likely..):

- mAb clone MRQ-50 (Roche/Cell Marque)
- pAb 10336-1-AP (Protein Tech group)
- pAb A363 (Cell Marque)
- pAb CP 379 (Biocare)

PAX8 antibodies towards C-terminal (most likely..):

- mAb clone BC12 (Biocare)
- mAb clone PAX8R1 (Abcam)
- rmAb clone ZR1 (Zeta)
- rmAb EP298 (Epitomics/Cell Marque)

IHC – Protocols and controls – GYN, GI, Liver

PAX8 antibodies towards N-terminal (most likely..):

Fallopian tube – secretory & ciliated cells

Kidney – epithelial cells lining the proximal tubules

Thyroid – epithelial cells lining the follicles

Tonsil – B-lymphocytes

Pancreas – neuroendocrine cells

PAX8 antibodies towards C-terminal (most likely..):

Fallopian tube – secretory & ciliated cells

Kidney – epithelial cells lining the proximal tubules

Thyroid – epithelial cells lining the follicles

IHC – Protocols and controls – GYN, GI, Liver

PAX8

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb MRQ-50*	HIER High	1:25-200	3-step	*Ventana*	*3-step (OP)*
mAb BC12*	HIER High	1:20-30	3-step	-	-
rmAb EP298	**HIER High**	**1:50-150**	**3-step**	-	-
rmAb ZR-1	HIER High**	1:25-800	3-step	-	-
pAb 10336-1-AP	HIER High	1:100-800	3-step	-	-

* Inferior performance on VMS & Dako Omnis stainer platform

** VMS: P3 4 min + HIER CC1 32M

Table 3. Proportion of optimal results for PAX8 using concentrated antibodies on the 3 main IHC systems*

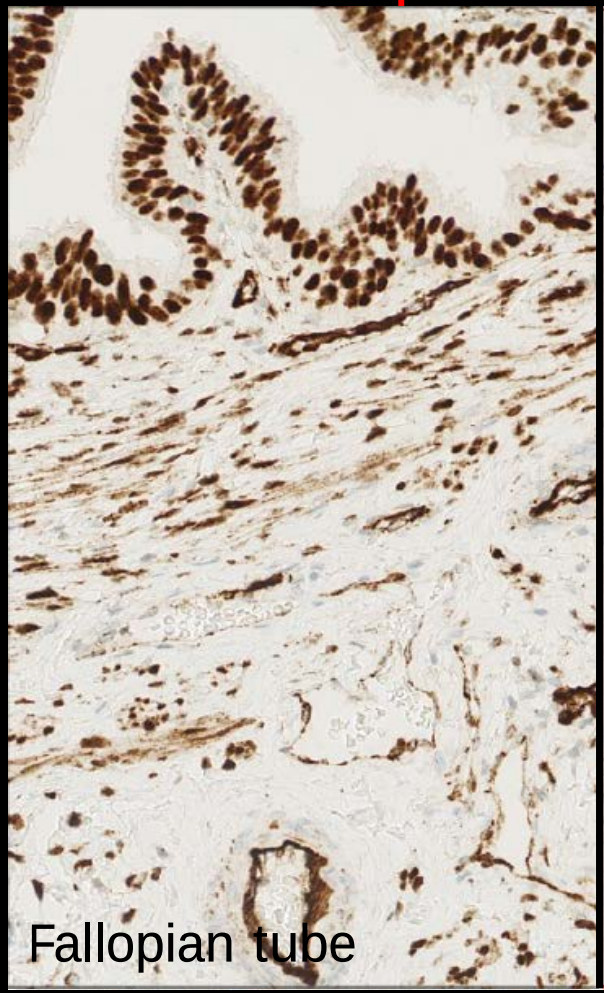
Concentrated antibodies	Dako Autostainer Link / Classic / Omnis		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **MRQ-50**	6/7 (85%)**	0/2	1/10 (10%)	-	6/6 (100%)	1/1
mAb clone **BC12**	0/2	-	0/2	-	0/1	-
pAb **10336-1-AP**	1/2	0/1	2/3	1/2	0/2	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as recommended by the vendors of the respective platforms.

** (number of optimal results/number of laboratories using this buffer)

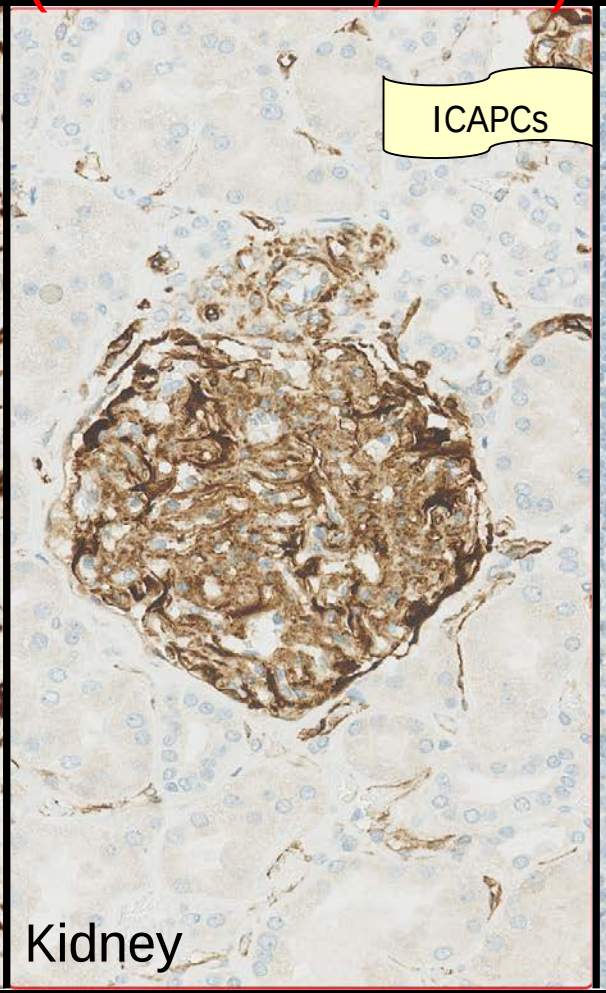
IHC – Protocols and controls – GYN, GI, Liver

WT1 reaction pattern (mAb 6F-H2, HIER)



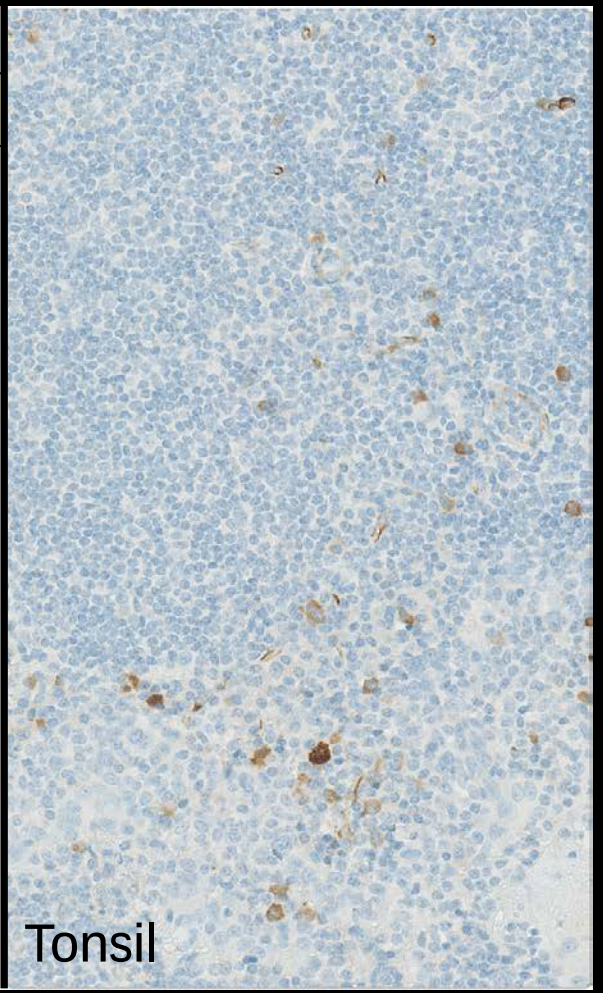
Fallopian tube

A moderate to strong nuclear staining reaction of virtually all epithelial (and stromal) cells.
A weak to moderate cytoplasmic staining in many cells will be seen.



Kidney

A moderate to strong nuclear staining reaction in parietal epithelial cells and podocytes of the Bowman capsule.....

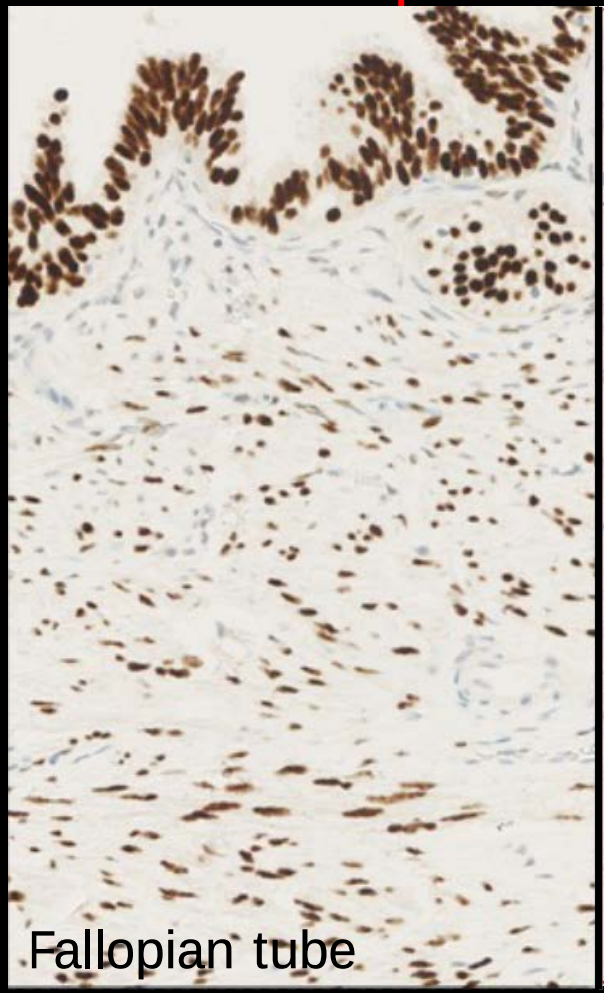


Tonsil

No nuclear staining reaction of lymphocytes, endothelial cells etc.

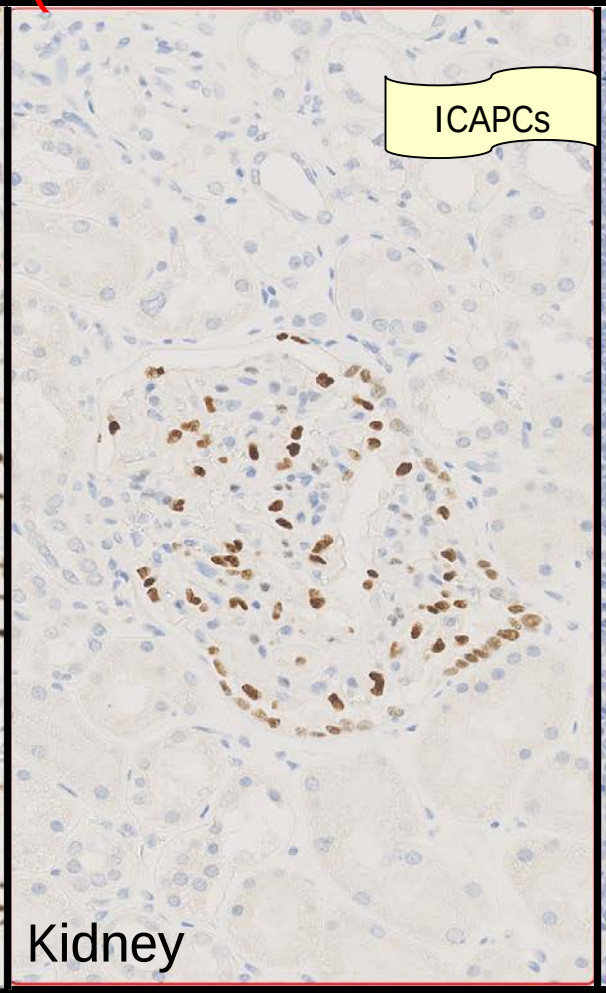
IHC – Protocols and controls – GYN, GI, Liver

WT1 reaction pattern (mAb WT49 and 6F-H2, HIER+proteolysis)



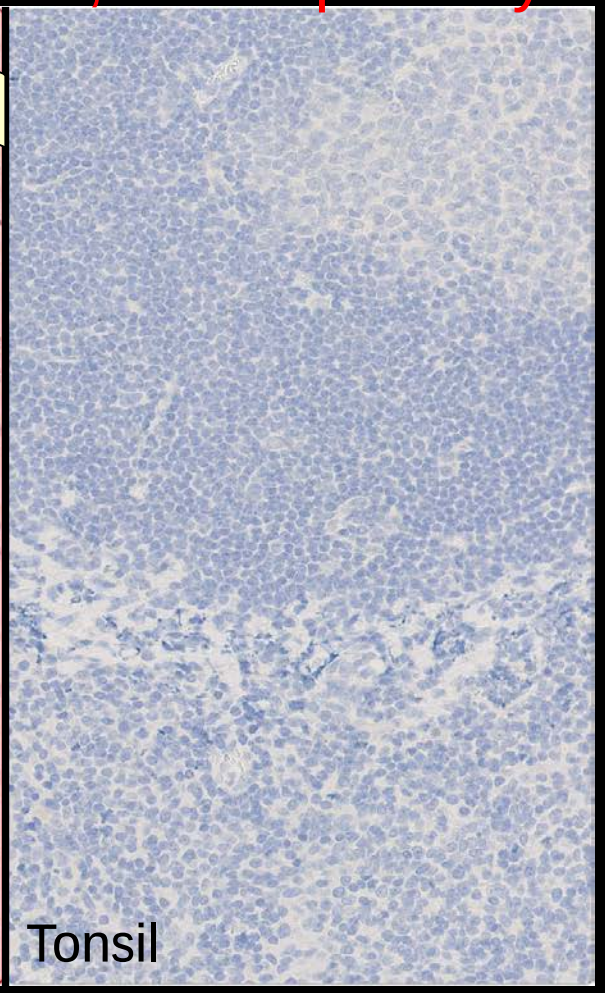
Fallopian tube

A moderate to strong nuclear staining reaction of virtually all epithelial (and stromal) cells.



Kidney

A moderate to strong nuclear staining reaction in parietal epithelial cells and podocytes of the Bowman capsule.



Tonsil

No nuclear staining reaction of lymphocytes, endothelial cells etc.

IHC – Protocols and controls – GYN, GI, Liver

Table 1. **Antibodies and assessment marks for WT1, run 43**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mmAb clone **6F-H2**	70 7 2 1 1 1 1 1	Dako Cell Marque Immunologic BioSB Genemed Novous Thermo Fisher Zeta	23	41	17	3	76%	81%
mmAb clone **WT49**	20 1	Leica/Novocastra Monosan	9	8	2	2	81%	88%
rmAb clone **EP122**	1	Epitomics	0	1	0	0	-	-
pAb, **C-19**	1	Santa Cruz	0	0	1	0	-	-
pAb, **RB-9267-P1**	1	Thermo Fisher	0	0	0	1	-	-
Ready-To-Use antibodies								
mmAb clone **6F-H2 IR055/IS055**	51	Dako	40	8	2	1	94%	100%
mmAb clone **6F-H2 760-4397**	45	Ventana/Cell Marque	4	33	5	3	82%	97%
mmAb clone **6F-H2 348M-98**	3	Cell Marque	0	2	1	0	-	-
mmAb clone **6F-H2 PM258**	1	BioCare	0	0	1	0	-	-
mmAb clone **6F-H2 MAD-005671QD**	1	Master Diagnostica	0	1	0	0	-	-
mmAb clone **6F-H2 MON-RTU1210**	1	Monosan	0	0	1	0	-	-
mmAb clone **WT49 PA0562**	8	Leica/Novocastra	5	2	1	0	88%	100%
mmAb clone **MX012 MAB-0678**	1	Maixin	0	1	0	0	-	-
rmAb clone **EP122 AN828-5M**	1	Biogenex	1	0	0	0	-	-
Total	220		82	97	31	10	-	
Proportion			37%	44%	14%	5%	81%	

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

Optimal but challening to read

Optimal and easy to read

Insufficient:
- Too low titre
- Short incubation time
- (*WT49 on VMS)*

IHC – Protocols and controls – GYN, GI, Liver

WT49 – HIER ER2, 3-step polymer
1:10 – 25 min. 1:25 – 15 min.

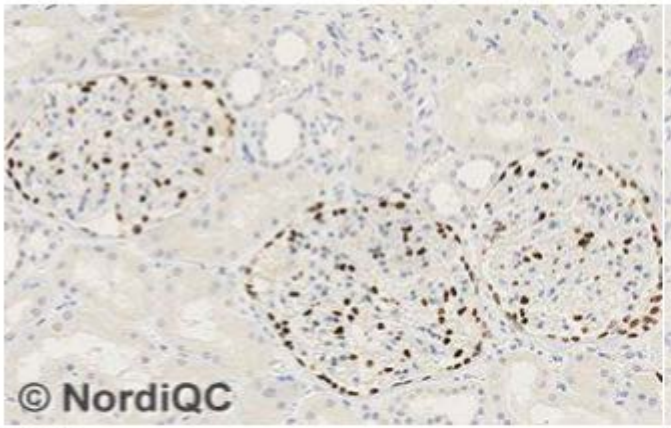


Fig. 2a
Optimal WT1 staining of the kidney using the same protocol as in Fig. 1a. A strong, distinct nuclear staining of the podocytes and the epithelial cells lining the Bowman capsule is seen. Compare with Fig. 2b.

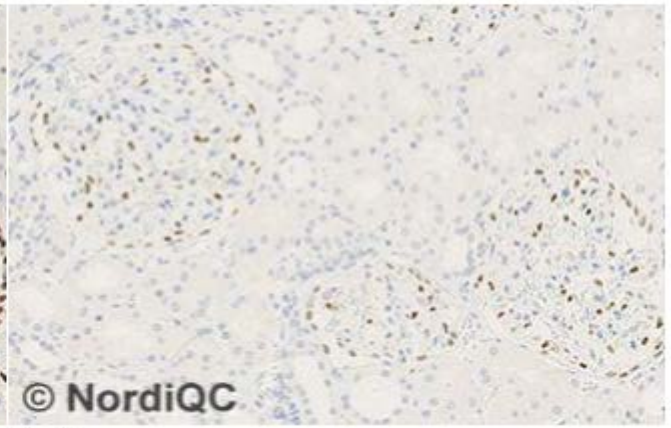


Fig. 2b
Insufficient WT1 staining of the kidney using the same protocol as in Fig. 1b. Only a weak nuclear staining of the podocytes and the epithelial cells lining the Bowman capsule is seen. Compare with Fig. 2a. - same field.

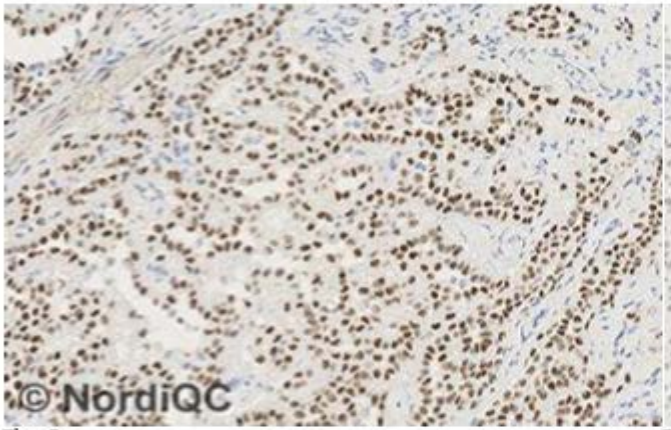


Fig. 3a
Optimal WT1 staining of the mesothelioma using the same protocol as in Figs. 1a & 2a. A strong, nuclear staining is seen in virtually all the neoplastic cells of the mesothelioma. Compare with Fig. 3b.

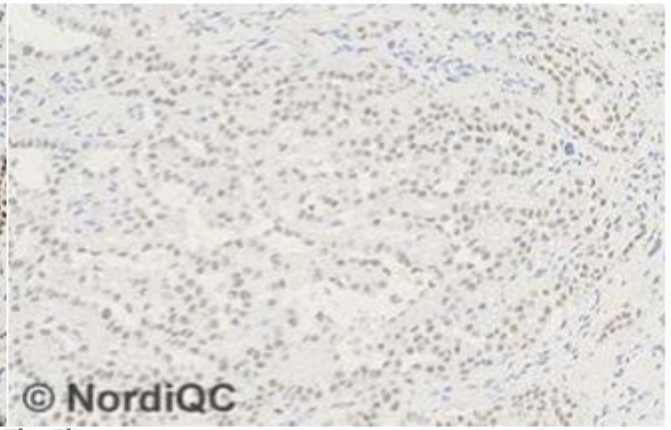


Fig. 3b
Insufficient WT1 staining of the mesothelioma using the same protocol as in Figs. 1b & 2b. The majority of neoplastic cells display only a moderate to weak nuclear staining reaction. Compare with Fig. 3a. – same field.

IHC – Protocols and controls – GYN, GI, Liver

6F-H2 – RTU, 3-step multimer

HIER + Proteolysis

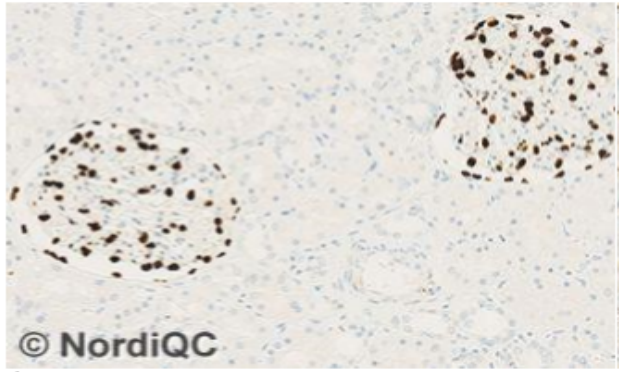


Fig. 5a
Optimal WT1 staining of the kidney using the mmAb 6F-H2 (Ventana/Cell Marque, RTU) with HIER in an alkaline buffer (CC1, Ventana) followed by protease treatment in Protease 3 (Ventana) using a 3-step multimer system (OptiView, Ventana) and performed on the BenchMark Ultra. A strong, distinct nuclear staining of the podocytes and the epithelial cells lining the Bowman capsule is seen. No cytoplasmic staining of endothelial and muscle cells is seen. Compare with Fig. 5b.

HIER

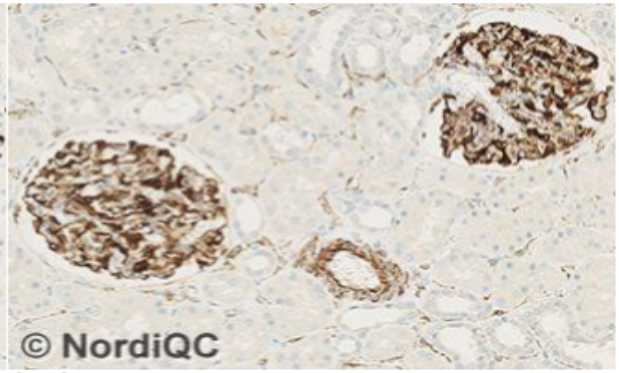


Fig. 5b
Good WT1 staining of the kidney using the mmAb 6F-H2 (Ventana/Cell Marque, RTU) with HIER in an alkaline buffer (CC1, Ventana) using a 3-step multimer system (OptiView, Ventana) and performed on the BenchMark Ultra. A moderate nuclear staining of the podocytes and the epithelial cells lining the Bowman capsule is seen. Moderate cytoplasmic staining of endothelial and muscle cells is also seen, making interpretation more challenging. Compare with Fig. 5a. - same field.

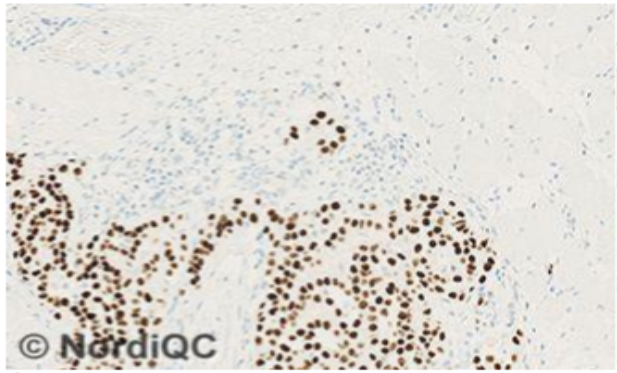


Fig. 6a
Optimal WT1 staining in the mesothelioma using the same protocol as in Figs. 5a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. No cytoplasmic reaction is seen. Compare with Fig 6b.

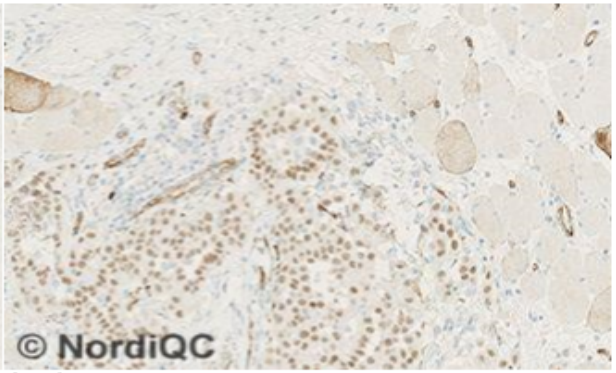


Fig. 6b
Good WT1 staining in the mesothelioma using the same protocol as in Fig. 5b. The majority of the neoplastic cells show a moderate nuclear staining reaction. A moderate cytoplasmic reaction is seen in the endothelial cells and smooth muscle cells. A minor proportion of skeletal muscle cells exhibit weak to moderate cytoplasmic reaction. Compare with Fig. 6a - same field.

IHC – Protocols and controls – GYN, GI, Liver

UPT III: WT1

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb 6F-H2	HIER high pH	1:30-400	2- & 3-step	Dako	2- & 3-step
mAb 6F-H2	HIER high pH + proteolysis*	1:200-250	3-step	*Ventana*	*3-step (OP)*
mAb WT1	HIER high pH	1:10-25	3-step	Leica	3-step

* e.g. VMS: HIER in CC1 32 min + P3 for 4-8 min.

IHC – Protocols and controls – GYN, GI, Liver

MMR

Basic protocol settings for an optimal staining result (NQC)

	Recommendable clones (conc.)*	Less successful clones (conc.)	RTU ”plug and play”** giving optimal result
MLH1	**mAb ES05** mAb G168-15 mAb GM011	mAb G168-728	Dako: mAb ES05 Leica: mAb ES05
MSH2	**mAb FE11** mAb G219-1129	mAb 25D12	Dako: mAb FE11 VMS: G219-1129
MSH6	**rmAb EP49** rmAb EPR3945	mAb 44	Dako: rmAb EP49
PMS2	mAb 16-4 **rmAb EP51** rmAb EPR3947		Dako: rmAb EP51 VMS: rmAb EPR3947

* Potential to provide optimal result by a laboratory developed test (LDT)

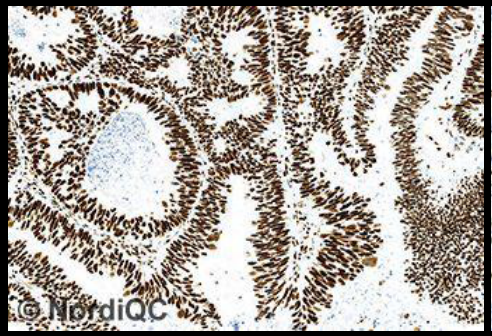
** Using the protocol settings as recommended by the vendor – incubation, retrieval, detection kit.

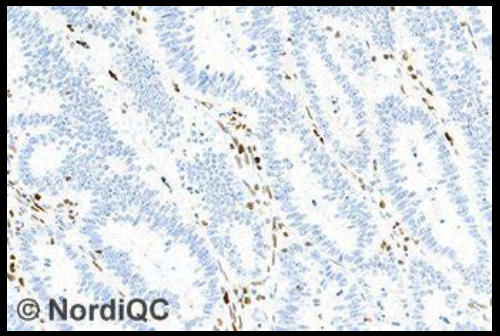
IHC test: Fit for purpose –

All IHC tests both laboratory developed assays and RTU systems must be calibrated for the diagnostic use

E.g. IHC assays for mismatch repair proteins (MMR)

Purpose	Diagnostic utility	Tool	Application
Disease screening of patients with Lynch syndrome	IHC results have been shown to have high concordance to mutation analysis	IHC panel for 4 MMR proteins; MLH1, MSH2, MSH6 & PMS2	Identification of a reliable IHC protocol and interpretation guidelines for the pathologist
Diagnostic relevant	Diagnostic validity	Technically possible	Diagnostic possible





MMR General patttern

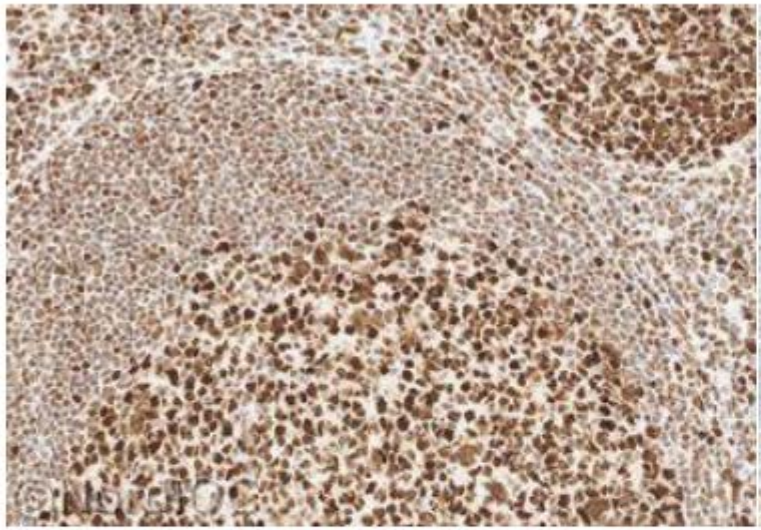


Fig. 1a
Optimal staining reaction for MLH1 of the tonsil using the mAb clone ES05, HIER in an alkaline buffer and a 3-step polymer based detection system. Virtually all the mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction.
Also compare with Figs. 2a and 3a, same protocol.

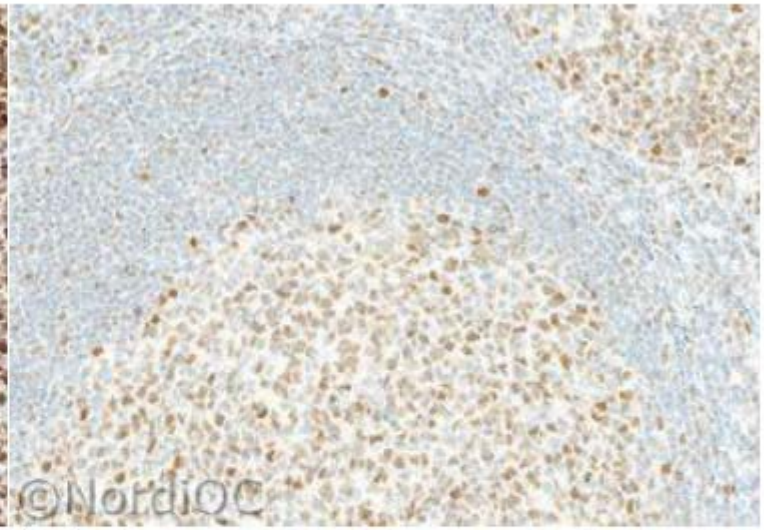


Fig. 1b
Insufficient staining reaction for MLH1 of the tonsil using the mAb clone ES05 with a protocol providing a too low sensitivity (2-step multimer based detection system and/or a too low concentration of the primary Ab) - same field as in Fig. 1a.
Only the germinal centre B-cells are demonstrated, while the mantle zone B-cells expressing a low level of MLH1 are virtually unstained.
Also compare with Figs. 2b and 3b, same protocol.

Interpretation based on internal tissue control.
Negative IHC result in neoplastic cells must be confirmed by identification of stromal cells being positive !

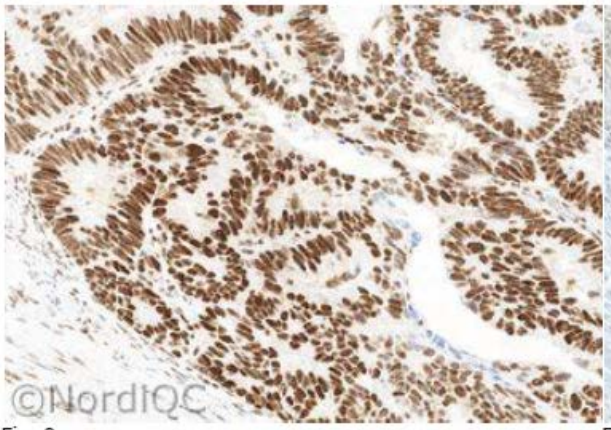


Fig. 2a
Optimal staining reaction for MLH1 of the colon adenocarcinoma tissue core no. 5 with normal MLH1 expression using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. A high signal-to-noise ratio is obtained. No background staining is seen and a distinct nuclear staining reaction in the stromal cells is seen.

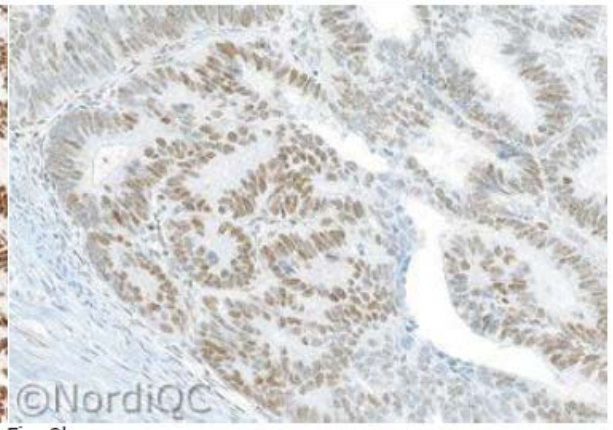


Fig. 2b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma tissue core no. 5 using same protocol as in Fig. 1b - same field as in Fig. 2a.
The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result in Fig. 2, especially note the stromal cells are virtually negative. Also compare with Fig. 3b, same protocol.

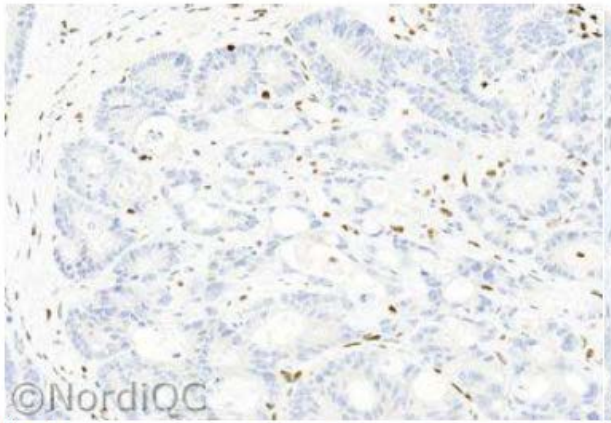


Fig. 3a
Optimal staining reaction for MLH1 of the colon adenocarcinoma no. 3 with loss of MLH1 using same protocol as in Figs. 1a & 2a. The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

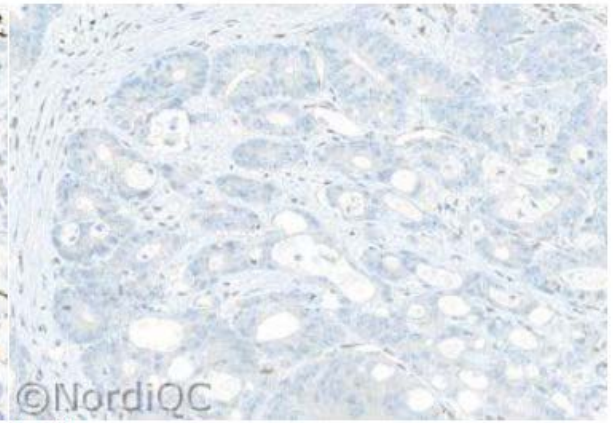


Fig. 3b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma no. 3 with loss of MLH1 using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
No staining reaction in the neoplastic cells is seen, but as also virtually no nuclear staining reaction is seen in the normal stromal cells, the staining pattern can not reliably be interpreted.

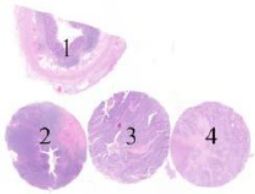
Assessment Run 41 2014

Mismatch Repair Protein MSH2 (MSH2)

Recommended MSH2 protocols Recommended MSH2 control tissue

Material

The slide to be stained for **MSH2** comprised:



1. Appendix, 2. Tonsil, 3. Colon adenocarcinoma with normal MSH2 expression, 4. Colon adenocarcinoma with loss of MSH2 expression.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a MSH2 staining as optimal were:

- An at least weak to moderate, distinct nuclear staining reaction of virtually all cells in the appendix.
- An at least weak to moderate, distinct nuclear staining reaction of virtually all mantle zone B-cells and a moderate to strong, distinct nuclear staining reaction of the germinal centre B-cells in the tonsil.
- A moderate to strong, distinct nuclear staining reaction in virtually all neoplastic cells of the colon adenocarcinoma no. 3.
- No nuclear staining reaction of the neoplastic cells of the colon adenocarcinoma no. 4, but a distinct nuclear staining reaction in the vast majority of other cells (stromal cells, lymphocytes etc.).

A weak cytoplasmic staining reaction was accepted.

Participation

Number of laboratories registered for MSH2, run 41	155
Number of laboratories returning slides	143 (92%)

Results

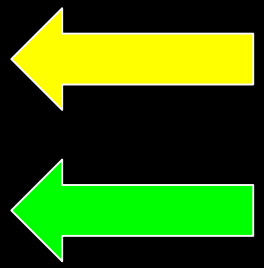
143 laboratories participated in this assessment. Of these, 96 (67%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

NordiQC

Table 1. **Antibodies and assessment marks for MSH2, run 41**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **25D12**	12 1 1	Leica/Novocastra Diagnostic BioSystems Thermo/NeoMarkers	0	0	12	2	-	-
mAb clone **FE11**	10 6 6	Biocare Dako Millipore/Calbiochem	3	10	9	0	59%	80%
mAb clone **G219-1129**	11 8 1	BD Biosciences Cell Marque Monosan	4	6	6	4	50%	90%
mAb clone **GB12**	1	Millipore/Calbiochem	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **25D12 PA0048**	3	Leica/Novocastra	0	0	3	0	-	-
mAb clone **FE11 IR085**	23	Dako	20	2	1	0	96%	100%
mAb clone **FE11 PM219**	2	Biocare	0	2	0	0	-	-
mAb clone **FE11 MSG031**	1	Zytomed	1	0	0	0	-	-
mAb clone **G219-1129 760-4265**	50	Ventana/Cell Marque	26	19	3	2	90%	93%
mAb clone **G219-1129 286M-18**	5	Cell Marque	2	1	2	0	60%	-
mAb clone **G219-1129 MAD-000371QD**	2	Master Diagnostica	0	0	2	0	-	-
Total	143		56	40	39	8	-	
Proportion			39%	28%	27%	6%	67%	

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

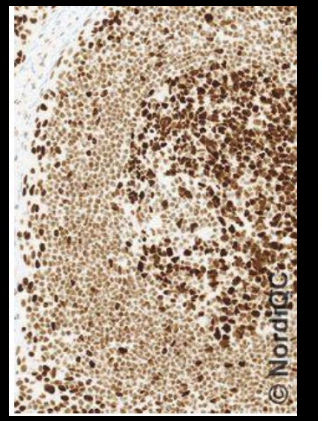


Clone

Titre

RTU > In-house

NordiQC



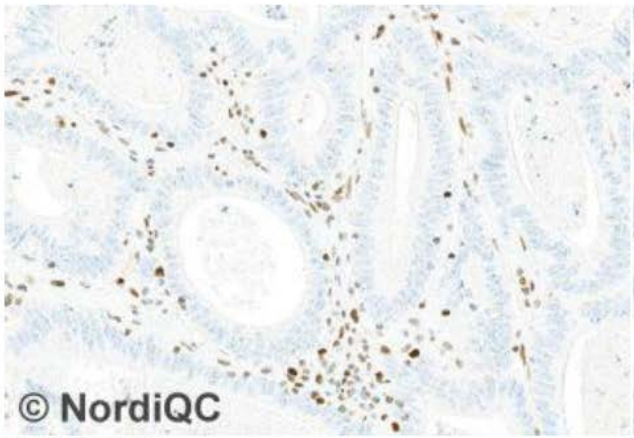


Fig. 3a (X200)
Optimal MSH2 staining of the colon adenocarcinoma no. 4 with loss of MSH2 expression using same protocol as in Figs. 1a & 2a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

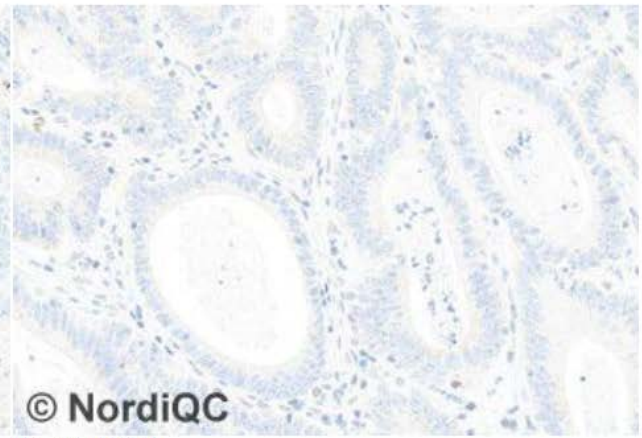
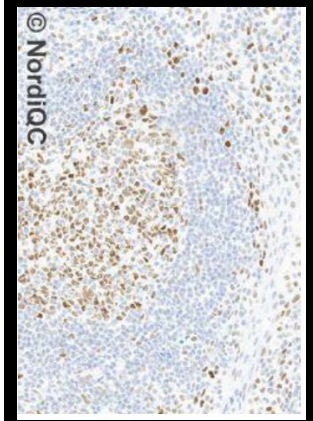


Fig. 3b (2400)
Insufficient MSH2 staining of the colon adenocarcinoma no. 4 with loss of MSH2 expression using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a. No staining reaction in the neoplastic cells is seen, but as no nuclear staining reaction in the normal stromal cells is present, the staining pattern cannot reliably be interpreted.



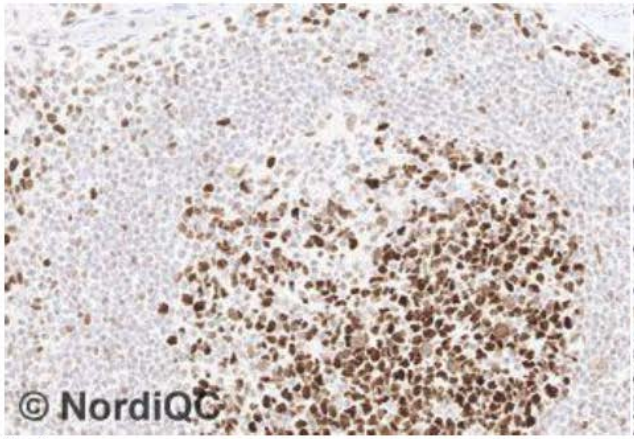


Fig.4a
Insufficient staining reaction for MSH2 using the mAb clone 25D12 with HIER in an alkaline buffer and a 3-step polymer based detection system. Mantle zone B-cells only show a faint or equivocal nuclear staining reaction, whereas germinal centre B-cells show a strong nuclear staining reaction. Also compare with Fig. 4b, same protocol.

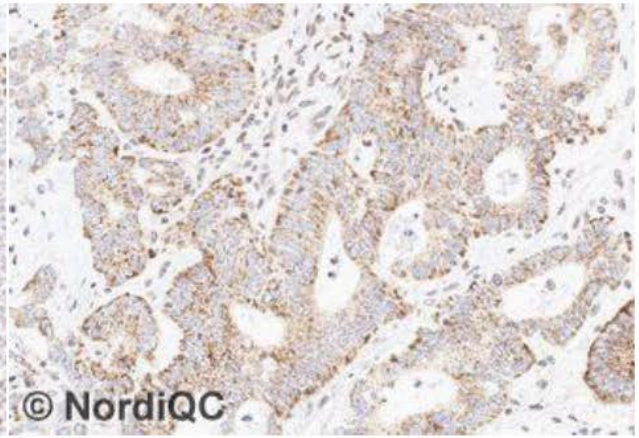


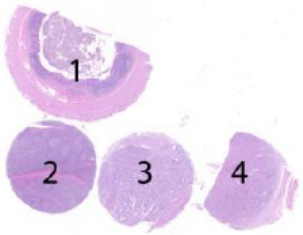
Fig. 4b
Insufficient staining reaction for MSH2 of the colon adenocarcinoma no. 4 with loss of MSH2 expression using same protocol as in Fig. 4a. The combination of an excessive granular cytoplasmic staining reaction in the neoplastic cells and a faint nuclear staining reaction of the stromal cells complicates the interpretation.
In this run all protocols (n=17) based on the mAb clone 25D12 gave an insufficient result.

Assessment Run 43 2015

MSH6

Recommended MSH6 protocols Recommended MSH6 control tissue

Material
The slide to be stained for **MSH6** comprised:



1. Appendix 2. Tonsil fixed for 24 hours, 3. Colon adenocarcinoma with normal MSH6 expression, 4. Colon adenocarcinoma with loss of MSH6 expression.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing MSH6 staining as optimal included:

- An at least weak to moderate, distinct nuclear staining reaction of virtually all cells in the appendix
- An at least weak to moderate, distinct nuclear staining reaction of virtually all mantle zone B-cells and a moderate to strong, distinct nuclear staining reaction of the germinal centre B-cells in the tonsil
- A moderate to strong, distinct nuclear staining reaction of virtually all neoplastic cells in the colon adenocarcinoma no. 3
- No nuclear staining reaction of the neoplastic cells in the colon adenocarcinomas no. 4, but a distinct nuclear staining reaction in the vast majority of other cells (stromal cells, lymphocytes etc).
- A generally weak cytoplasmic staining reaction was accepted.

Participation

Number of laboratories registered for MSH6, run 43	173
Number of laboratories returning slides	153 (88%)

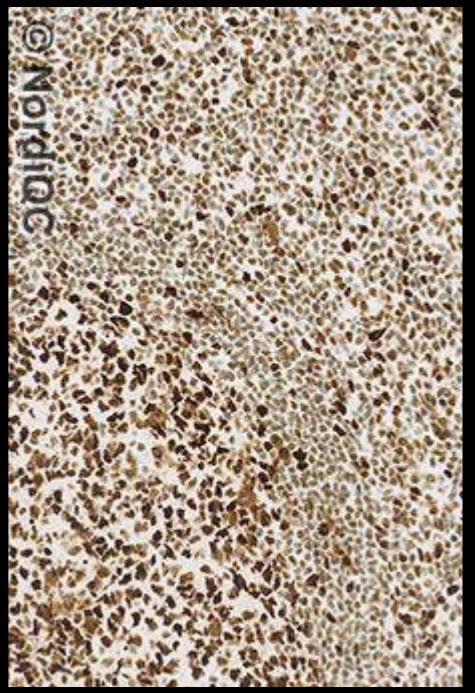
Results
153 laboratories participated in this assessment. 96 (63%) of these achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

Table 1. **Antibodies and assessment marks for MSH6, run 43**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **44**	12 2 1 1	BD Biosciences Cell Marque Diagnostic Biosystems Zeta	0	1	14	1	6%	-
mAb clone **BC/44**	6	Biocare	1	3	2	0	67%	67%
mAb clone **PU29**	6	Leica/Novocastra	0	1	4	1	17%	-
mAb clone **SPM525**	1	Zytomed Systems	0	0	0	1	-	-
rmAb clone **EP49**	20 12	Epitomics Dako	22	7	3	0	91%	91%
rmAb clone **EPR3945**	4 2 1 1	Abcam Epitomics Gene Tex Nordic Biosite	5	3	0	0	100%	100%
rmAb clone **SP93**	2 1	Cell Marque Spring Bioscience	3	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **44** **790-4455**	33	Ventana	1	9	22	1	30%	40%
mAb clone **44** **287M**	2	Cell Marque	0	0	2	0	-	-
mAb clone **44** **PDM 147**	1	Diagnostic Biosystems	0	0	1	0	-	-
mAb clone **44** **081374**	1	Invitrogen/Life Technologies	0	0	1	0	-	-
mAb clone **44** **MAB-0643**	1	Maixin	1	0	0	0	-	-
mAb **BC/44** **M265**	2	Biocare	0	1	1	0	-	-
rmAb clone **EP49** **IR086**	35	Dako	24	8	3	0	91%	96%
rmAb clone **EP49** **MAD-000635QD**	2	Master Diagnostica	1	1	0	0	-	-
rmAb clone **EP49** **AN780-5M**	1	Biogenex	1	0	0	0	-	-
rmAb clone **SP93** **287R**	2	Cell Marque	1	1	0	0	-	-
rmAb **SP93** **M3931**	1	Spring Bioscience	1	0	0	0	-	-
Total	153		61	35	53	4	-	
Proportion			40%	23%	35%	2%	63%	

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

Choice of clone……..



NordiQC

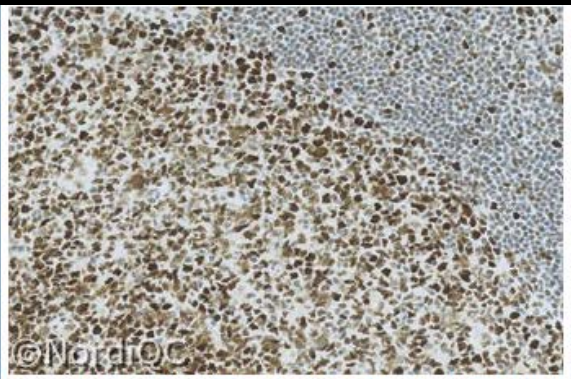


Fig. 1a. Optimal staining for MSH6 of the tonsil using the rmAb clone EP49 optimally calibrated, HIER in an alkaline buffer and a 3-step polymer based detection system. Virtually all the mantle zone B-cells show a distinct, moderate to strong nuclear staining, while the germinal centre B-cells show a strong nuclear staining.

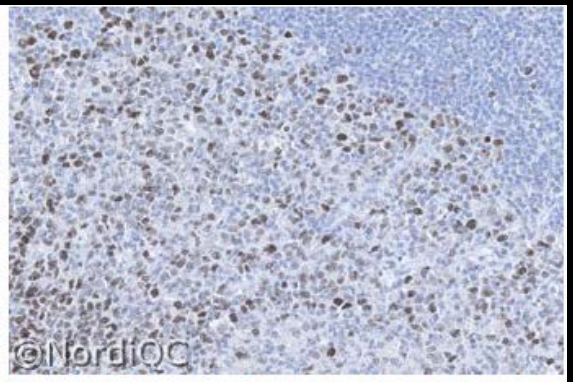


Fig. 1b. Insufficient staining for MSH6 of the tonsil using the mAb clone 44. by a protocol with a too low sensitivity (2-step polymer and too low. conc. of the primary Ab), same field as in Fig. 1a.
Only the germinal centre B-cells are demonstrated, while the mantle zone B-cells expressing limited MSH6 are virtually unstained.
Also compare with Figs. 2b. & 3b., same protocol.

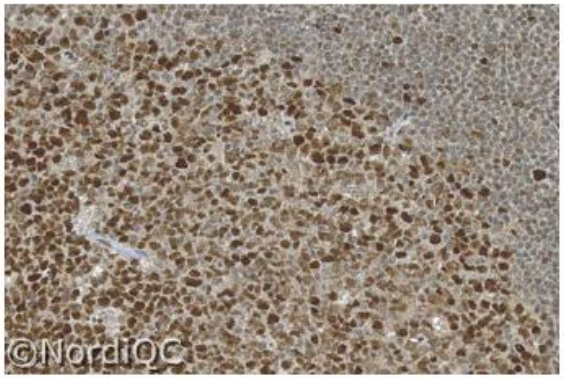


Fig. 4a. Staining for MSH6 of the tonsil using the mAb clone 44 by HIER in an alkaline buffer and a 3-step polymer based detection system – same field as in Fig. 1a. Virtually all the mantle zone B-cells show a moderate to strong nuclear staining, while the germinal centre B-cells show a strong nuclear staining. However also compare with Fig. 4b, same protocol.

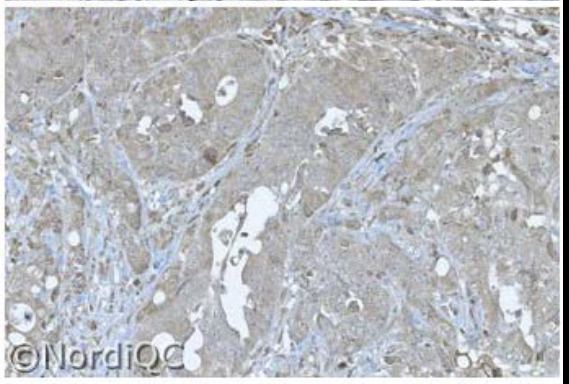
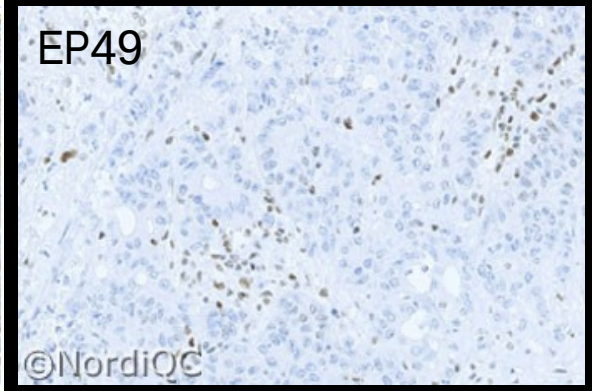


Fig. 4b. Insufficient staining for MSH6 of the colon adenocarcinoma no. 5 with loss of MSH6 protein using same protocol as in Fig. 4a.
The excessive cytoplasmic staining in both the neoplastic cells and in the stromal cells obscures the interpretation of the nuclear staining.
This staining pattern was typically seen when the mAb clone 44 was applied with a high sensitive protocol.

MSH6 ”issues”:

<u>mAb clone 44 used</u>
Difficult to calibrate



IHC – Protocols and controls – GYN, GI, Liver

IHC – Protocols and controls – GYN, GI, Liver

	Positive tissue control HE*	Positive tissue control LE**	Negative tissue control NE***
HEPA	Liver: Hepatocytes	Appendix: Scattered epithelial cells	Tonsil: Lymphocytes,...
Arginase	Liver: Hepatocytes	.....	Tonsil: Lymphocytes,...
Glyp 3	Placenta: Trophoblasts	Appendix: Nerves	Liver: Hepatocytes

* HE = High expression

** LE = Low expression

*** NE = No expression

IHC – Protocols and controls – GYN, GI, Liver

	Recommendable clones (conc.)*	Less successful clones (conc.)	RTU ”plug and play”** giving optimal result
HEPA	mAb OCH1E5		Dako: mAb OCH1E5 VMS: mAb OCH1E5
Arginase	rmAb SP156***		
Glyp 3	mAb 1G12		VMS: mAb OCH1E5

* Potential to provide optimal result by a laboratory developed test (LDT)
** Using the protocol settings as recommended by the vendor – incubation, retrieval, detection kit.
*** Aalborg University Hospital data

IHC – Protocols and controls – GYN, GI, Liver

	Positive tissue control HE*	Positive tissue control LE**	Negative tissue control NE***
HEPA	Liver: Hepatocytes	Appendix: Scattered epithelial cells	Tonsil: Lymphocytes,...
Arginase	Liver: Hepatocytes	.....	Tonsil: Lymphocytes,...
Glyp 3	Placenta: Trophoblasts	Appendix: Nerves	Liver: Hepatocytes

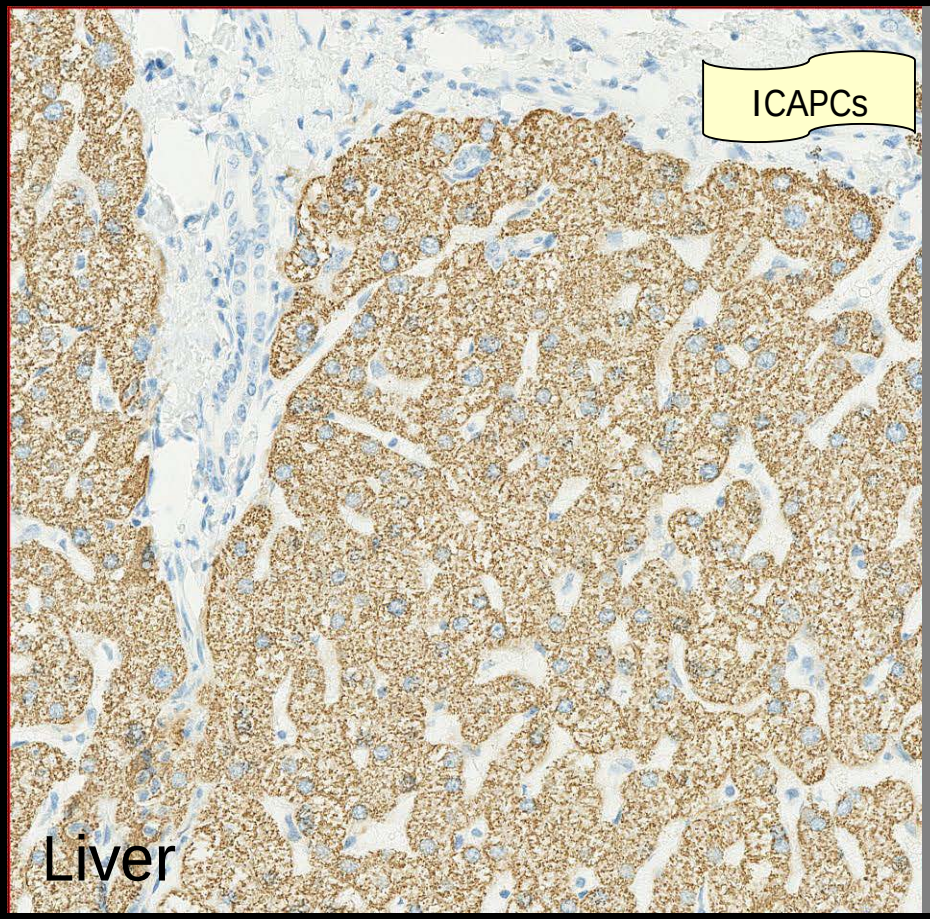
* HE = High expression

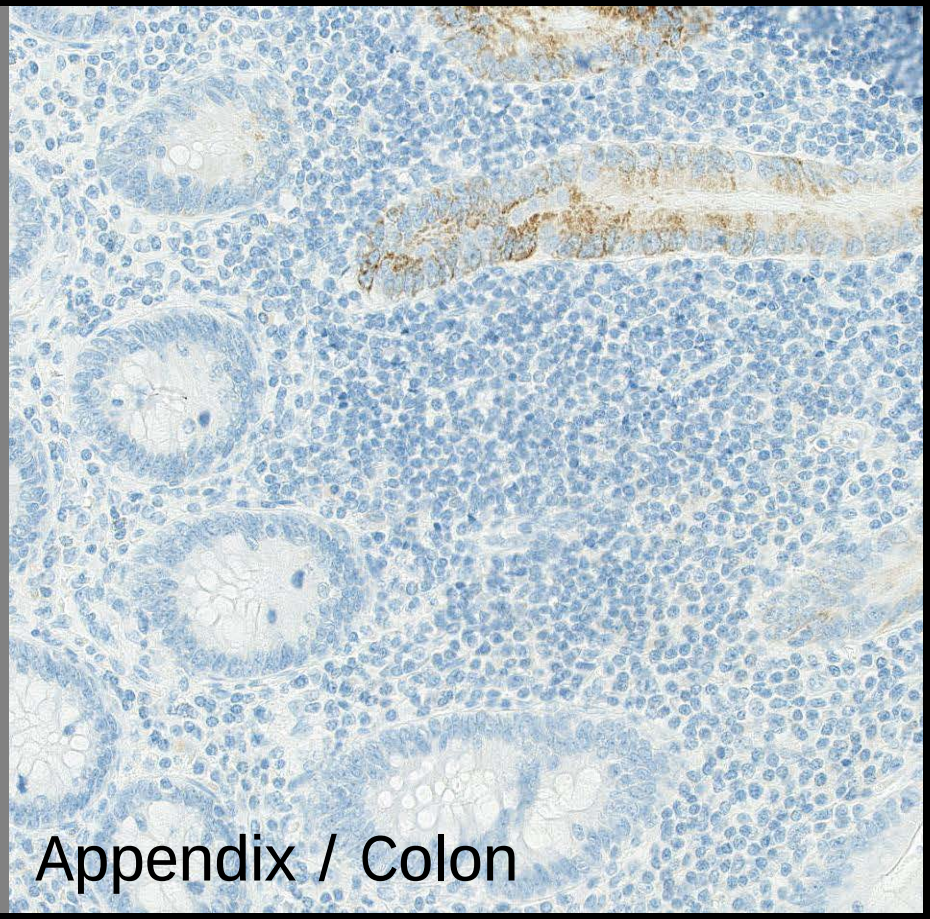
** LE = Low expression

*** NE = No expression

IHC – Protocols and controls – GYN, GI, Liver

HEPA reaction pattern





A moderate to strong granular cytoplasmic staining reaction in virtually all hepatocytes (mitochodria)
No-go for biotin based detection systems.

No staining reaction of lymhocytes, stromal cells, muscle cells and vast majority of epithelial cells.

IHC – Protocols and controls – GYN, GI, Liver

Table 1. **Abs and assessment marks for Hepa, run 36**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **OCH1E5**	93 6 5 2 1 1	Dako Leica/Novocastra Thermo/NeoMarkers Cell Marque Diagnostic Biosystems Maxim	48	38	19	3	80 %	90 %
Ready-To-Use Abs								
mAb clone **OCH1E5 IS/IR624**	25	Dako	21	4	0	0	100 %	100 %
mAb clone **OCH1E5 760-4350**	20	Ventana/Cell Marque	18	2	0	0	100 %	100 %
mAb clone **OCH1E5 264M-97/98**	3	Cell Marque	2	1	0	0	-	-
mAb clone **OCH1E5 BSB 5629**	1	BioSB	1	0	0	0	-	-
mAb clone **OCH1E5 113-03**	1	Master Diagnostica	0	1	0	0	-	-
Total	158		90	46	19	3	-	
Proportion			57 %	29 %	12 %	2 %	86 %	

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

+ HIER

+ Non-biotin system

RTU superior

IHC – Protocols and controls – GYN, GI, Liver

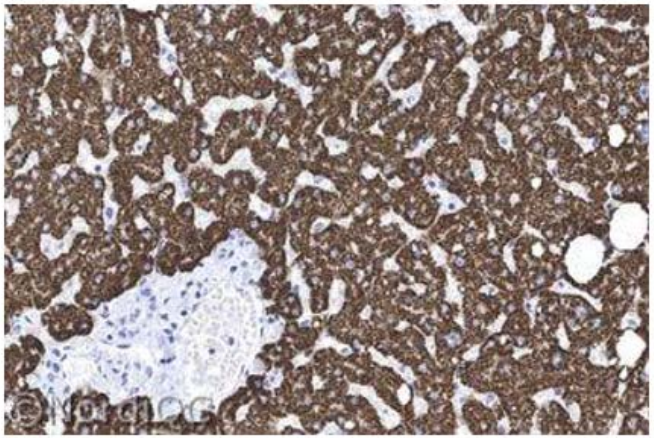


Fig. 1a
Optimal Hepa staining of the liver using the mAb clone OCH1E5 optimally calibrated and with HIER. Virtually all the hepatocytes show a strong, granular cytoplasmic staining reaction. No background staining or staining of the bile ductal epithelial cells is seen.

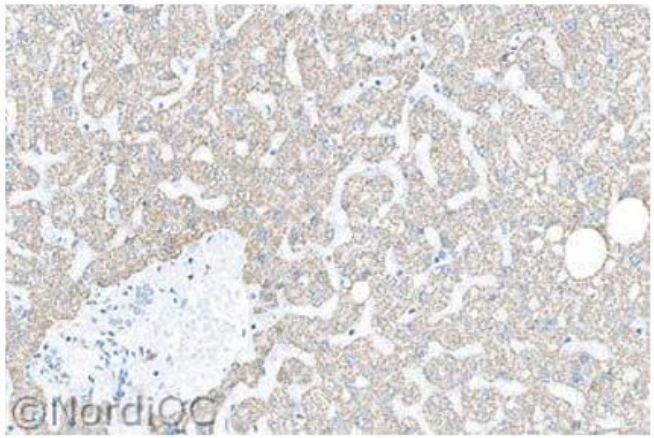


Fig. 1b
Insufficient staining for Hepa of the liver, using the mAb clone OCH1E5 with protocol settings giving a too low sensitivity (too low concentration of the primary Ab) - same field as in Fig. 1a. The intensity of the cells demonstrated is significantly reduced. Also compare with Fig. 2b – same protocol.

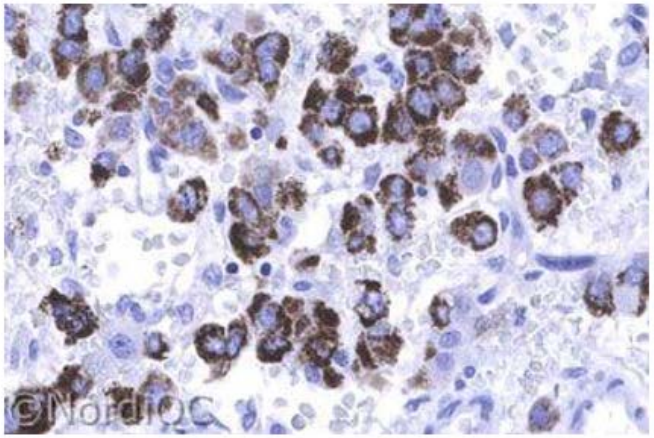


Fig. 2a
Optimal Hepa staining of the hepatocellular carcinoma, tissue no. 4 in the NordiQC multiblock using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining reaction. No background staining is seen.

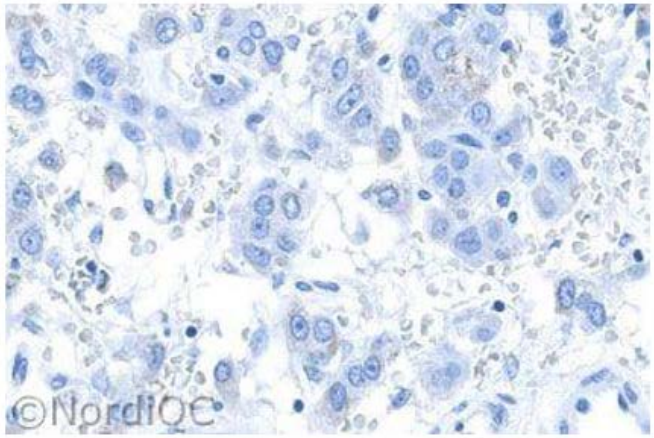
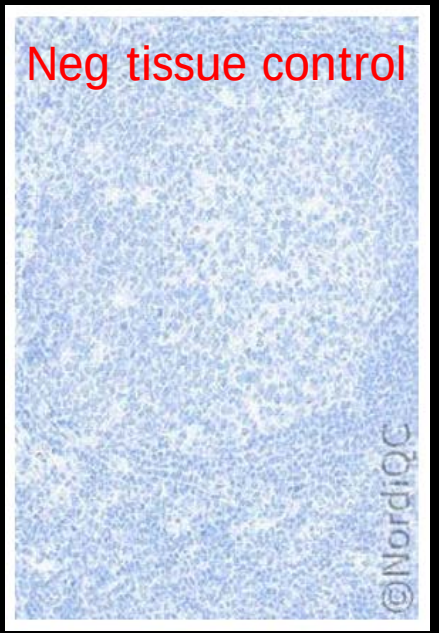


Fig. 2b
Insufficient staining for Hepa of the hepatocellular carcinoma, tissue no. 4 in the NordiQC multiblock using same protocol as in Fig. 1b – same field as in Fig. 2a.
Only scattered neoplastic cells show a weak and equivocal staining reaction.

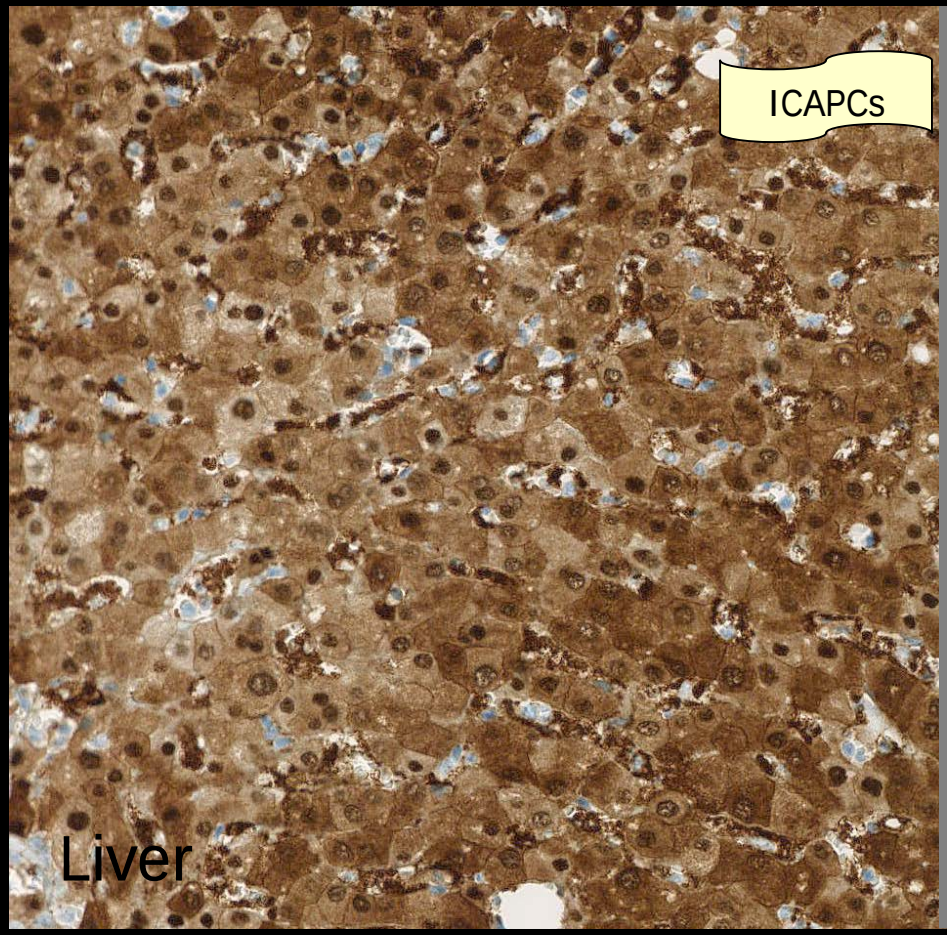
ASAP....

As Strong
As Possible

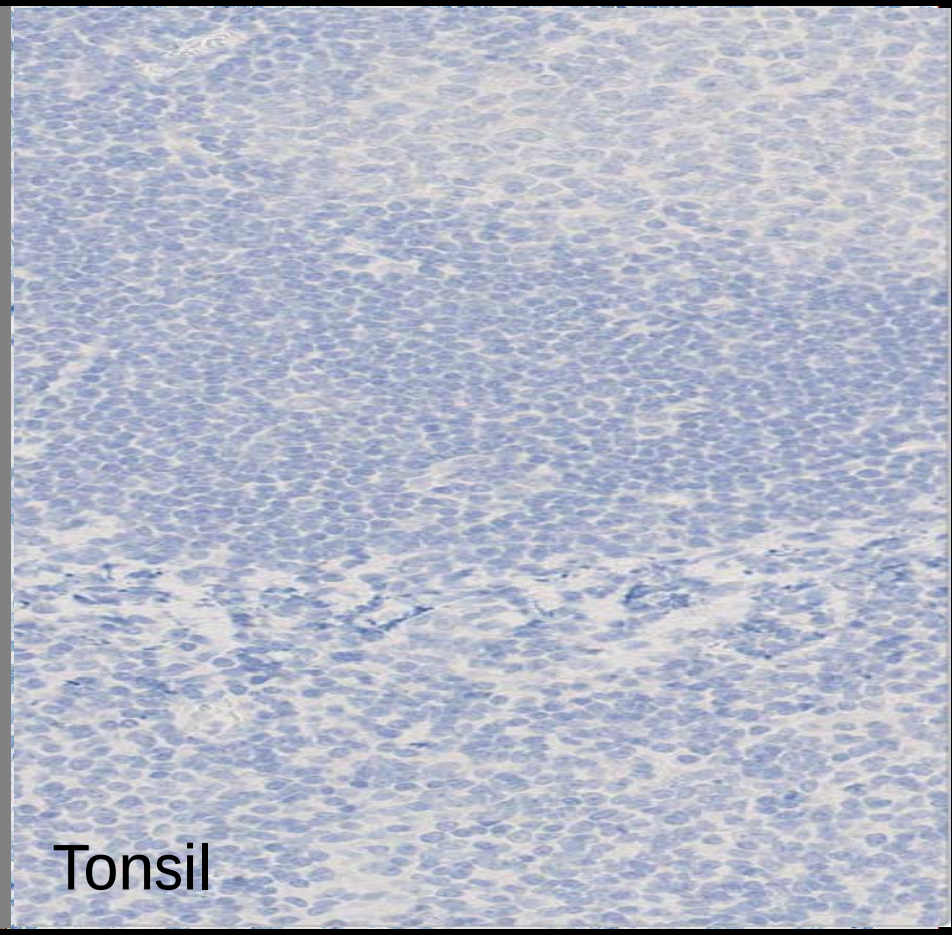


IHC – Protocols and controls – GYN, GI, Liver

Arginase reaction pattern

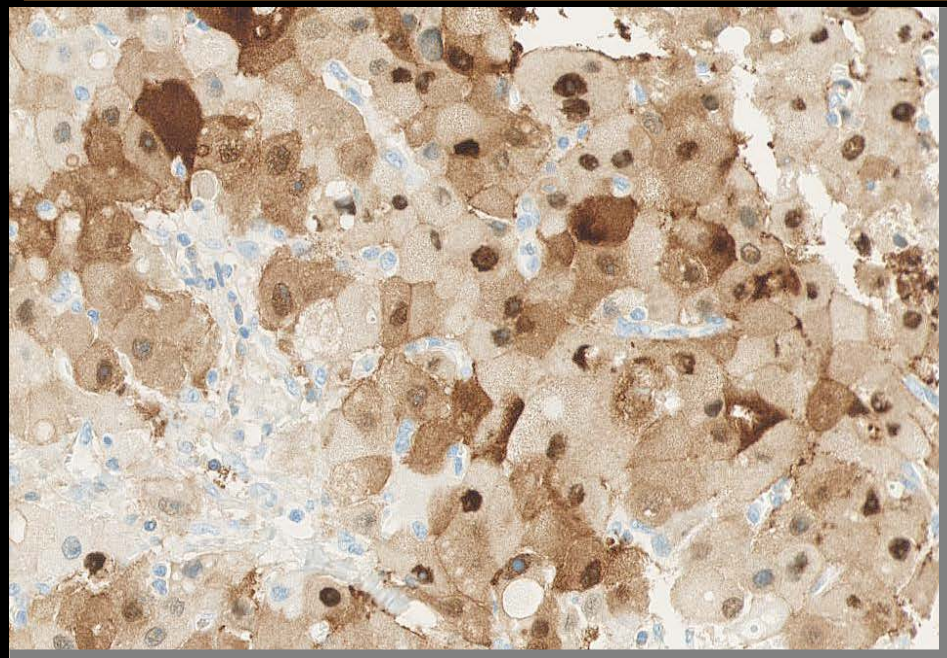


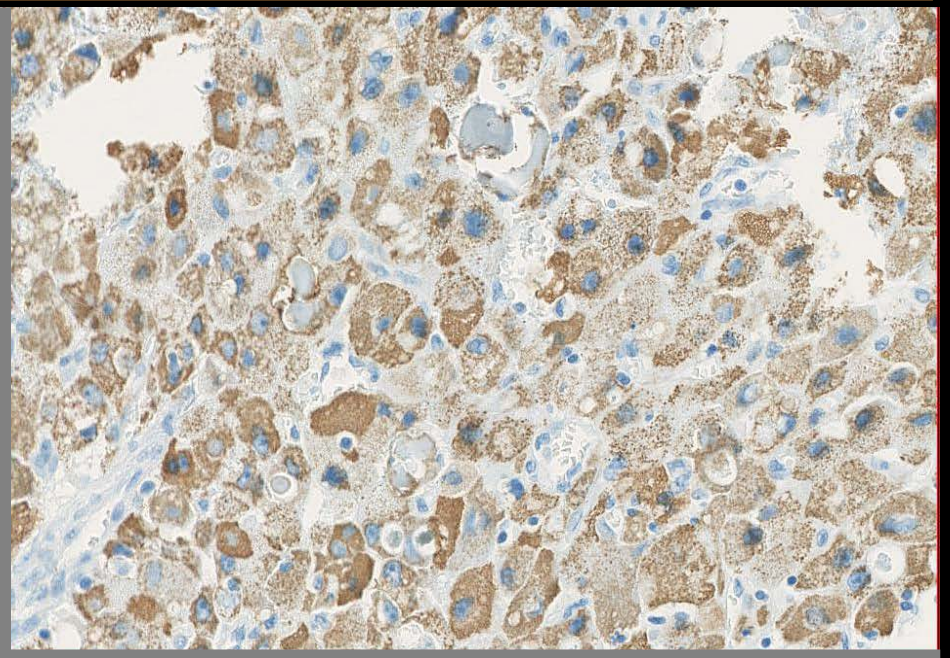
A moderate to strong nuclear and cytoplasmic staining reaction in virtually all hepatocytes).

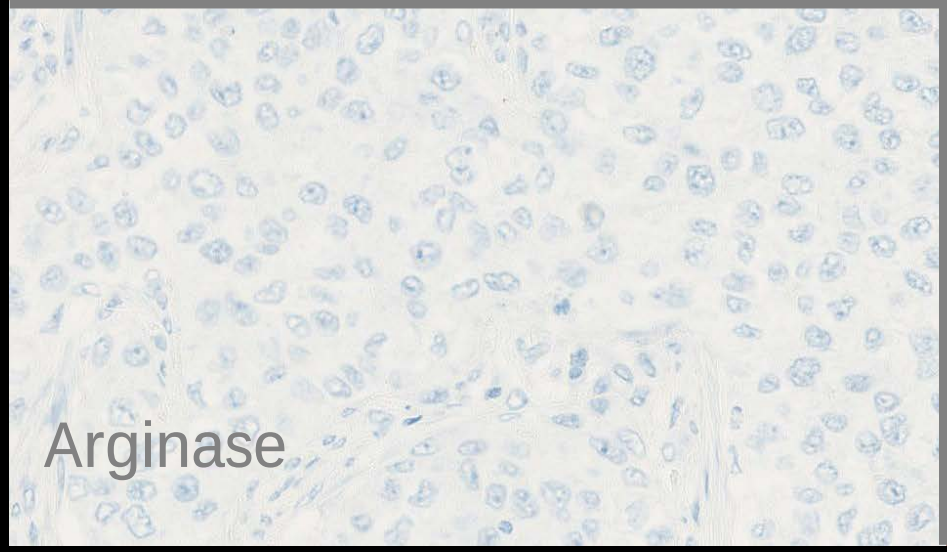


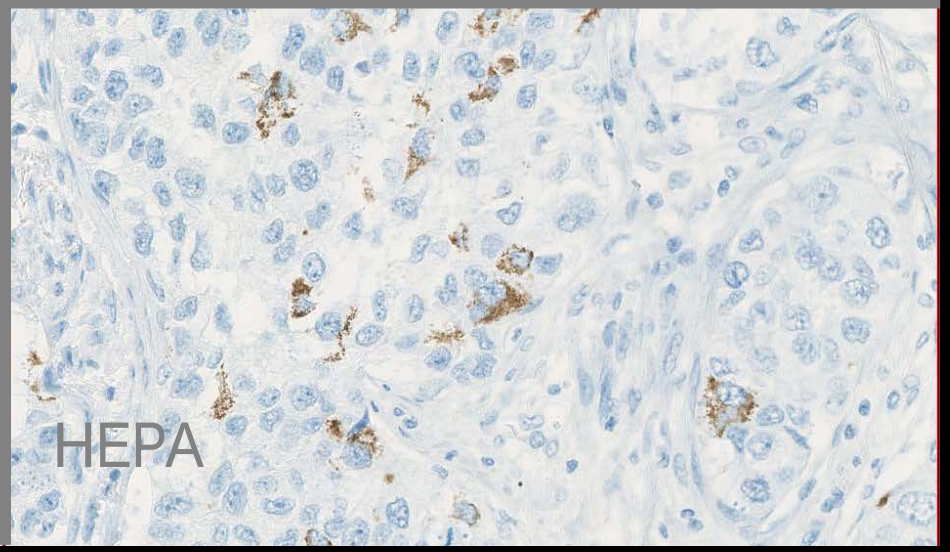
No staining reaction.

IHC – Protocols and controls – GYN, GI, Liver





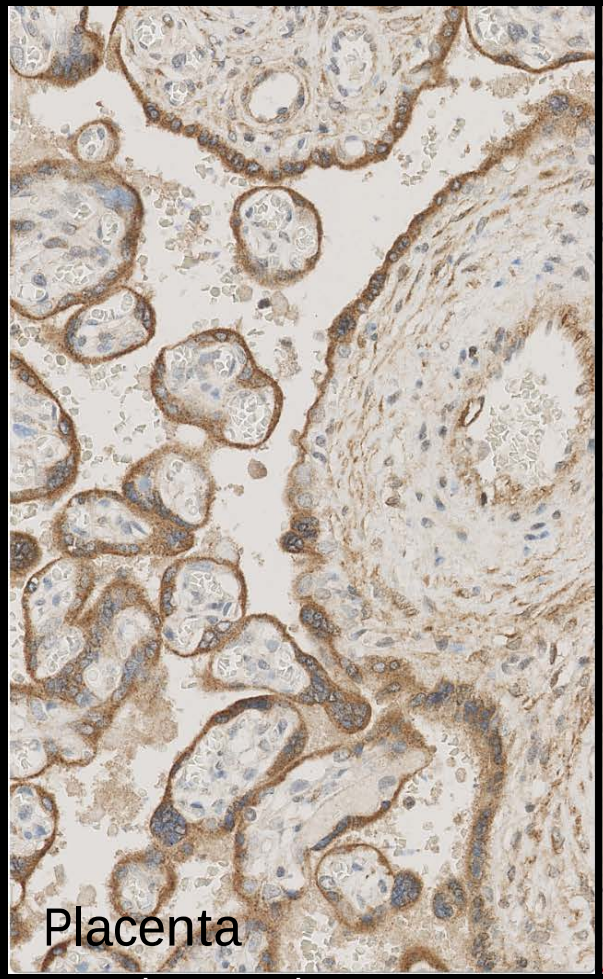




Hepatocelluar carcinoma & undifferentiated carcinoma

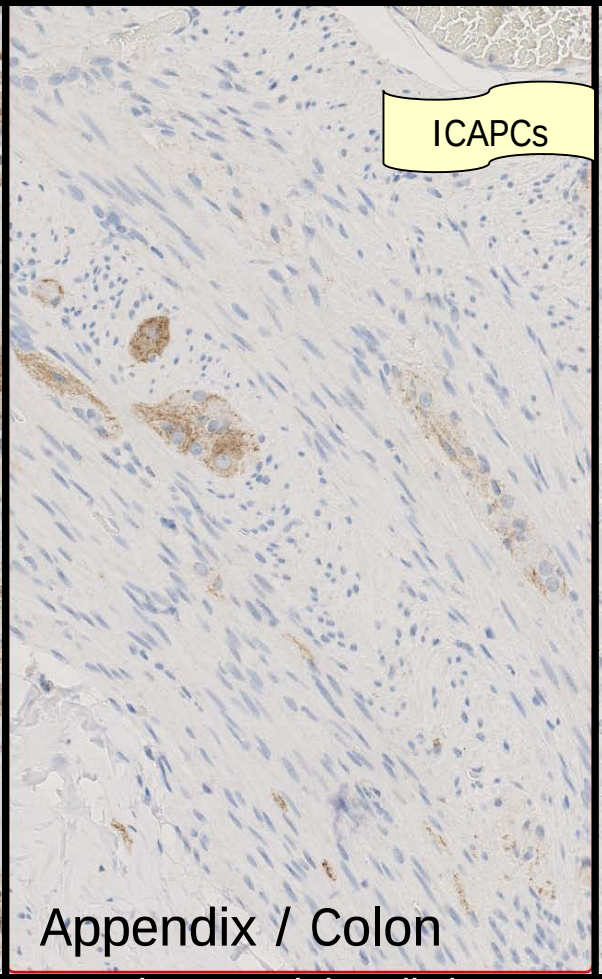
IHC – Protocols and controls – GYN, GI, Liver

Glypican 3 reaction pattern



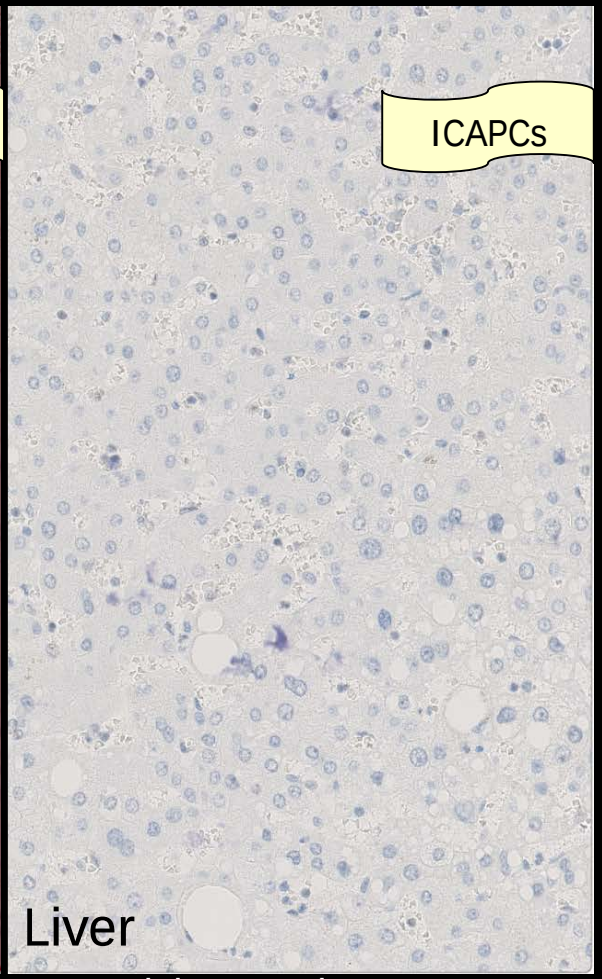
Placenta

An at least moderate predominantly cytoplasmic staining reaction of the vast majority of throphoblastic cells. Weak reaction in stromal and endothelial cells.



Appendix / Colon

An at least weak but distinct cytoplasmic staining reaction of peripheral nerves.



Liver

No staining reaction.

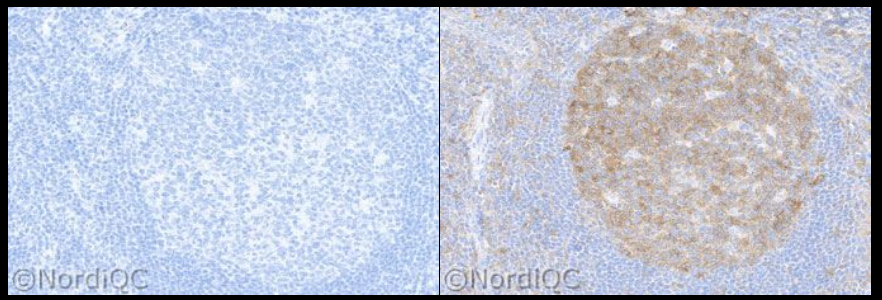
IHC – Protocols and controls – GYN, GI, Liver

UPT II: HEPA

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb OCH1E5*	HIER	1:30-1.200	2- & 3-step	Dako Ventana	2- & 3-step 2- & 3- step

* Less successful performance on Bond stainer



UPT II: Glypican 3 & Arginase*

*NordiQC data and In-house pre-liminary data**

	Retrieval	Titre	Detection	RTU	Detection
mAb 1G12	HIER	1:20-200	2- & 3-step	Ventana	2- & 3-step
rmAb SP156	HIER TE	1:25-50	3-step	-	-