Lung tumours

Optimization of antibodies, selection, protocols and controls

NQC Workshop 2018

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Lung tumours: Antibodies, protocols and controls

Lung markers in NordiQC assessments:

- Iu-ALK (NQC in 2017)
- PD-L1 (NQC in 2018)
- p63 (NQC in 2016)
- p40 (NQC in 2016)
- Napsin A (NQC in 2015)
- TTF-1 (NQC in 2016)
- SYP (NQC in 2018)
- WT1 (NQC in 2015)
- CEA (NQC in 2016)
- Calretinin (NQC in 2018)
- CGA (NQC in 2016)*
- Podoplanin (NQC in 2012)
- CD56 (NQC in 2013)

** In current run (2018)*

Virchows Arch (2016) 468:19–29
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ANNUAL REVIEW ISSUE

Proficiency testing in immunohistochemistry—experiences from Nordic Immunohistochemical Quality Control (NordiQC)

Mogens Vyberg[1,2] · **Søren Nielsen**[1]

Table 3 Major causes of insufficient staining reactions

1. Less successful antibodies (17 %)
   a. Poor antibodies[a]
   b. Less robust antibodies[b]
   c. Poorly calibrated RTUs
   d. Stainer platform dependent antibodies
2. Insufficiently calibrated antibody dilutions (20 %)
3. Insufficient or erroneous epitope retrieval (27 %)
4. Error-prone or less sensitive visualization systems[c] (19 %)
5 Other (17 %)
   a. Heat-induced impaired morphology
   b. Proteolysis induced impaired morphology
   c. Drying out phenomena
   d. Stainer platform-dependant protocol issues
   e. Excessive counterstaining impairing interpretation

Lung tumours: Antibodies, protocols and controls

Target	High scoring clones*	Low scoring clones*
lu-ALK	rmAb: D5F3, mAb: OTI1A4	mAb: ALK1 mAb: 5A4
PD-L1	mAb: 22C3, rmAb: 28-8 and rmAb SP263	
p63	mAb: DAK-p63 and 4A4	mAb: 7JUL
p40	mAb: BC28 and rmAb: ZR8	Many pAbs
Napsin A	mAb: IP64 and MRQ-60	pAb: 760-4446 and 352A-7x
TTF1	mAb: SPT24 and SP141	mAb: 8G7G3/1
SYP	mAb: 27G12, rmAb MRQ-40 and DAK-SYNAP	mAb: SY38
WT1	mAb: WT49 and 6F-H2	
CEA	mAb: CEA31 and COL-1	mAb: TF3H8-1 and II-7
CGA	pAb: A0430§ / IR502§, mAb: LK2H10	rmAb: SP12, mAb DAK-A3
Calretinin	rmAb: SP65, mAb DAK-Calret1, pAb 18-0211	rmAb: SP13
Podoplanin	mAb: D2-40	mAb: D2-40 #
CD56	rmAb: MRQ-42, mAb: CD564 and 123C3	mAb: 123C3 #

Ventana platform § Products discontinued * on the basis of the assessments in NordiQC

Recommended protocols - p63

Search:

Epitope	Staining Platform	Clone name	Clone format	Version date	View
p63	Autostainer, LabVision	DAK-p63	CONC	23 Sep 2015	PDF
p63	Dako Autostainer Link 48 +	DAK-p63	CONC	13 Sep 2015	PDF
p63	Dako Autostainer Link 48 +	4A4	CONC	29 Aug 2016	PDF
p63	Dako Omnis	DAK-p63	CONC	05 Oct 2016	PDF
p63	DBS Montage 360 system	DBR16.1	Other	23 Aug 2016	PDF
p63	Gene Stainer, Gene Tech	4A4	CONC	23 Sep 2015	PDF
p63	Gene Tech Genestainer	4A4	Other	19 Aug 2016	PDF
p63	Leica BOND III	4A4	CONC	12 Sep 2015	PDF
p63	Leica BOND III	4A4	Other	25 Aug 2016	PDF
p63	Leica BOND III	DAK-p63	CONC	30 Aug 2016	PDF
p63	Thermo Autostainer 36/48/72	DAK-p63	CONC	05 Sep 2016	PDF
p63	Ventana Benchmark Ultra	DAK-p63	CONC	16 Sep 2015	PDF
p63	Ventana Benchmark Ultra	4A4	CONC	16 Sep 2015	PDF
p63	Ventana Benchmark Ultra	DAK-p63	CONC	29 Aug 2016	PDF
p63	Ventana Benchmark Ultra	4A4	CONC	02 Sep 2016	PDF

Recommended protocol for p63

Obtained in run 48

29 Aug 2016

Immunostainer

Type:	Ventana Benchmark Ultra

Primary antibody

Clone:	DAK-p63
Producer:	Dako
Product no. / lot no.:	M7317 / 20032413
Diluent:	Da Vinci Green
Dilution factor:	1:100
Incubation time / temperature:	32 min. / 36°C

Epitope retrieval, HIER

Device:	On Board / On Machine
Buffer:	Ventana Ultra CC1
Heating time at max. temp.:	56 min.
Maximum heating temp.:	100°C

Visualization system

Producer:	Ventana
Product / no:	OptiView DAB IHC Detection Kit / 760-700
Incubation time linker:	8 min.
Incubation time polymer:	8 min.
Incubation temperature:	36°C

Chromogen

Producer:	Ventana
Product / no:	OptiView DAB IHC Detection Kit / 760-700
Incubation time / temperature:	8 min. / 22°C
Enhancement:	CuSO4

Lung tumours: Antibodies, protocols and controls

Target	Controls, positive	Controls, negative
lu-ALK	Colon/appendix (LE*), lung carc. (lu-ALK pos)	Lung carcinoma (lu-ALK neg)
PD-L1	Tonsil (HE** and LE), placenta (HE) and kit controls	Kit controls
p63	Tonsil (HE and LE) or prostate (LE)	Prostate and tonsil
p40	Tonsil (HE) and placenta (LE)	Tonsil
Napsin A	Kidney (LE) and lung (HE)	Colon/appendix
TTF1	Lung terminal bronchioles (HE and LE)	Liver
SYP	Colon/appendix (HE and LE)	Liver
WT1	Fallopian tube (LE and HE) and kidney (HE)	Kidney
CEA	Colon/appendix (HE and LE)	Liver
CGA	Colon/appendix (HE and LE) and pancreas (HE)	Liver
Calretinin	Adrenal gland (LE) and appendix (HE and LE)	Appendix
Podoplanin	Tonsil (HE and LE)	
CD56	Tonsil (LE) colon/appendix (HE)	Tonsil

"Onslide" control

*Low Expresser **High Expresser LE = LLOD (Low limit of detection)

Lung tumours: Antibodies, protocols and controls

Target	High scoring clones*	Low scoring clones*
lu-ALK	rmAb: D5F3, mAb: OTI1A4	mAb: ALK1 mAb: 5A4
PD-L1	mAb: 22C3, rmAb: 28-8 and rmAb SP263	
p63	mAb: DAK-p63 and 4A4	mAb: 7JUL
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TTF1	mAb: SPT24 and SP141	mAb: 8G7G3/1
SYP	mAb: 27G12, rmAb MRQ-40 and DAK-SYNAP	mAb: SY38
WT1	mAb: WT49 and 6F-H2	
CEA	mAb: CEA31 and COL-1	mAb: TF3H8-1 and II-7
CGA	pAb: A0430§ / IR502§, mAb: LK2H10	rmAb: SP12, mAb DAK-A3
Calretinin	rmAb: SP65, mAb DAK-Calret1, pAb 18-0211	rmAb: SP13
Podoplanin	mAb: D2-40	mAb: D2-40 #
CD56	rmAb: MRQ-42, mAb: CD564 and 123C3	mAb: 123C3 #

Ventana platform § Products discontinued * on the basis of the assessments in NordiQC

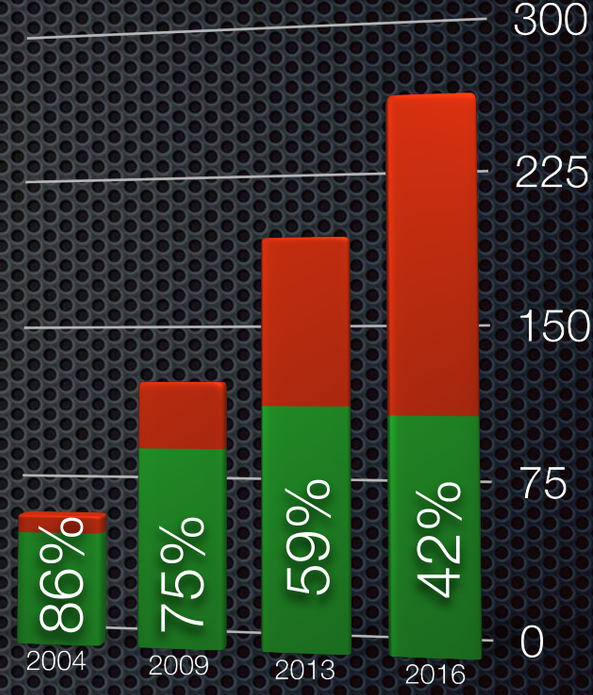
Lung tumours: Antibodies, protocols and controls

CEA / RUN 47 2016

Pass: 42 %

Table 1. **Antibodies and assessment marks for CEA, run 47**

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **12-140-10**	3	Leica/Novocastra	0	0	0	3	-	-
mAb clone **CEA31**	9 1	Cell Marque BioSB	6	0	3	1	67%	75%
mAb **COL-1**	6 5 5 2 1 1	Thermo/Neomarkers Invitrogen/Zymed Biocare Immunologic Zytomed GeneTex	11	7	2	0	90%	94%
mAb **II-7**	85	Dako/Agilent	2	19	60	4	25%	58%
mAb **CEA88**	2	BioGenex	0	0	1	1	-	-
mAb **PARLAM 4**	1	Monosan	0	0	1	0	-	-
mAb **BS33**	1	Nordic Biosite	0	0	1	0		
Ready-To-Use Antibodies								
mAb clone **CEA31 760-4594**	53	Ventana/Cell Marque	22	26	5	0	91%	100%
mAb clone **CEA31 236M**	4	Cell Marque	1	2	1	0	-	-
mAb clone **COL-1 MAD-002095QD**	2	Master Diagnostica	0	0	1	1	-	-
mAb clone **COL-1 PM058**	1	Biocare	0	0	1	0	-	-
mAb clone **COL-1 Kit-0008**	1	Maixin	1	0	0	0	-	-
mAb clone **II-7 IR/IS622/GA622**	47	Dako/Agilent	0	6	40	1	13%	-
mAb clone **II-7 PA0004**	12	Leica	0	5	6	1	42%	-
mAb clone **TF3H8-1 760-2507**	13	Ventana/Roche	0	0	0	13	0%	-
Total	255		43	65	122	25	-	
Proportion			17%	25%	48%	10%	42%	



mAb clone TF3H8-1 cross reacts with BGP and NCA

mAb clone II-7 is difficult to optimise

Lung tumours: Antibodies, protocols and controls

CEA / RUN 47 2016

Controls / iCAPC

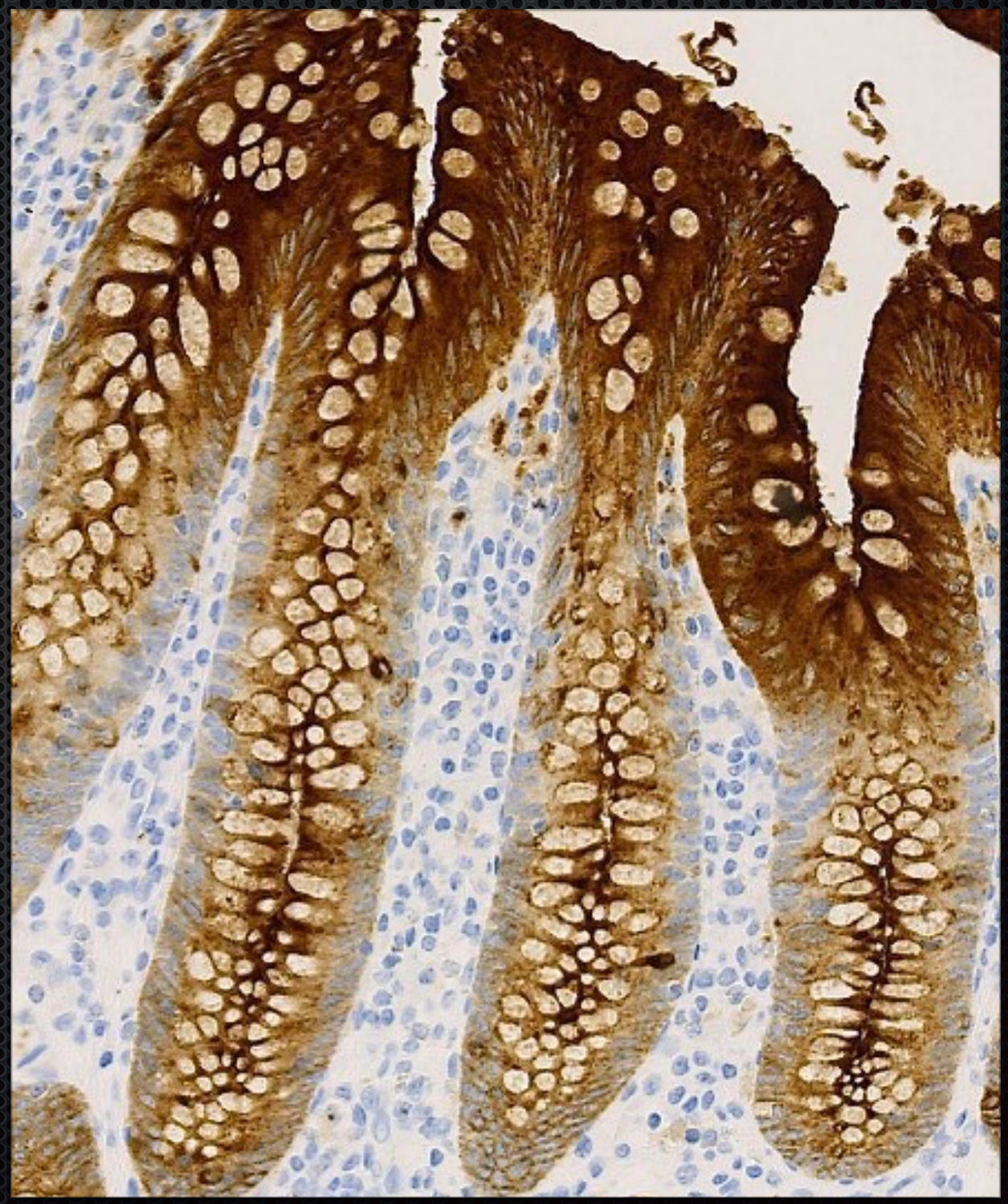
Positive: Appendix.

* The vast majority of the epithelial cells must show a moderate to strong cytoplasmic staining reaction.

Negative: Liver

* No cells must be positive.

iCAPC: immunohistochemistry
Critical Assay Performance Control



Lung tumours: Antibodies, protocols and controls

CEA / RUN 47 2016

Controls / iCAPC

Standardization of Positive Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Committee

Emina E. Torlakovic, MD, PhD,† Søren Nielsen, HT, CT,‡§ Glenn Francis, MBBS, FRCPA, MBA, FFSc (RCPA),‖¶# John Garratt, RT,†** Blake Gilks, MD, FRCPC,†† Jeffrey D. Goldsmith, MD,‡‡ Jason L. Hornick, MD, PhD,*§§ Elizabeth Hyjek, MD, PhD,* Merdol Ibrahim, PhD,‖‖ Keith Miller, FIBMS,‖‖ Eugen Petcu, MD, PhD,‖ Paul E. Swanson, MD,¶¶## Xiaoge Zhou, MD,***††† Clive R. Taylor, MD, PhD,‡‡‡ and Mogens Vyberg, MD‡§*

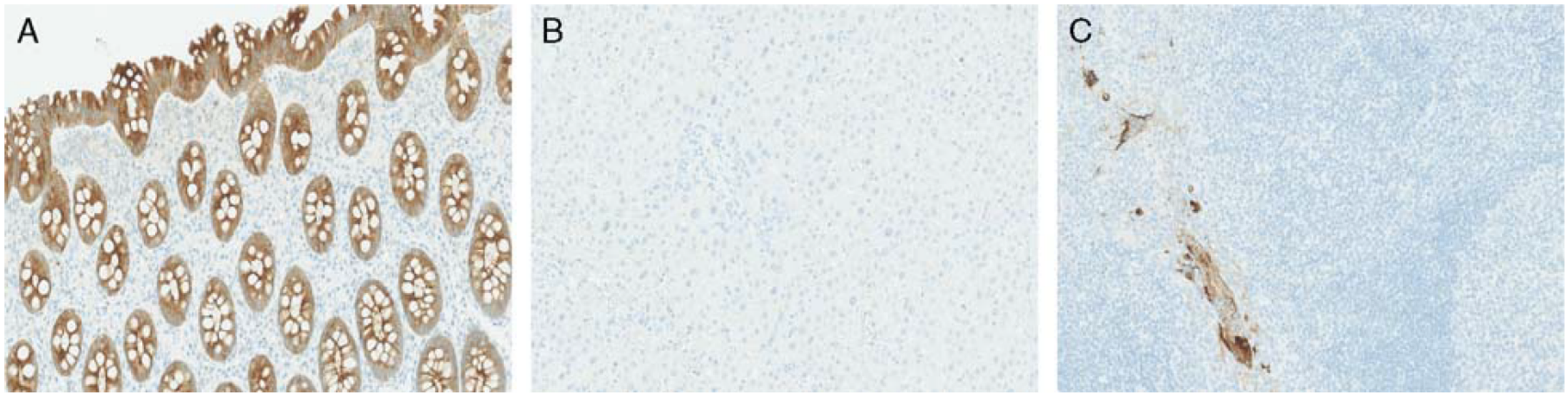


FIGURE 9. mCEA iCAP. A, A moderate to strong staining reaction must be seen in the brush border of the surface epithelial cells. Virtually all epithelial cells must show a weak to moderate cytoplasmic staining reaction (LLOD). If overexpression of the CEA is desirable target for detection in adenocarcinoma, the demonstration of the staining of only of the surface of mucosa can be selected as LLOD. B, Liver: no staining reaction must be seen. C, Tonsil: scattered squamous epithelial cells show a moderate to strong cytoplasmic staining reaction (number of cells demonstrated will vary from tonsil to tonsil).

Lung tumours: Antibodies, protocols and controls

NordiQC

CEA / RUN 47 2016

Recommend-able clones	Retrieval	Titre	Detection	RTU	Detection
mAb COL-1	HIER, High pH	1:100 - 1:400	2- or 3-step	-	-
mAb CEA31	HIER, High pH	1:100 - 1:400	2- or 3-step	Ventana	2- or 3-step

Table 3. **Optimal results for CEA for the three most commonly used 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 **II-7**	1/17** (6%)	0/2	0/35 (0%)	-	1/10 (10%)	0/4 (0%)
mAb clone **COL-1**	1/2	-	8/13 (62%)	-	1/1	-
mAb clone **CEA31**	3/3	-	3/6 (50%)	-	-	-

* 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)

Lung tumours: Antibodies, protocols and controls

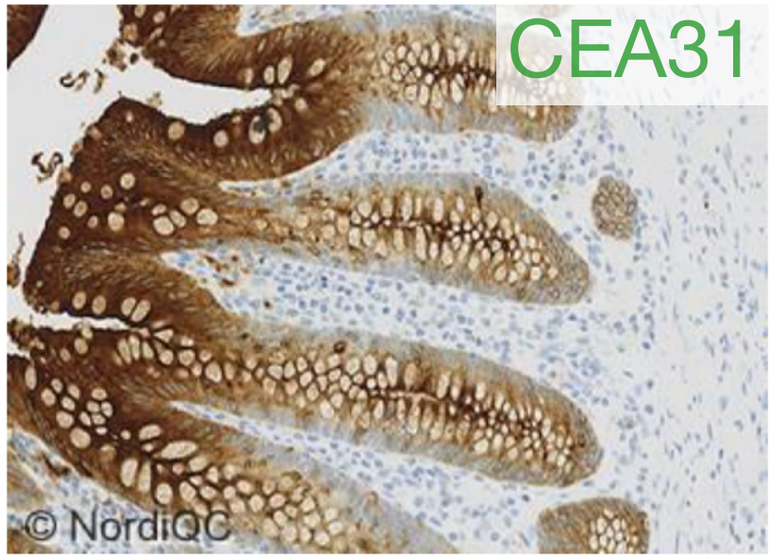


Fig. 1a (x200)
Optimal CEA staining of the appendix using the **mAb clone CEA31** diluted 1:100 and with an incubation time of 30 min. after HIER in an alkaline buffer (TRS pH 9, Dako). Staining was performed on the Dako Omnis using a 3-step polymer system (EnVision Flex+). A weak to moderate staining 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. No background staining is seen.

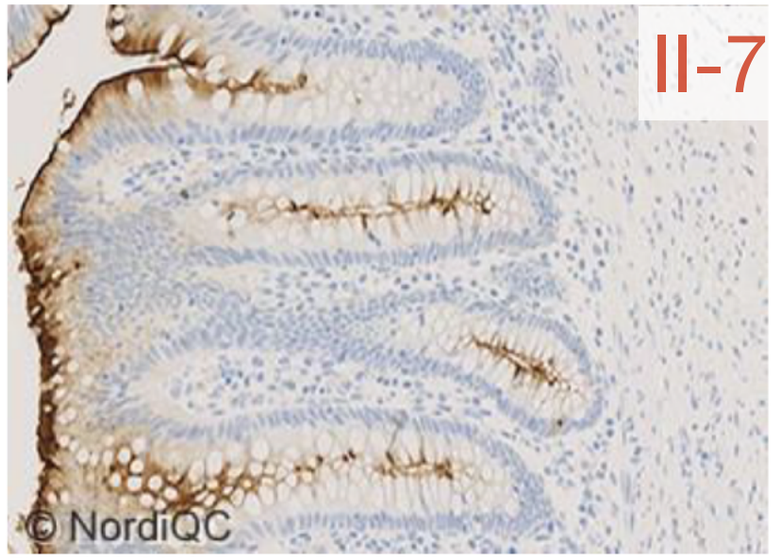


Fig. 1b (x200)
Insufficient CEA staining of the appendix using the **mAb clone II-7** in a RTU format (Dako GA622) with an incubation time of 25 min. after HIER in an alkaline buffer (TRS pH 9, Dako). Staining was performed on the Dako Omnis using a 3-step polymer system (EnVision Flex+). In spite of very similar protocol settings the "clone II-7"-protocol only demonstrates the glycocalyx distinctively, while the cytoplasmic compartment in the vast majority of epithelial cells is unstained - same field as in Fig. 1a. Also compare with Figs. 2b - 4b, same protocol.

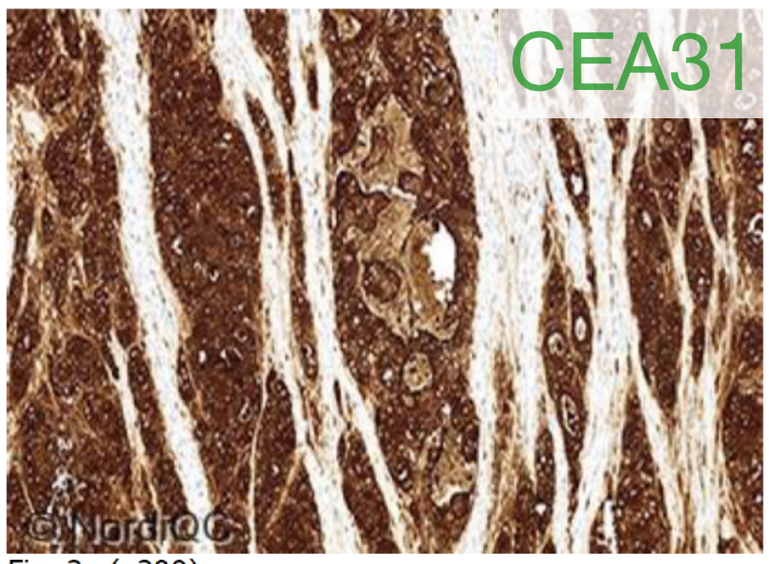


Fig. 2a (x200)
Optimal CEA staining of the colon adenocarcinoma with high level CEA expression using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining reaction. Weak background staining in the vicinity of the neoplastic cells, due to diffusion of antigen, is seen and accepted.

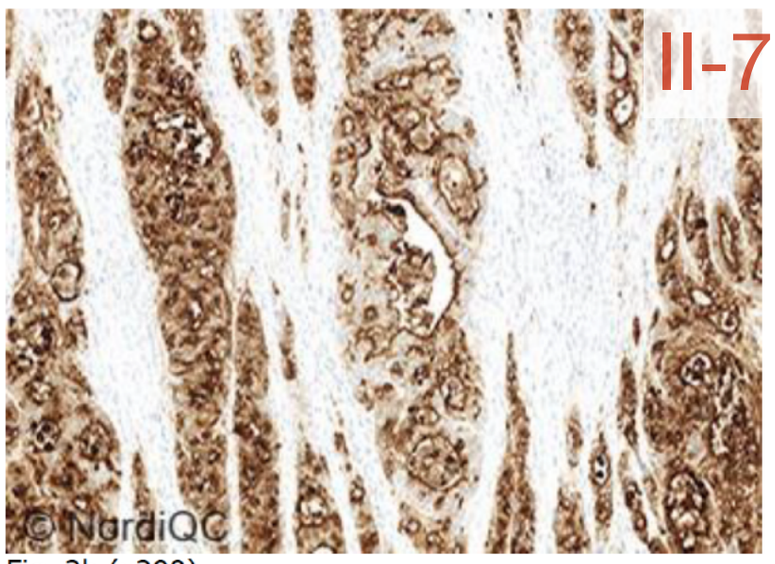


Fig. 2b (x200)
CEA staining of the colon adenocarcinoma with high level CEA expression using same insufficient protocol as in Fig. 1b – same field as in Fig. 2a. The intensity of the neoplastic cells demonstrated is reduced compared to the level expected and obtained in Fig. 2a.

Less successful primary antibody: mAb clone II-7

Lung tumours: Antibodies, protocols and controls

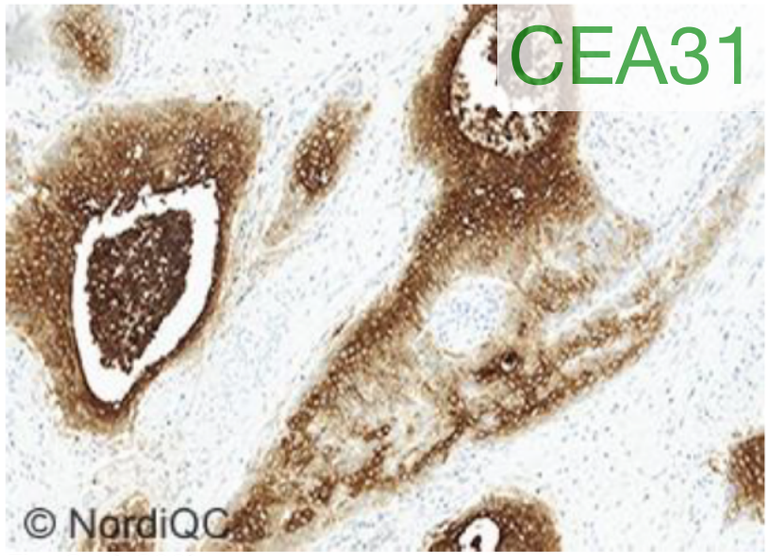


Fig. 3a (x200)
Optimal CEA staining of the urothelial carcinoma, tissue core no. 4, using same protocol as in Figs. 1a and 2a. The majority of the neoplastic cells show a strong and distinct staining reaction. No background staining is seen.

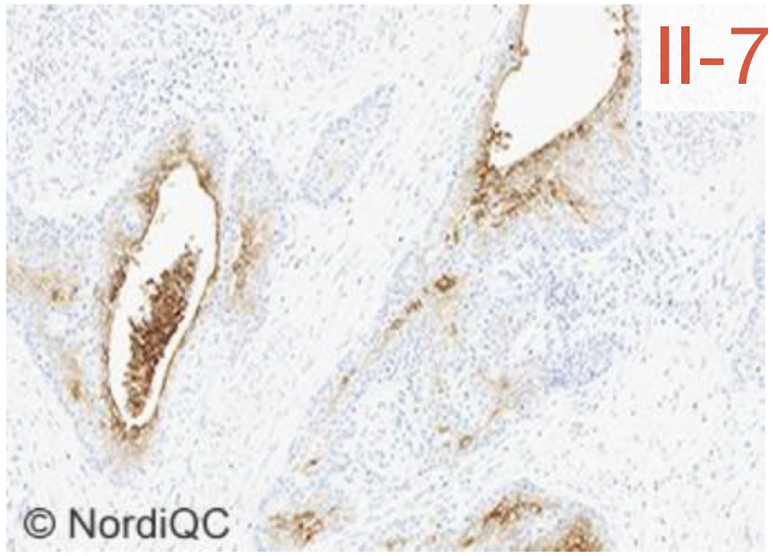


Fig. 3b (x200)
Insufficient CEA staining of the urothelial carcinoma, tissue core no. 4, using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 3a.

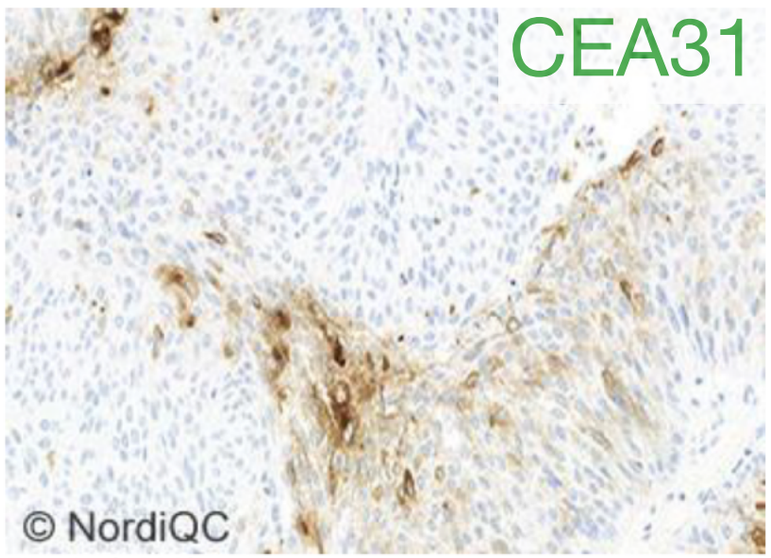


Fig. 4a (x200)
Optimal CEA staining of the urothelial carcinoma, tissue core no. 5, with low level CEA expression using same protocol as in Figs. 1a - 3a. Focally the neoplastic cells show a moderate to strong and distinct staining reaction. No background staining is seen.

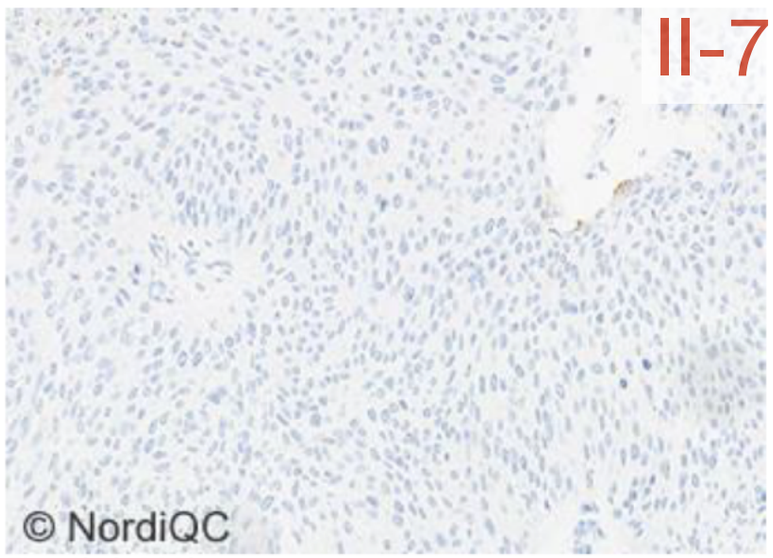


Fig. 4b (x200)
Insufficient CEA staining of the urothelial carcinoma, tissue core no. 5, with low level CEA expression using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
The neoplastic cells show no staining reaction and a false negative result of the tumour is seen.

Less successful primary antibody: mAb clone II-7

Lung tumours: Antibodies, protocols and controls

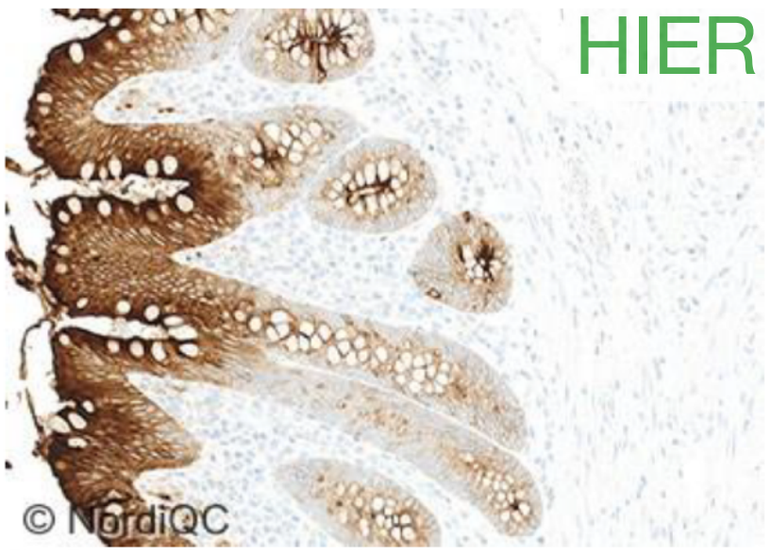


Fig. 5a (x200)
Optimal CEA staining of the appendix using the mAb clone CEA31 diluted 1:400 and with an incubation time of 30 min. after **HIER** in an alkaline buffer (CC1, Ventana). Staining was performed on the Ventana BenchMark using a 3-step multimer system (OptiView)
A weak to moderate staining reaction is seen in the vast majority of the luminal epithelial cells of the appendix, whereas the glycocalyx show an intense staining reaction. Compare also to Fig. 6a, same protocol.

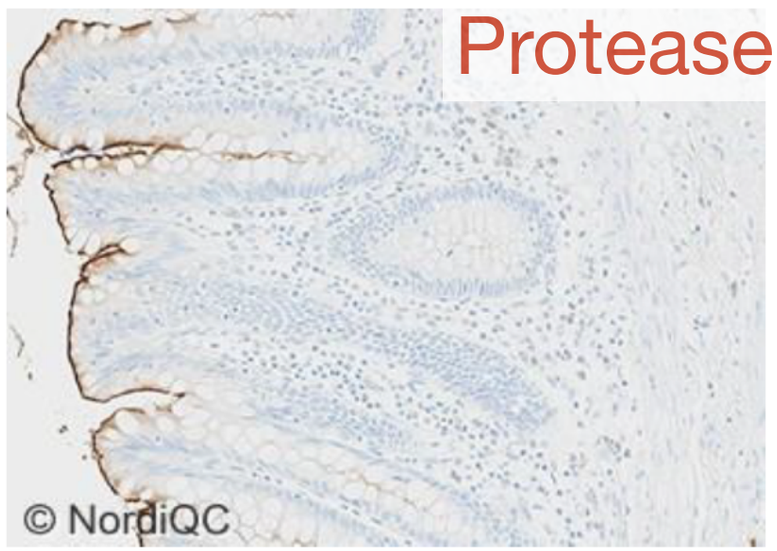


Fig. 5b (x200)
Insufficient CEA staining of the appendix using the mAb clone CEA31 with similar protocol settings as used in Fig. 5a. Only difference was the use of proteolytic pretreatment (Protease 1, Ventana for 8 min.) instead of HIER. Proteolytic pre-treatment results in a drastic reduction in staining intensity. Only the glycocalyx is distinctively demonstrated, while the cytoplasmic compartment of the epithelial cells is unstained - same field as in Fig. 5a. Compare also to Fig. 6b, same protocol.

Inappropriate retrieval - use of proteolysis

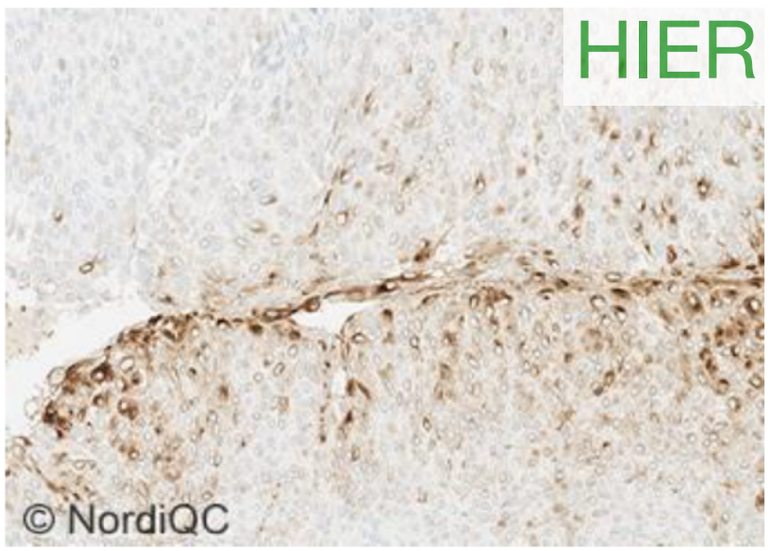


Fig. 6a (x200)
Optimal CEA staining of the urothelial carcinoma, tissue core no. 5, with low level CEA expression using same protocol as in Fig 5a. Focally the neoplastic cells show a moderate to strong and distinct staining reaction.

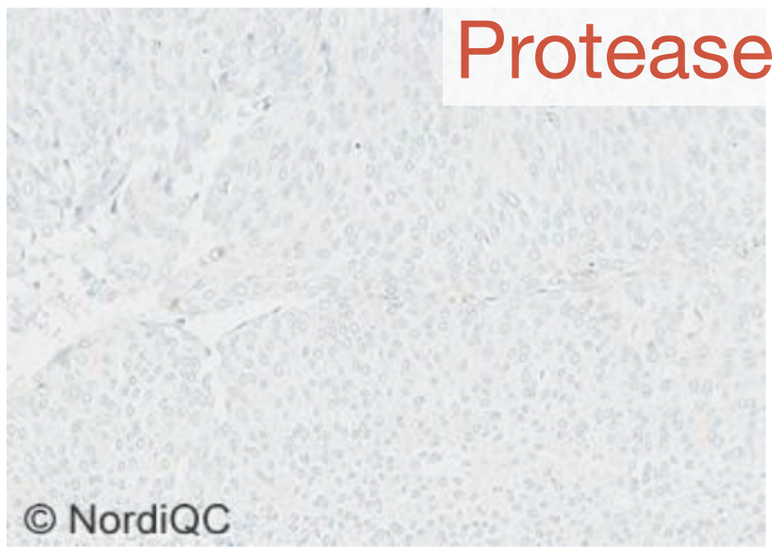


Fig. 6b (x200)
Insufficient CEA staining of the urothelial carcinoma, tissue core no. 5, with low level CEA expression using same protocol as in Fig. 5b – same field as in Fig. 6a. The neoplastic cells show no staining reaction and a false negative result in this tumour is seen.

Lung tumours: Antibodies, protocols and controls

Inappropriate antibody - NCA and BGP cross reaction

CEA31

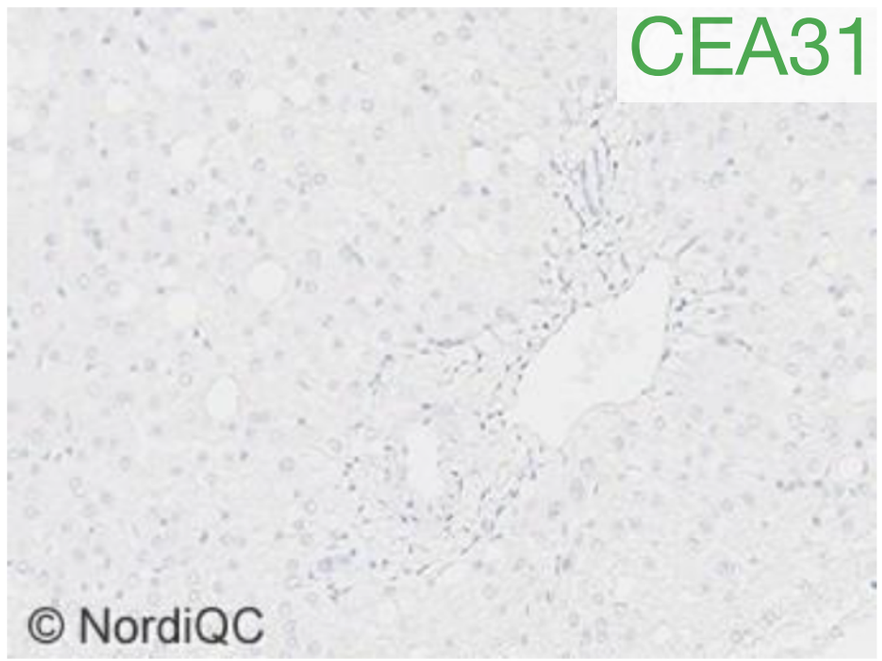


Fig. 7a (x200)
Optimal CEA staining of the liver using same protocol as in Figs. 5a and 6a based on the **mAb clone CEA31**. No staining reaction is seen in the Kupffer cells, leucocytes and the bile canaliculi. No background staining is seen.

TF3H8-1

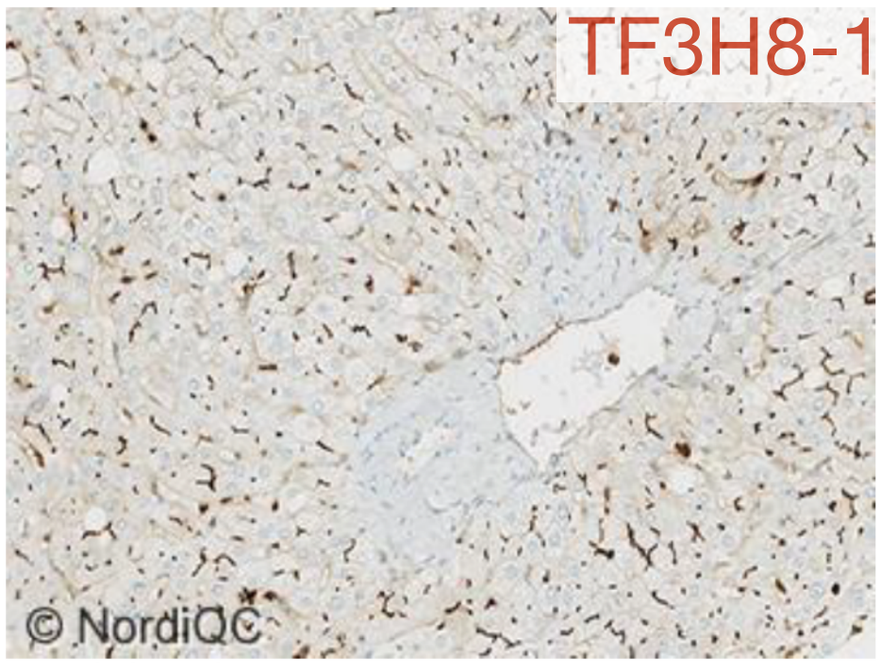


Fig. 7b (x200)
Insufficient CEA staining of the liver using the **mAb clone TF3H8-1.** Both the Kupffer cells, leucocytes and bile canaliculi are stained due to a cross reaction of the Ab to NCA (CEACAM6) and BGP (CEACAM1) – same field as in Fig. 7a.

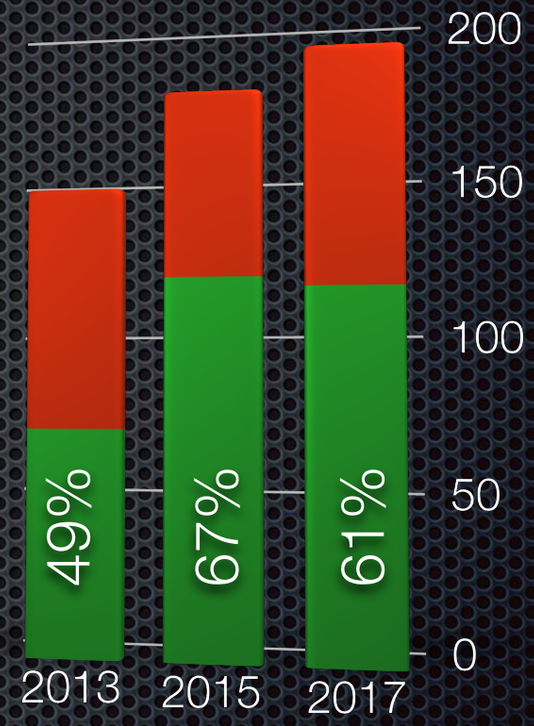
Lung tumours: Antibodies, protocols and controls

lu-ALK / RUN 51 2017

Pass: 61 %

Table 1. **Antibodies and assessment marks for lu-ALK, run 51**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **5A4**	43 1 1 1 1	Leica/Novocastra Abcam Biocare Monosan ThermoFisher	1	15	24	7	34%	22%
mAb clone **ALK1**	2 1	Dako Cell Marque	0	0	0	3	-	-
rmAb clone **D5F3**	23	Cell Signaling	6	12	3	2	78%	94%
mAb clone **OTI1A4**	13	ORIGENE	10	3	0	0	100%	100%
Ready-To-Use antibodies								
mAb clone **5A4 PA0306**	6	Leica/Novocastra	0	0	6	0	-	-
mAb clone **5A4 MAB-0281**	1	Maixin	0	0	1	0	-	-
mAb **5A4 MAD-001720QD**	1	Master Diagnostica	0	0	1	0	-	-
mAb clone **5A4 MS-1104-R7**	1	ThermoFisher	0	1	0	0	-	-
mAb **ALK1 IR641**	9	Dako	0	0	1	8	-	-
mAb clone **ALK1 GA641**	4	Dako	0	0	0	4	-	-
mAb clone **ALK1 790/800-2918**	7	Ventana	0	0	2	5	-	-
rmAb clone **SP8 AN770**	1	BioGenex	0	0	0	1	-	-
rmAb clone **D5F3 790-4796**	70	Ventana	53	12	4	1	93%	100%
rmAb clone **D5F3 790-4796**[3]	2	Ventana	1	0	1	0	-	-
mAb clone **OTI1A4 8344-C010**	1	Sakura Finetek	1	0	0	0	-	-
Total	189		72	43	43	31	-	
Proportion			38%	23%	23%	16%	61%	



mAb clone ALK1 is not “Fit for purpose”

mAb clone 5A5 is difficult to optimise

Lung tumours: Antibodies, protocols and controls

lu-ALK / RUN 51 2017

Recommend-able clones	Retrieval	Titre	Detection	RTU	Detection
mAb OTI1A4	HIER, High pH	1:100 - 1:1500	3-step	-	-
rmAb D5F3	HIER, High pH	1:50 - 1:200	3-step +/- amp	Ventana	3-step + amp
mAb 5A4	HIER, High pH	1:20	3-step + amp	No optimal	-

Table 3. **Proportion of optimal results for lu-ALK for the most commonly used antibodies as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	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 **5A4**	0/9** (0%)		0/3	-	1/22 (5%)	-	0/9 (0%)	0/1
mAb clone **OTI1A4**	2/2	-	5/5 (100%)	-	1/2	-	1/1	-
rmAb clone **D5F3**	2/3	0/1	0/3	-	2/6 (33%)	-	2/7 (29%)	0/1

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

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

Lung tumours: Antibodies, protocols and controls

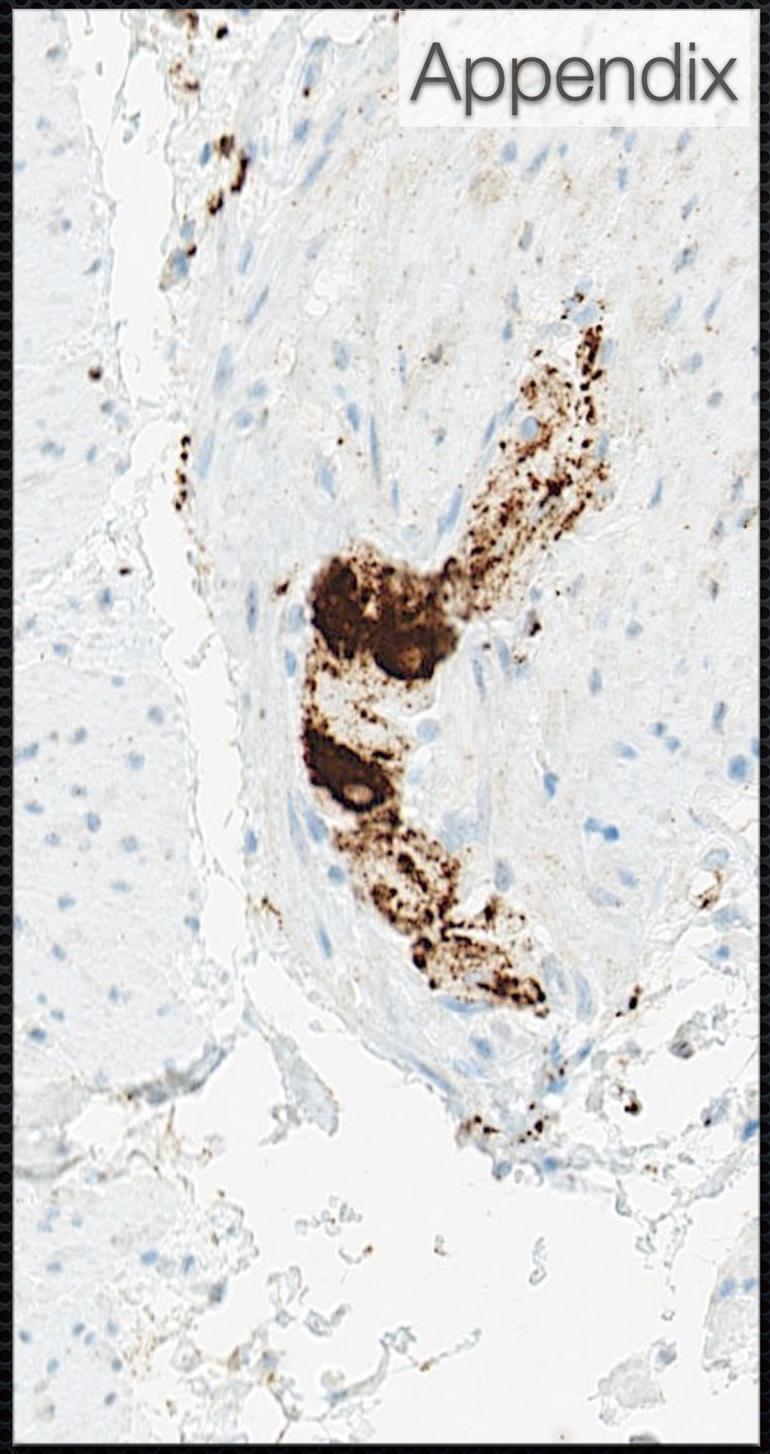
lu-ALK / RUN 51 2017

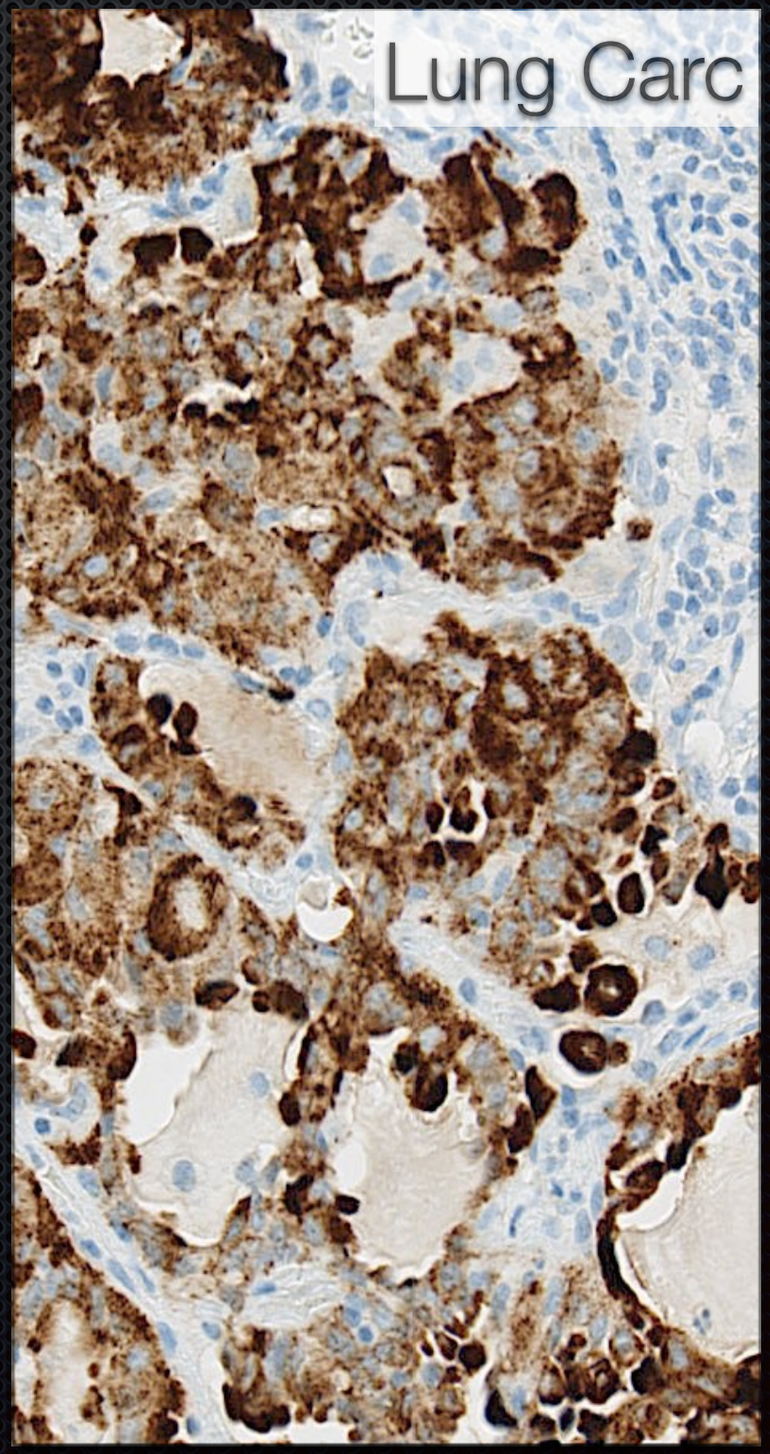
Controls / iCAPC

Positive: ALCL and lung adenocarcinoma with FISH verified ALK rearrangements and normal appendix.

* A weak to strong granular cytoplasmic staining reaction should be seen in the ganglion cells in appendix.

Negative: Lung cancer without ALK rearrangements





Lung tumours: Antibodies, protocols and controls

lu-ALK / RUN 51 2017

NordiQC

ALK staining using High pH HIER and a 3-step polymer based detection system (BOND)

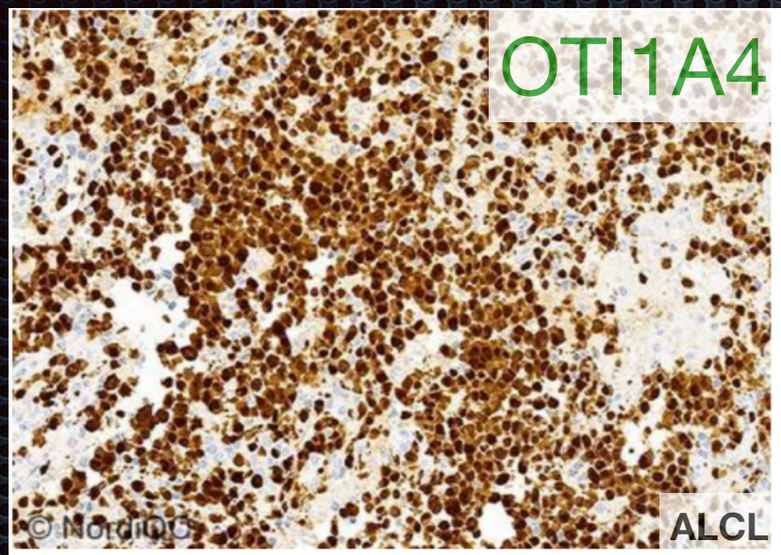


Fig. 1a

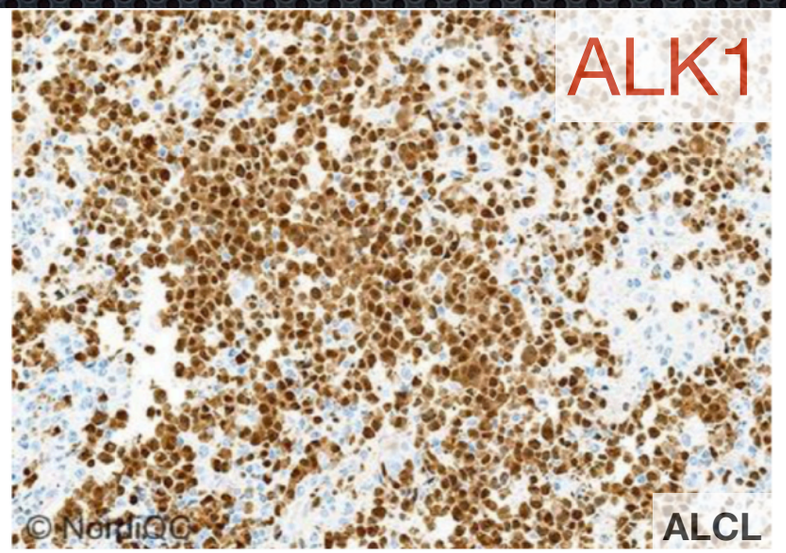


Fig. 1b

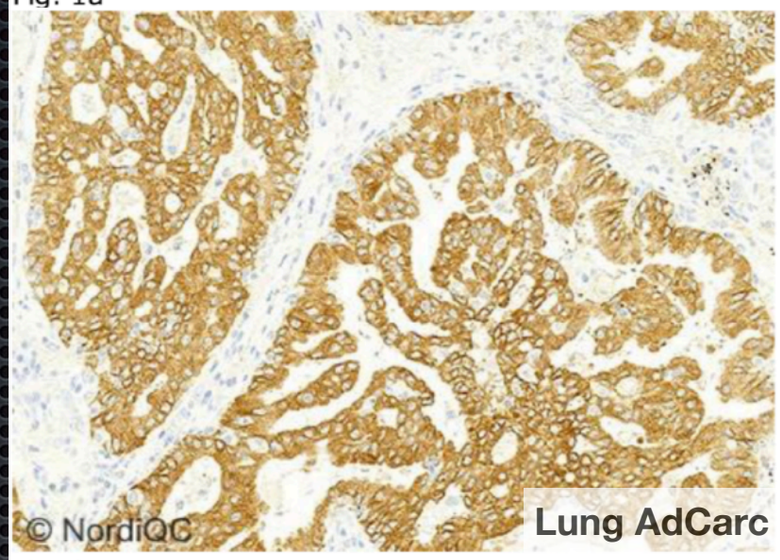


Fig. 2a

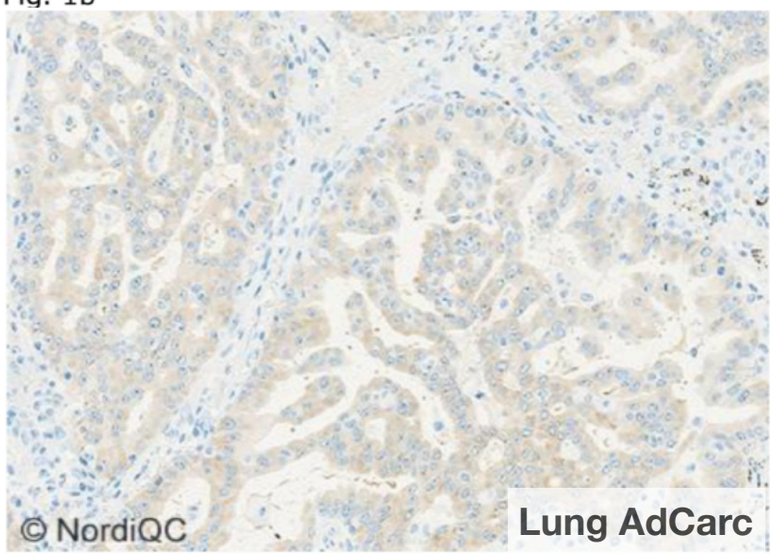


Fig. 2b

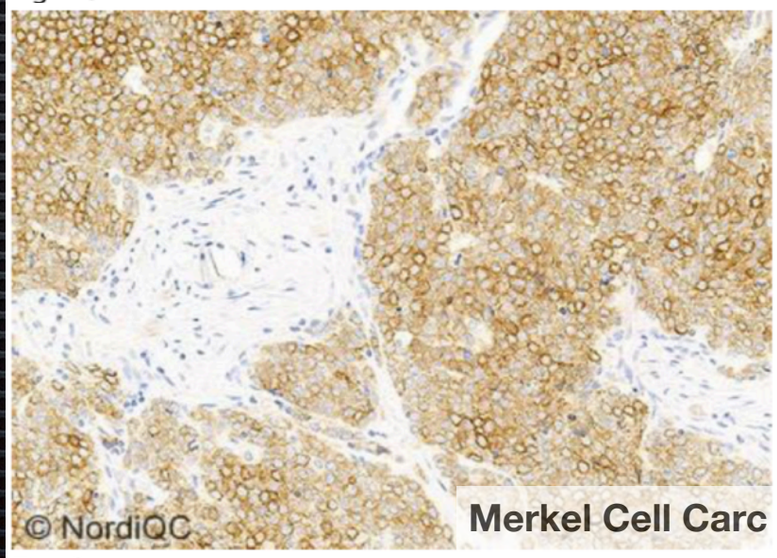


Fig. 3a

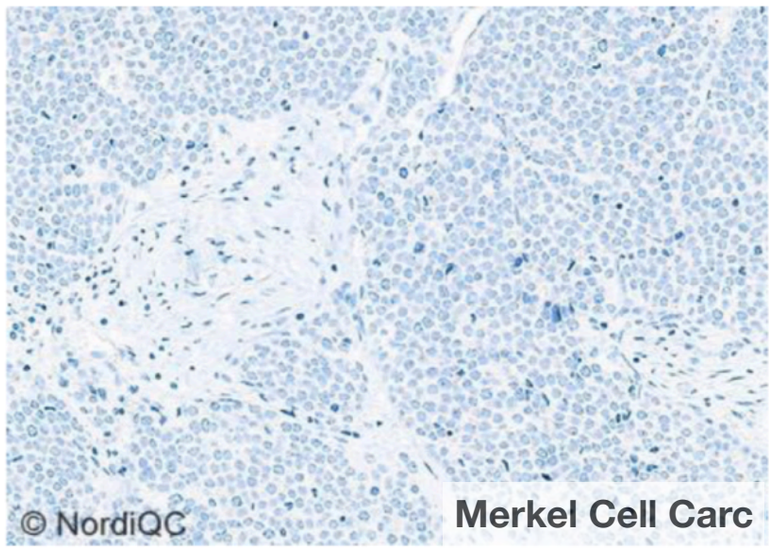


Fig. 3b

Less successful primary antibody: mAb clone ALK1

Lung tumours: Antibodies, protocols and controls

ALK staining based on the RTU systems from Ventana (D5F3) and Leica (5A4)

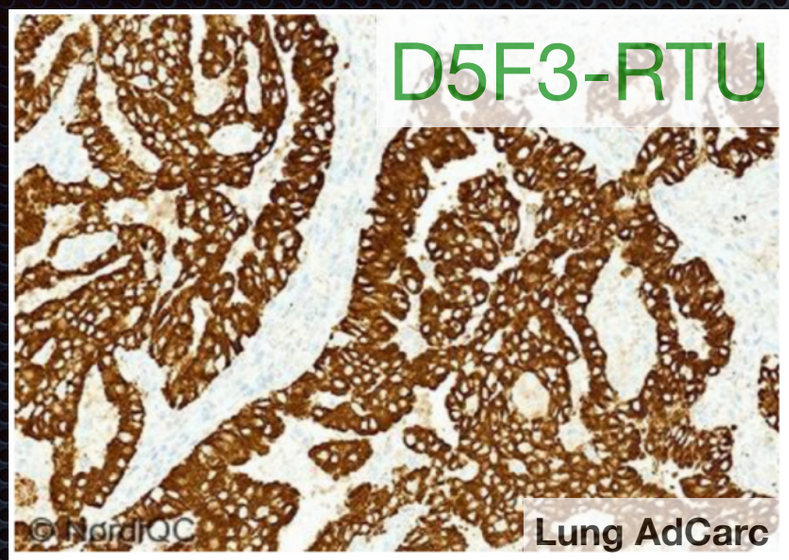


Fig.5a

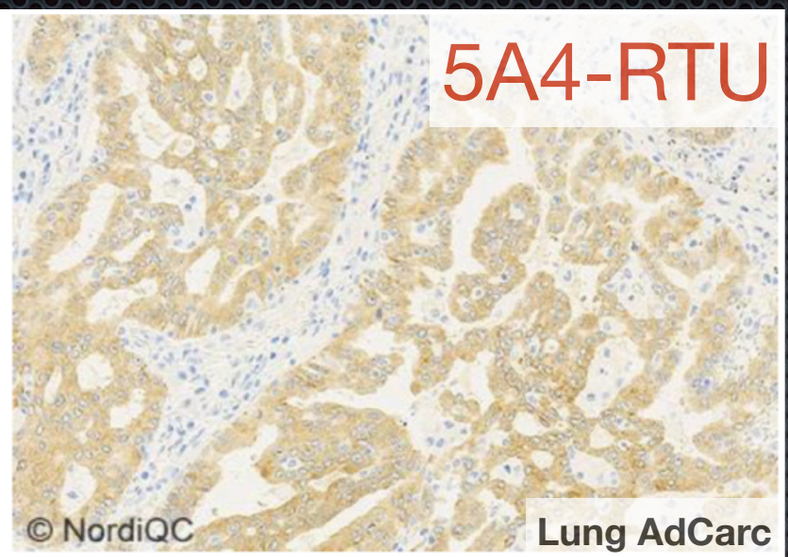


Fig. 5b

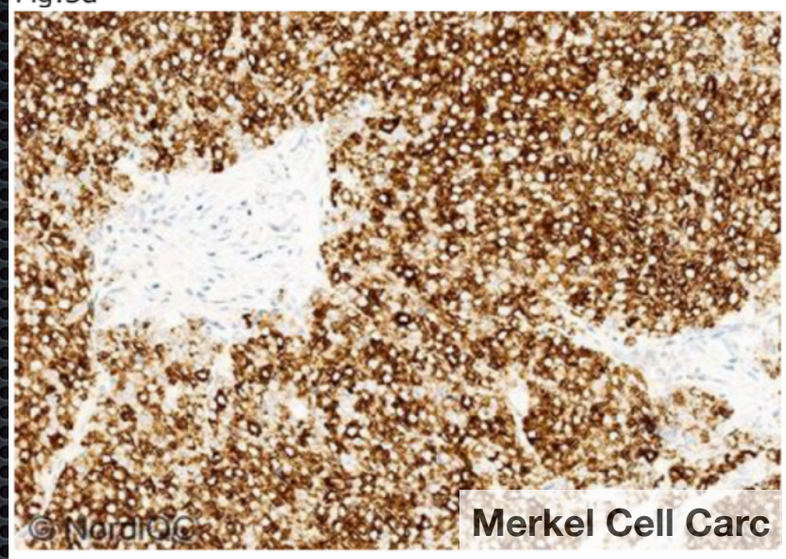


Fig. 6a

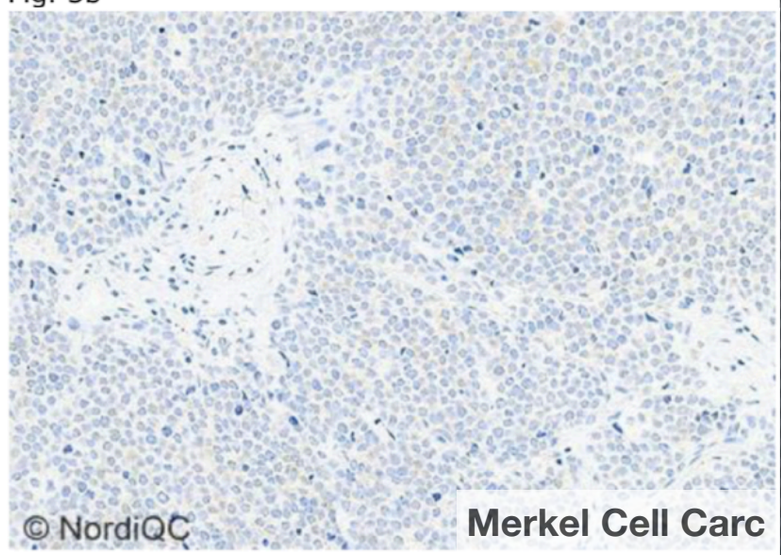


Fig. 6b

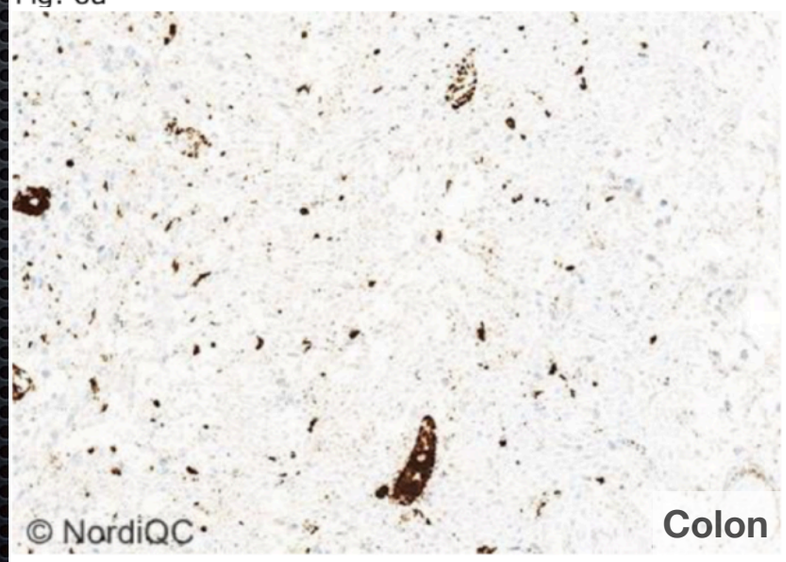


Fig. 7a

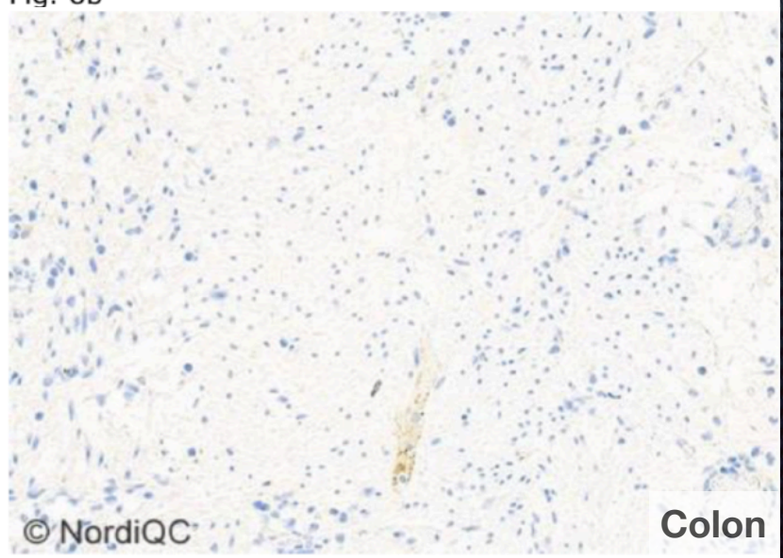


Fig. 7b

Less successful RTU system based on mAb clone 5A4

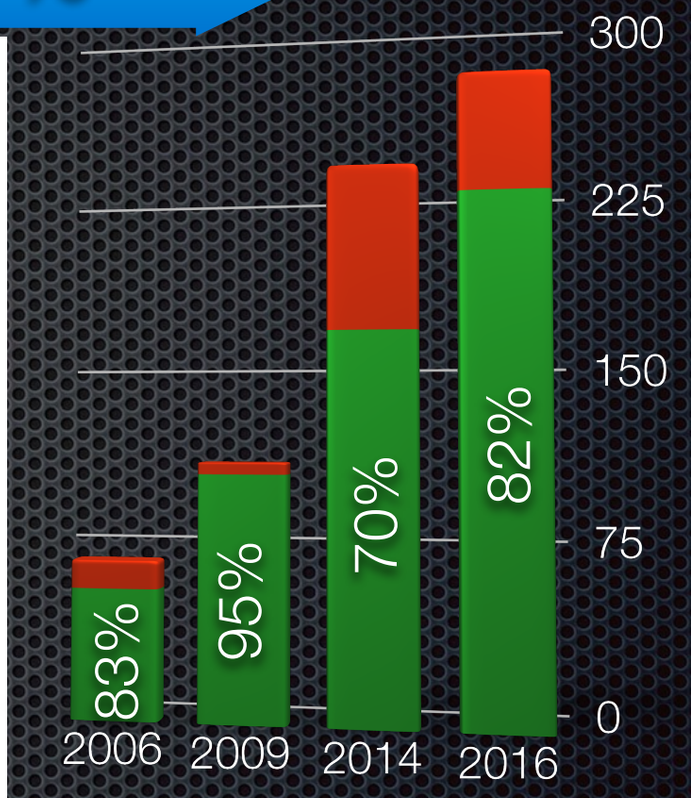
Lung tumours: Antibodies, protocols and controls

p63 / RUN 48 2016

Pass: 82 %

Table 1. **Antibodies and assessment marks for p63, run 48**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **4A4**	26 4 3 3 2 2 1 1 1 1 1	BioCare Medical ImmunoLogic Dako Zeta Corporation Thermo Scientific Zytomed Systems BioGenex Diagnostic BioSystems Klinipath Minarini Nordic Biosite Santa Cruz	13	20	11	2	72%	76%
mAb clone **DAK-p63**	47	Dako	20	21	6	0	87%	91%
mAb clone **7JUL**	12	Leica/Novocastra	0	1	3	8	8%	-
mAb clone **SFI-6**	2	DCS Immunoline	0	0	2	0	-	-
rmAb clone **BSR6**	1	Nordic Biosite	0	0	1	0	-	-
rmAb clone **DBR16.1**	1	Diagnostic Biosystems	1	0	0	0		
rmAb clone **EPR5701**	1	Epitomics	0	0	1	0	-	-
Unknown Ab	1	Unknown	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **4A4 790-4509**	102	Ventana	59	36	5	2	93%	95%
mAb clone **DAK-p63 IR662**	46	Dako	21	23	2	0	96%	94%
mAb clone **4A4 PM163**	3	BioCare	1	1	1	0	-	-
mAb clone **7JUL PA0103**	5	Leica/Novocastra	0	0	3	2	-	-
mAb clone **4A4 AM418**	2	BioGenex	0	1	0	1	-	-
mAb clone **4A4 ARB-56695**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **MX013 MAB-0694**	1	Maixin	0	1	0	0	-	-
mAb clone **4A4 MAD-000479QD**	3	Master Diagnostica SL	3	0	0	0	-	-
Total	274		120	104	35	15	-	
Proportion			44 %	38 %	13 %	5 %	82 %	



Lung tumours: Antibodies, protocols and controls

p63 / RUN 48 2016

Recommend-able clones	Retrieval	Titre	Detection	RTU	Detection
mAb 4A4	HIER, High pH	1:50 - 1:600	3-step	Ventana	3-step
mAb DAK-p63	HIER, High pH	1:50 - 1:300	2- or 3-step	Dako	2- or 3-step

Table 3. **Proportion of optimal results for p63 using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer / 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 **DAK-p63**	3/15 (20%)**	0/1	14/20 (70%)	-	1/4 (25%)	-
mAb clone **4A4**	0/6 (0%)	-	9/22 (41%)	-	2/8 (25%)	0/1

* 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)

p63 RTU antibodies / Plug-and-play ??

RTU: Ready To Use or Ready To Optimise??

Table 4 summarises the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance is evaluated both as a true plug-and-play system performed according to the recommendations provided by the vendor and by a laboratory modified system changing basal protocol settings. Only protocols performed on the specific IHC stainer device were included, whereas e.g. Dako RTU Ab formats applied on a Ventana stainer were excluded.

Table 4. **Proportion of sufficient and optimal results for p63 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS48 mAb DAK-p63 **IR662**	93% (14/15)	60% (9/15)	94% (16/17)	24% (4/17)
VMS Ultra/XT mAb 4A4 **790-4509**	60% (3/5)	20% (1/5)	95% (89/94)	60% (56/94)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer included.

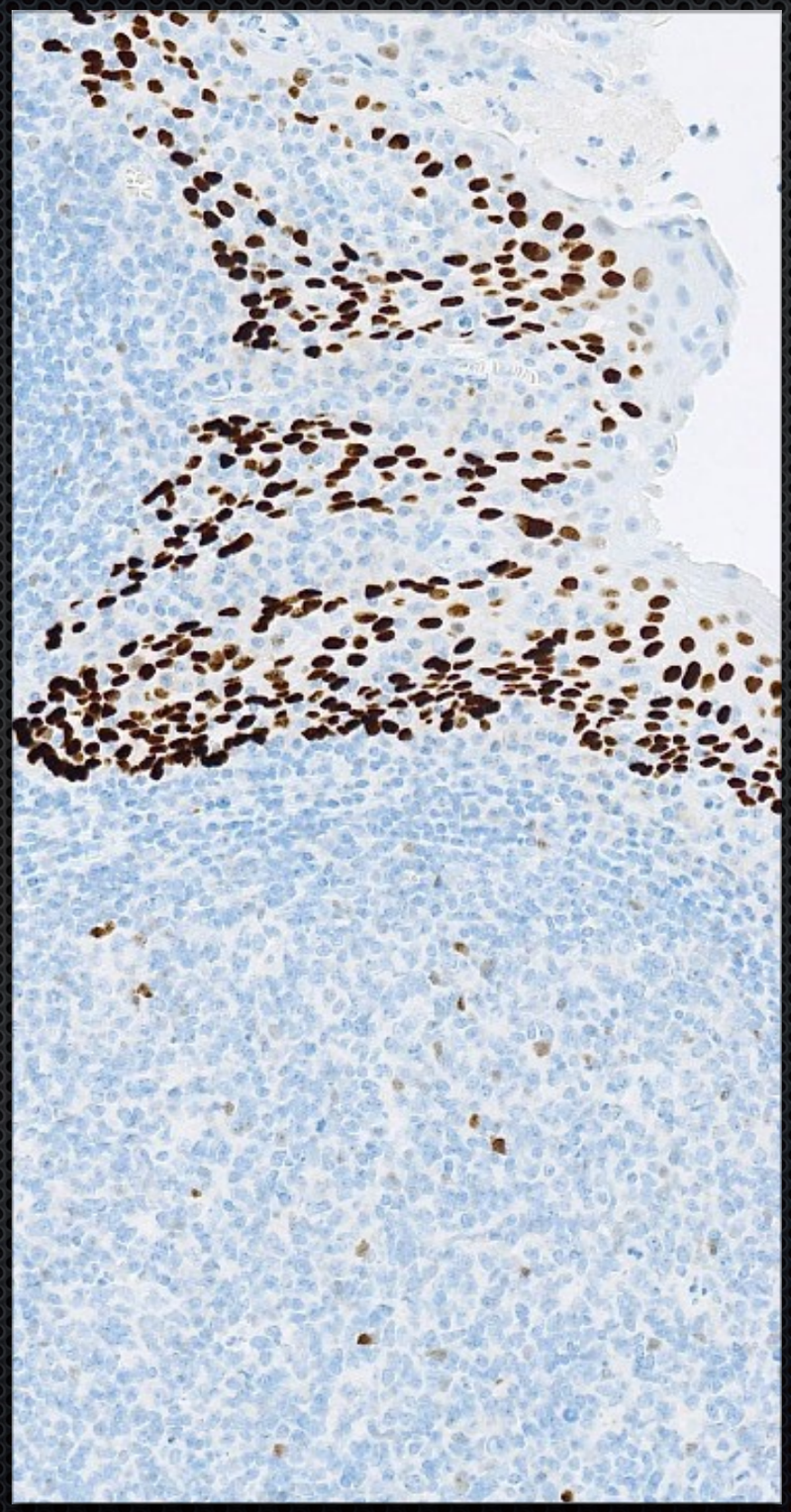
Lung tumours: Antibodies, protocols and controls

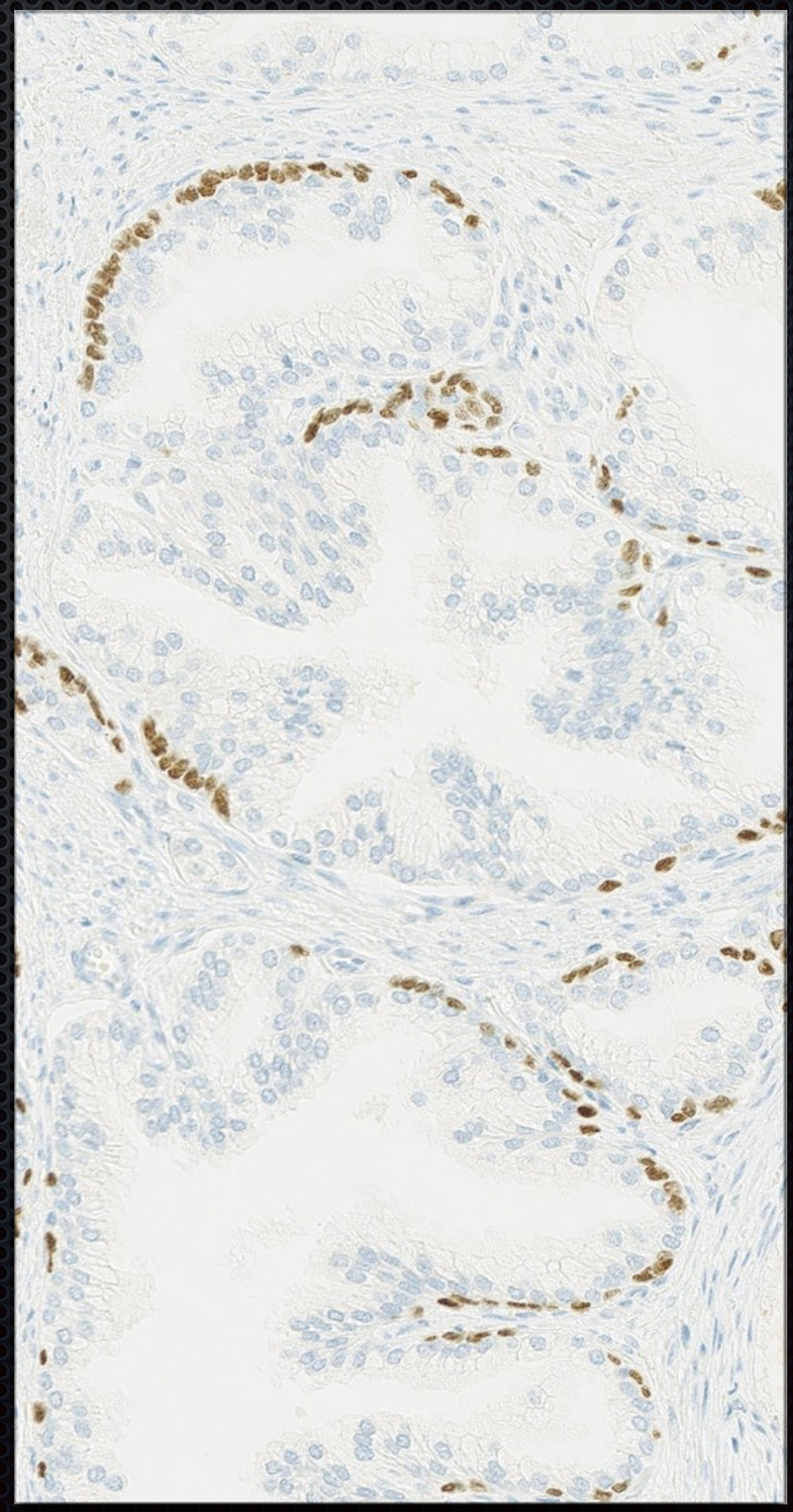
p63 / RUN 41 2014

Controls / iCAPC

Positive: Tonsil or prostate.

* Basal cells of prostate glands and squamous epithelial cells of tonsil must show a moderate to strong nuclear staining reaction.
* In the tonsil scattered lymphocytes must show a weak to moderate nuclear staining reaction.

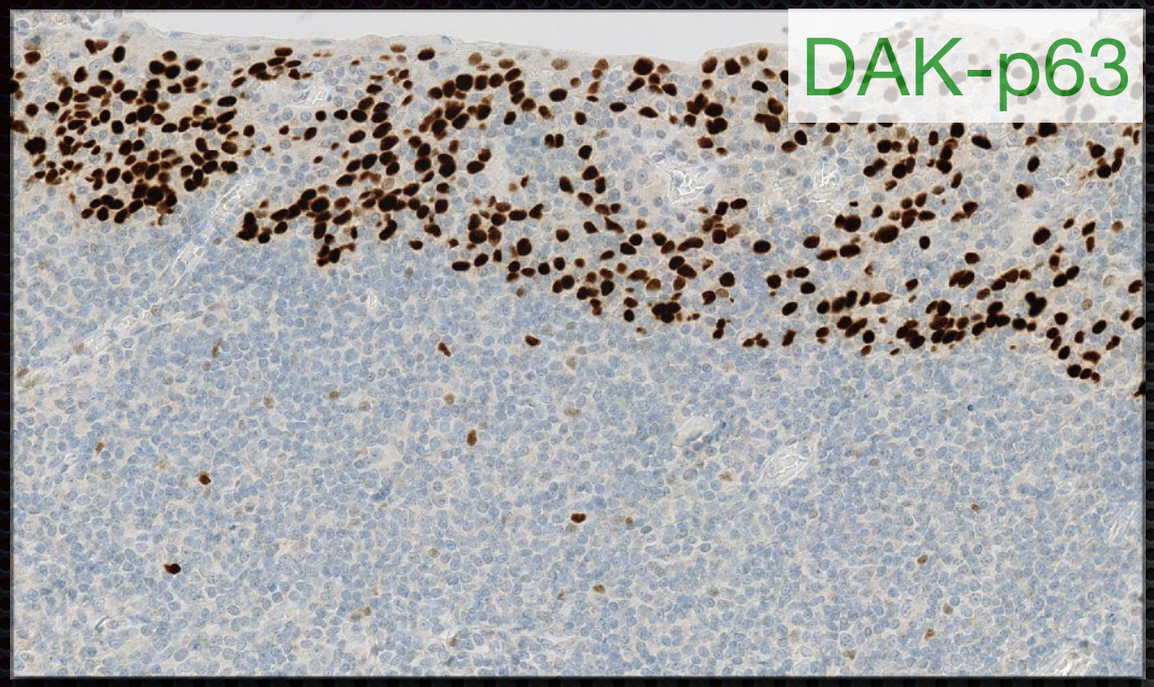


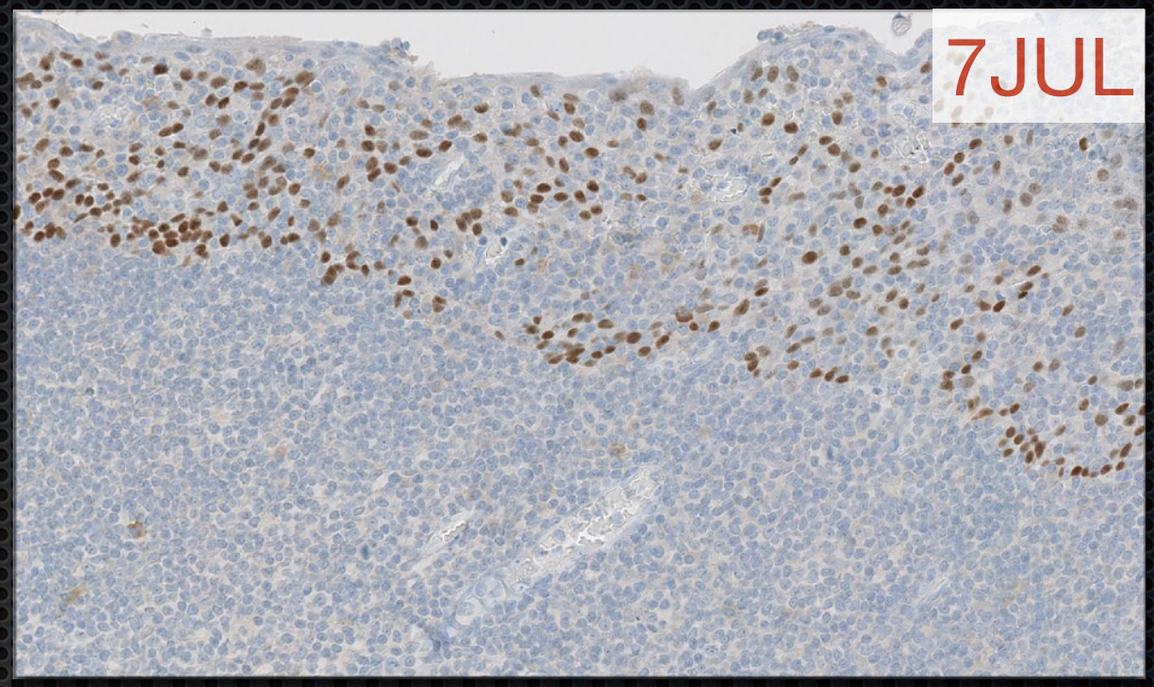


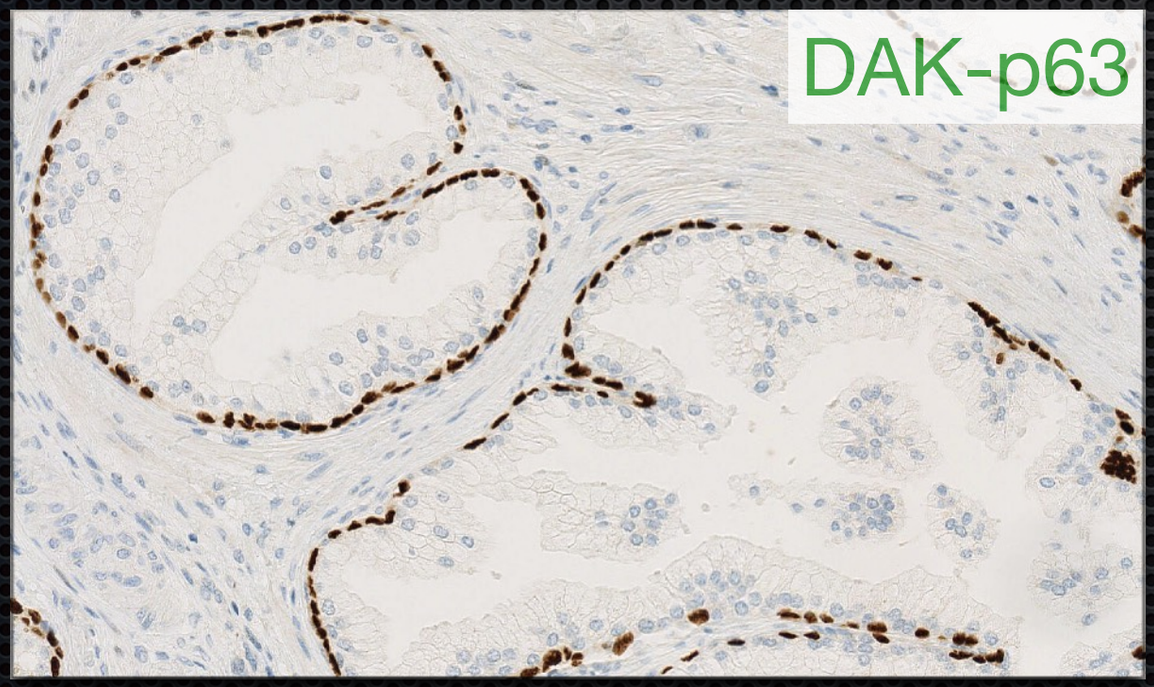
Lung tumours: Antibodies, protocols and controls

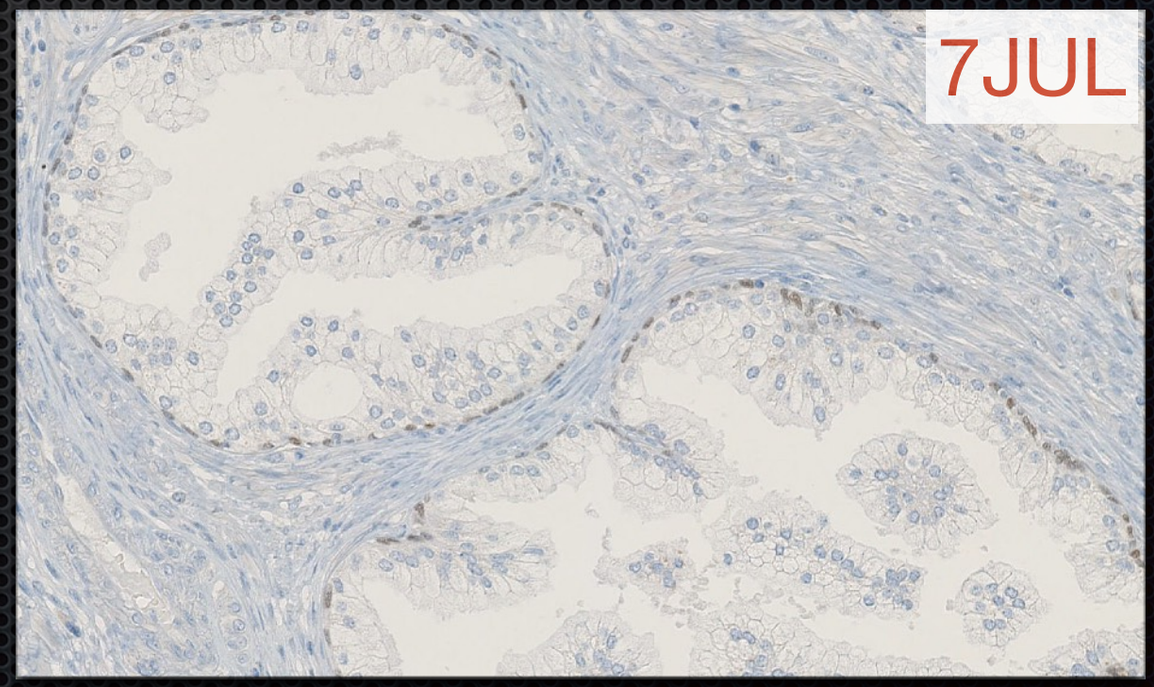
p63 / RUN 48 2016

NordiQC









mAb clone DAK-p63 optimally calibrated

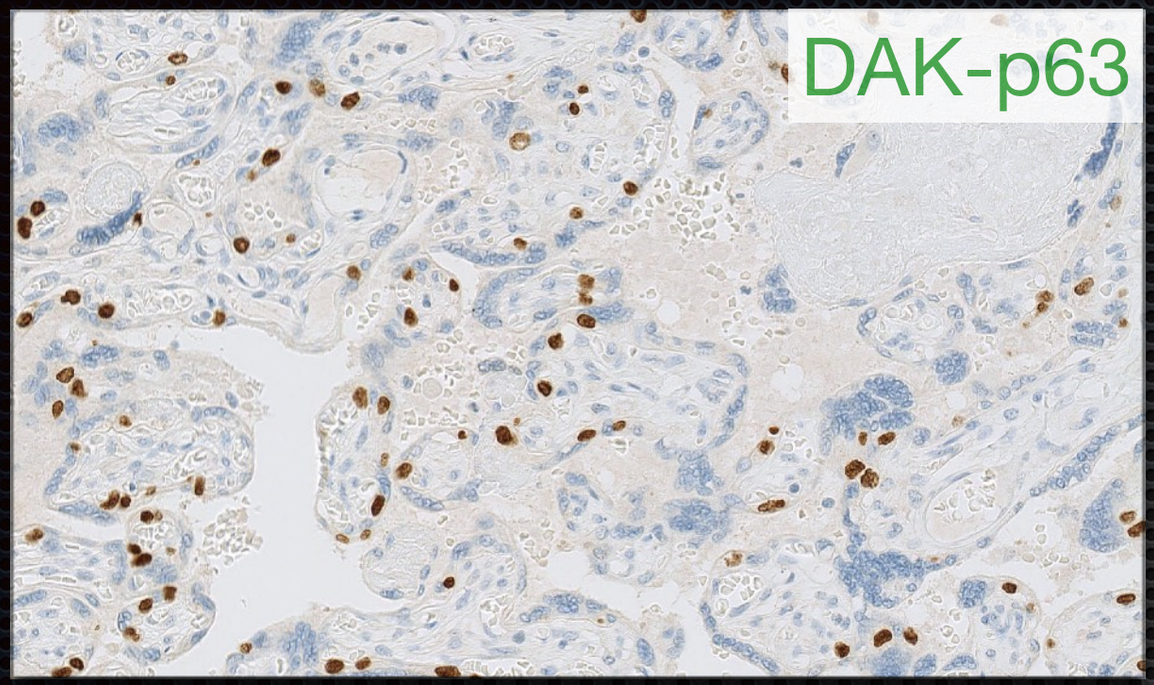
The use of a less successful primary Ab: 7JUL

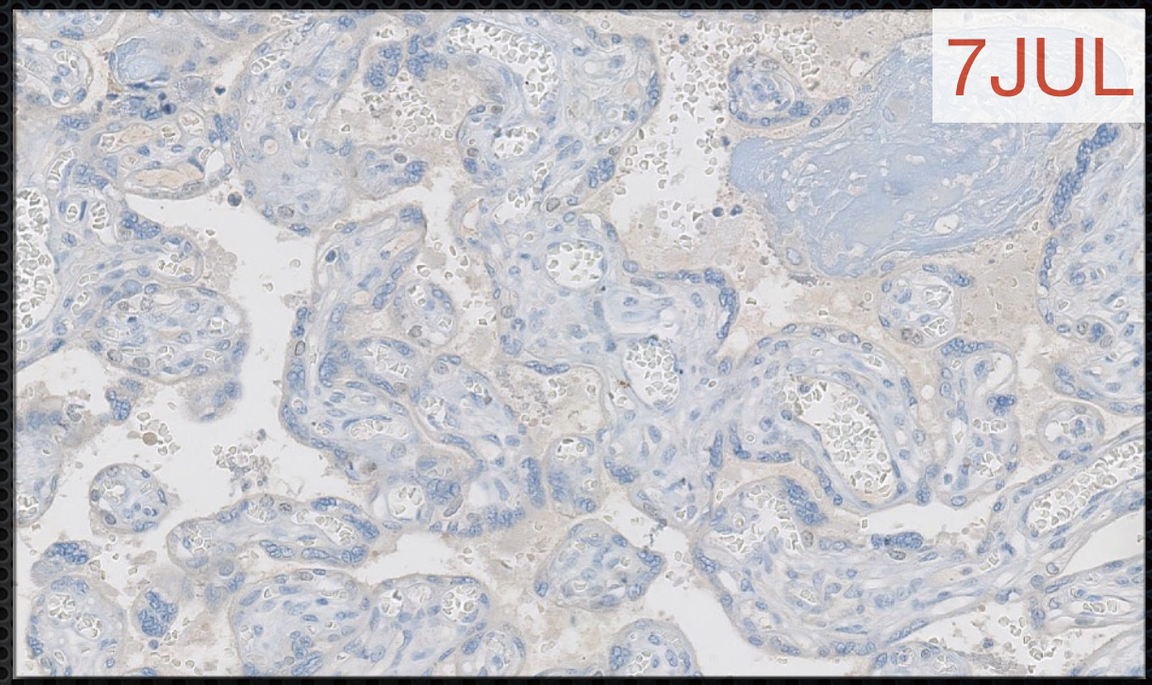
mAb clone **DAK-p63**	47	Dako	20	21	6	0	87%	91%
mAb clone **7JUL**	12	Leica/Novocastra	0	1	3	8	8%	-

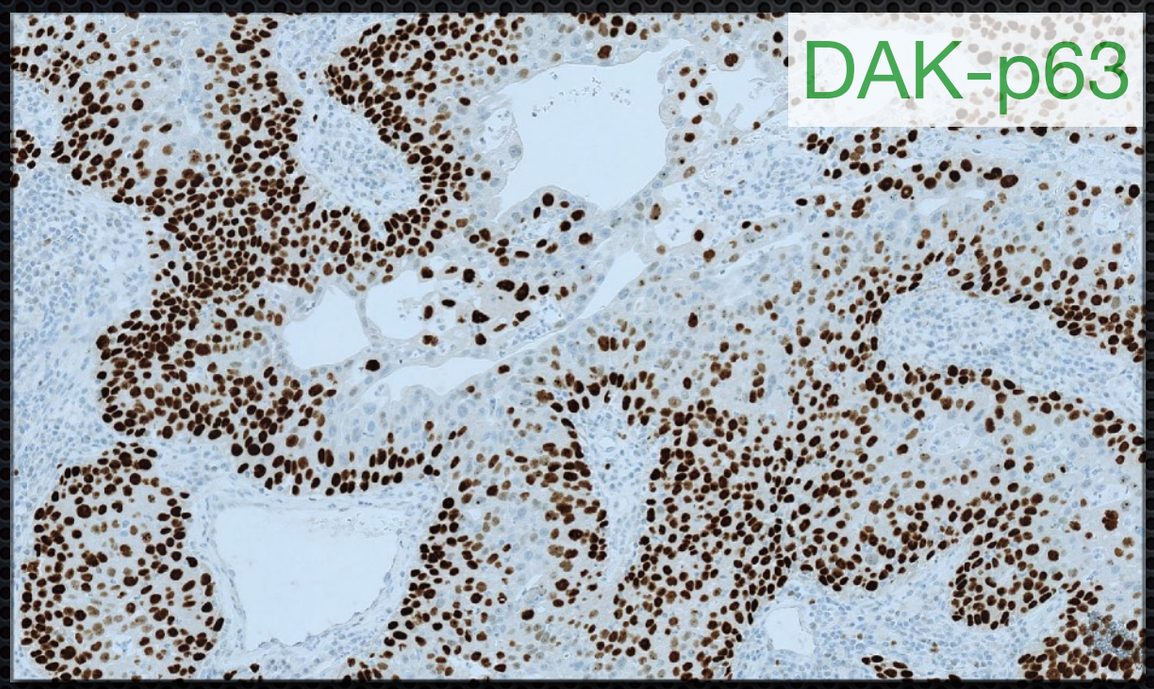
Lung tumours: Antibodies, protocols and controls

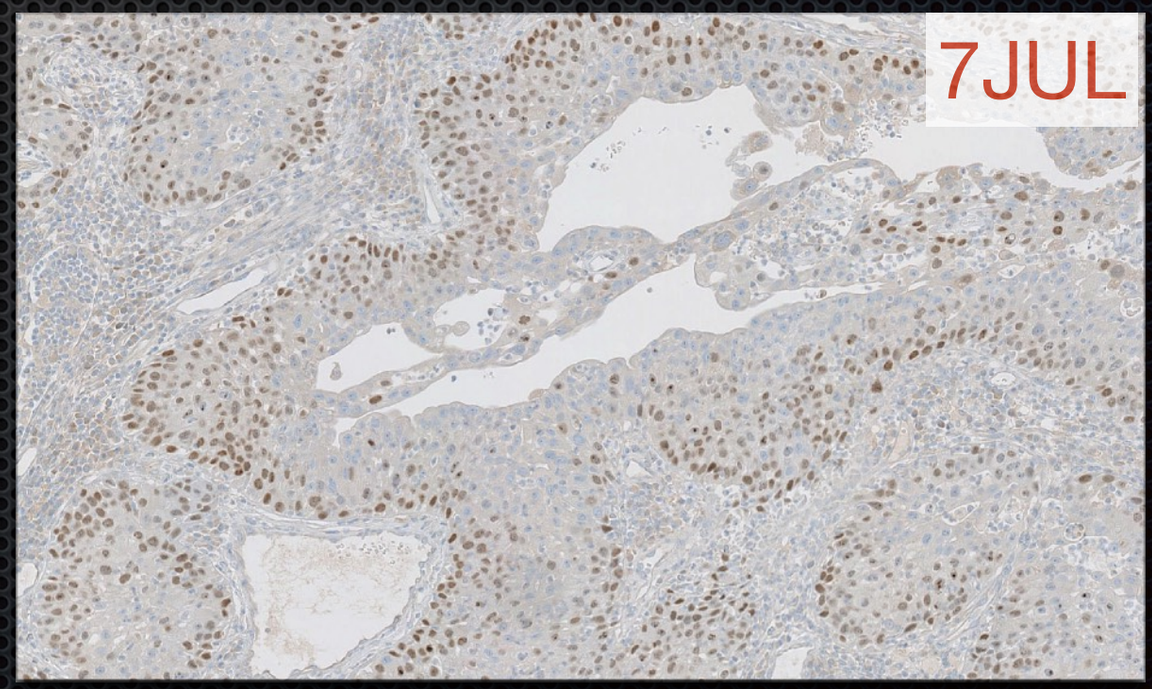
p63 / RUN 48 2016

NordiQC







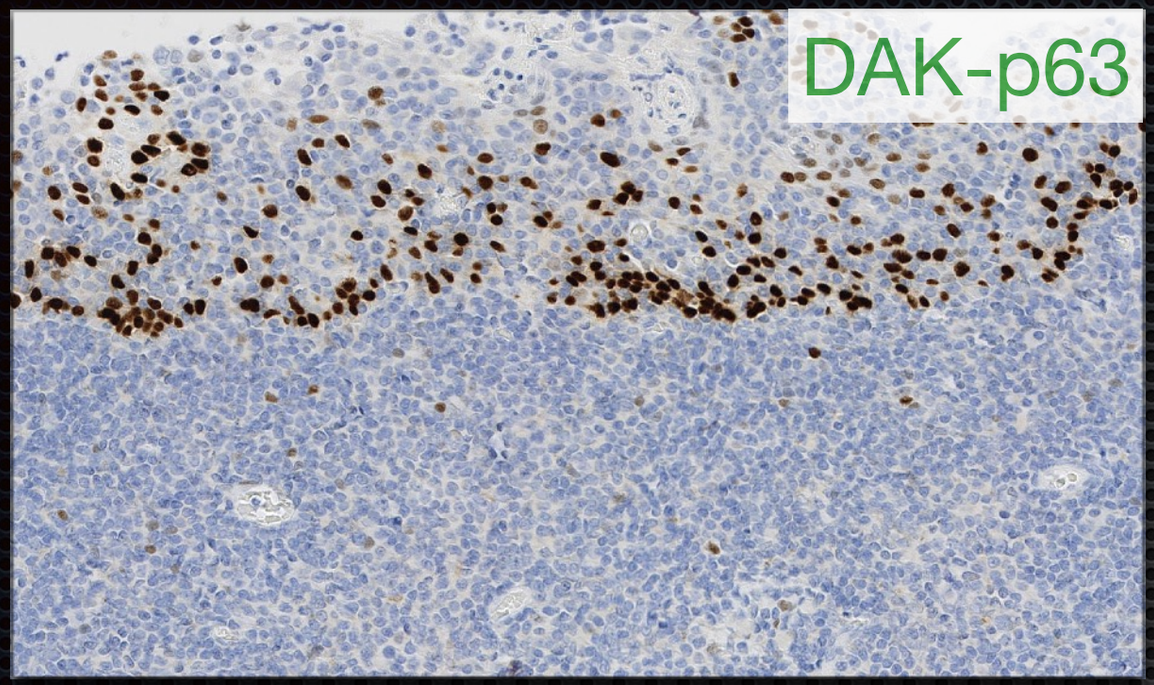


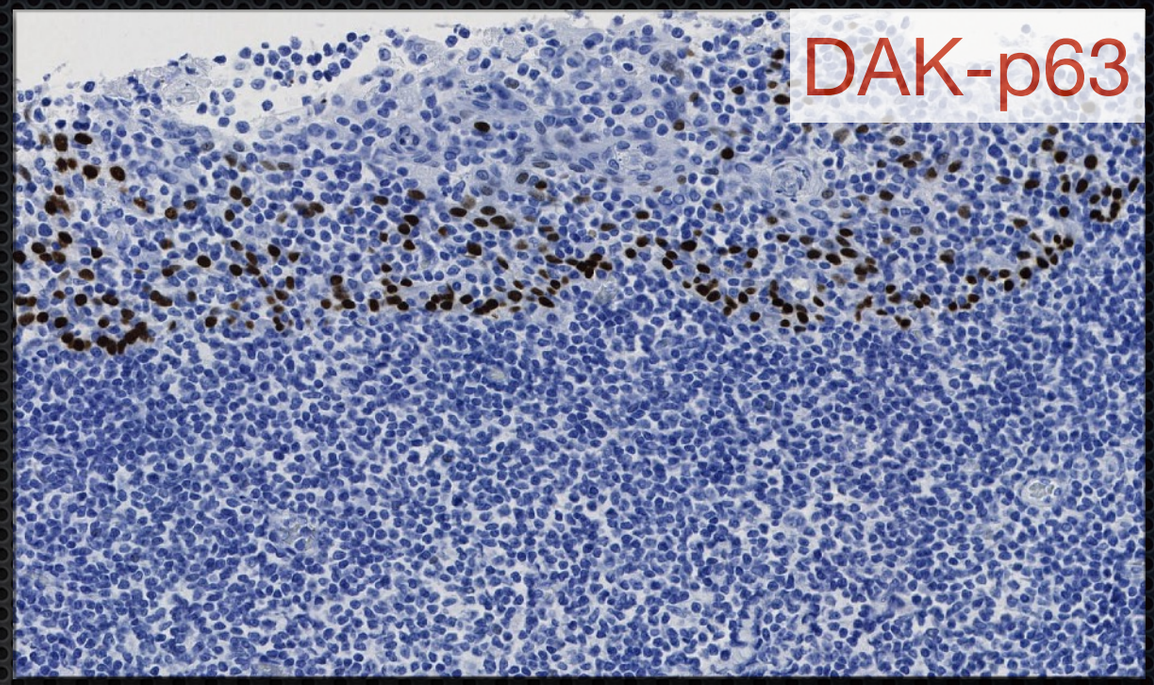
mAb clone DAK-p63 optimally calibrated

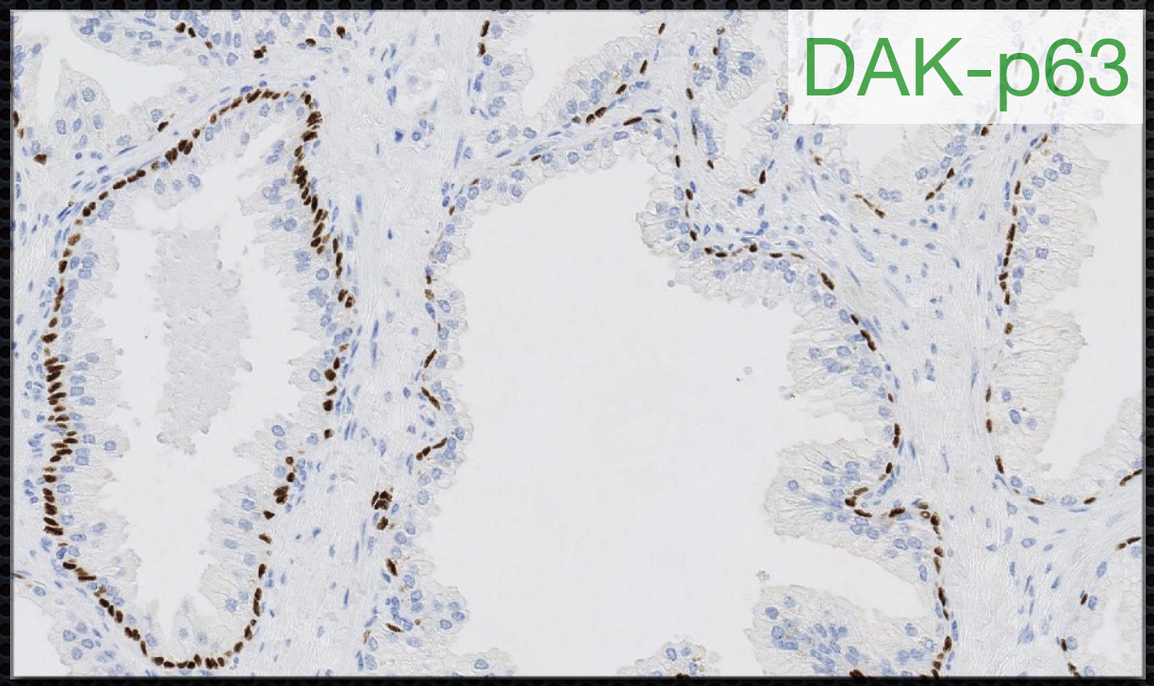
The use of a less successful primary Ab: 7JUL

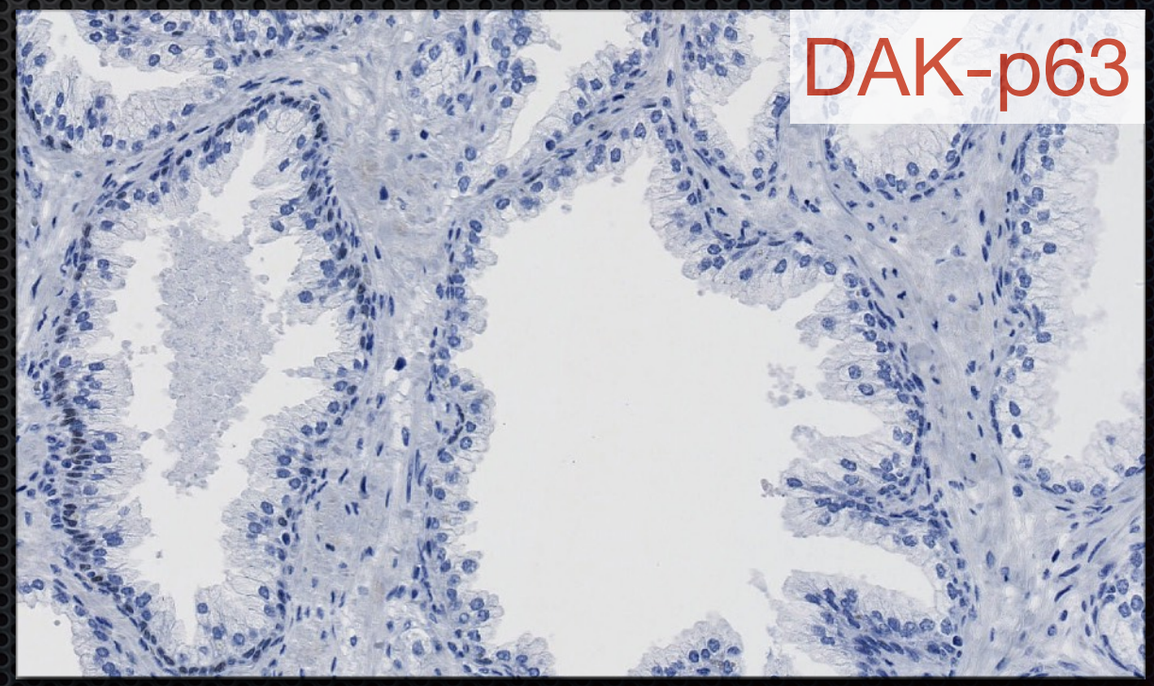
Lung tumours: Antibodies, protocols and controls

p63 / RUN 48 2016









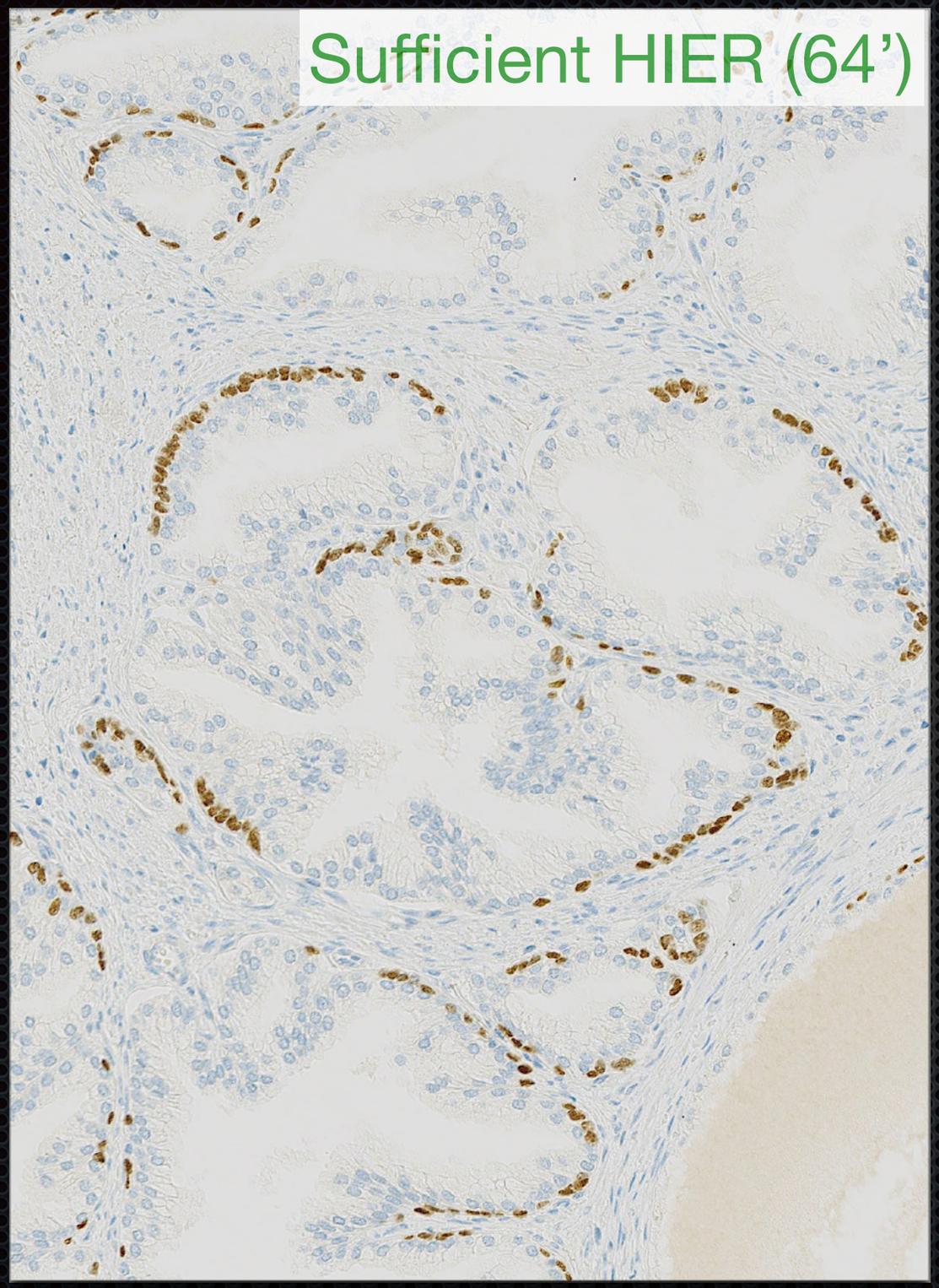
mAb clone DAK-p63 optimally calibrated in a sensitive 3-step polymer system.

Combination of the use of a less sensitive 2-step polymer based detection system and strong Hematoxylin counter stain

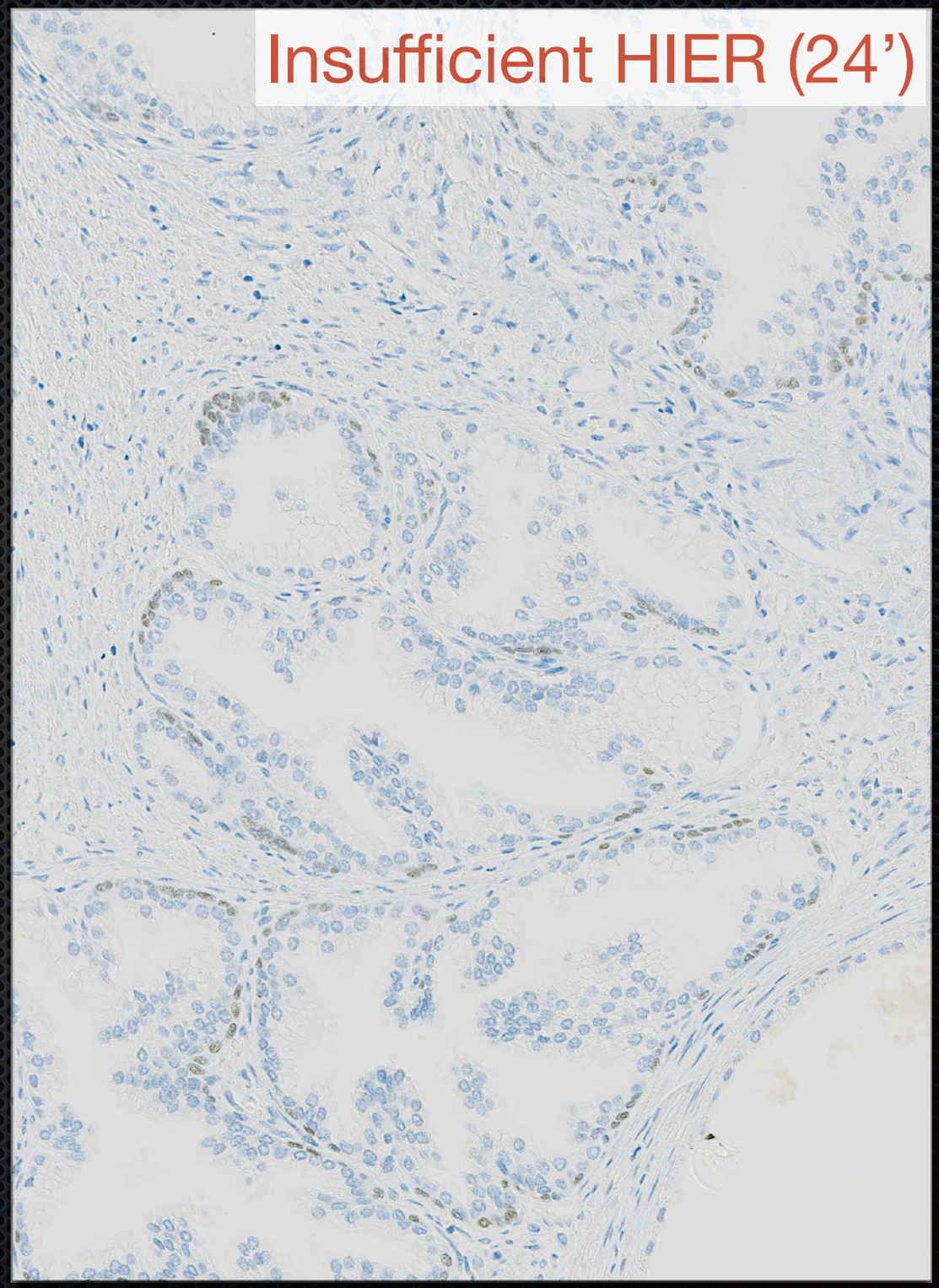
Lung tumours: Antibodies, protocols and controls

p63 / RUN 48 2016

Sufficient HIER (64’)



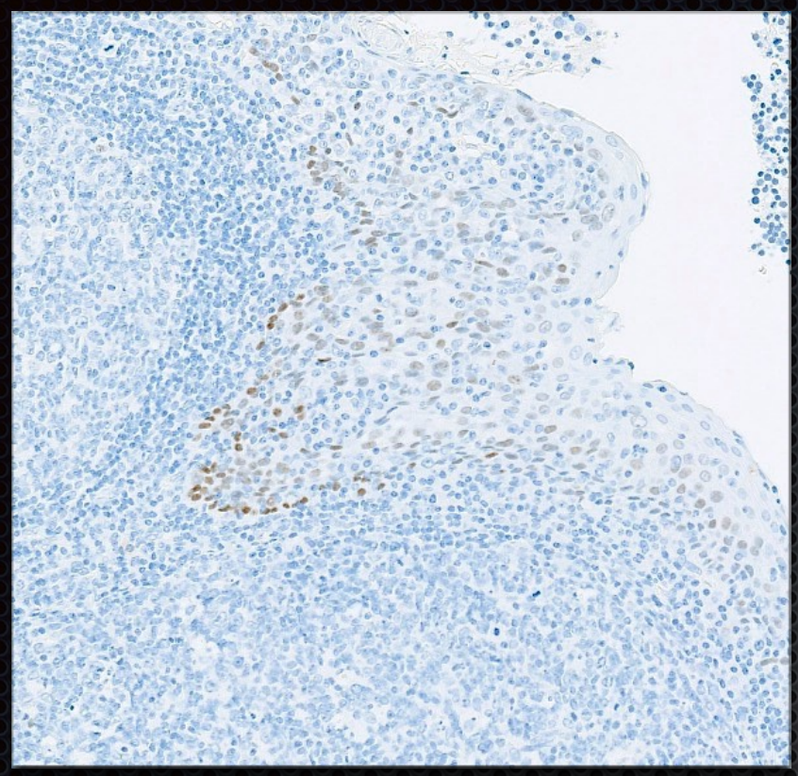
Insufficient HIER (24’)



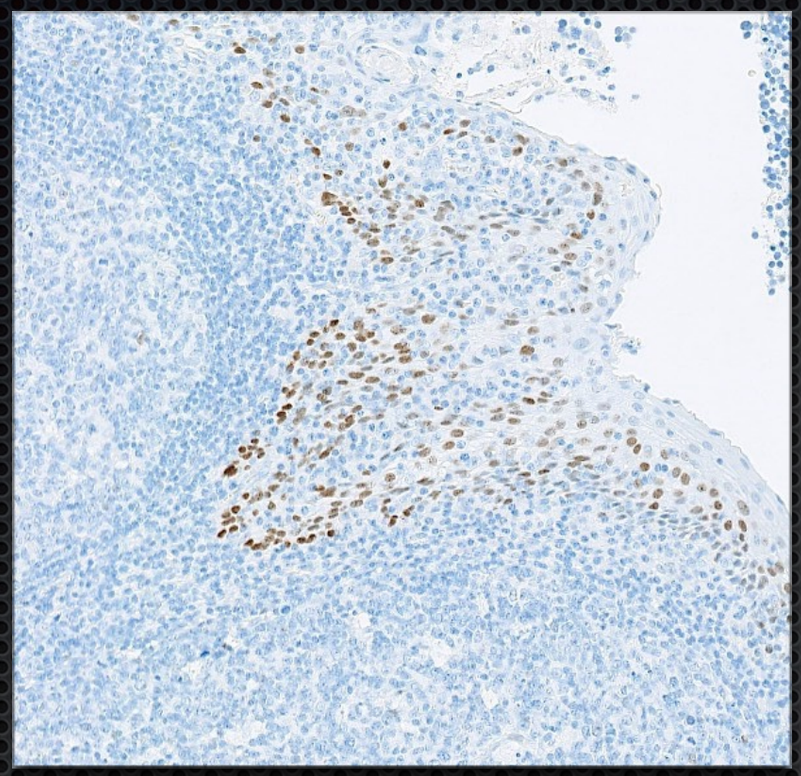
mAb clone 4A4 / CC1 64 min

mAb clone 4A4 / CC1 24 min

p63, 4A4 - OptiView (3-step) - Various HIER time



CC1_8_100°C



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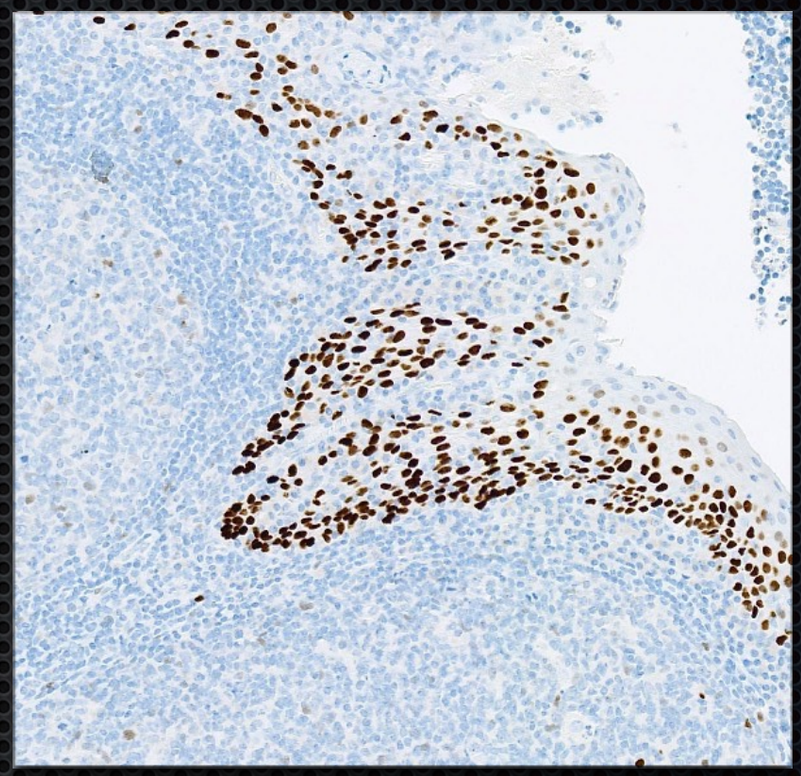
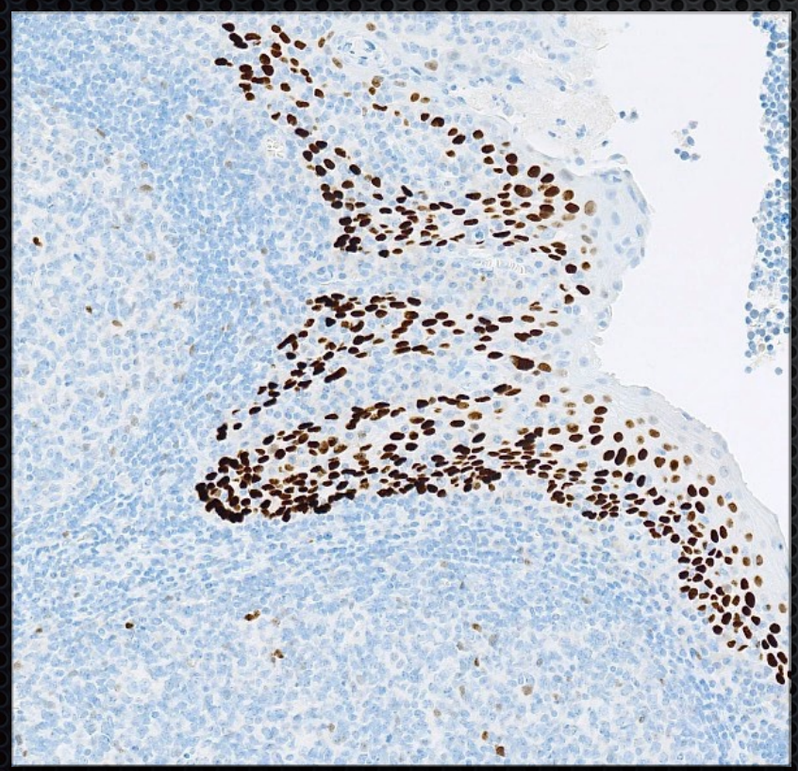
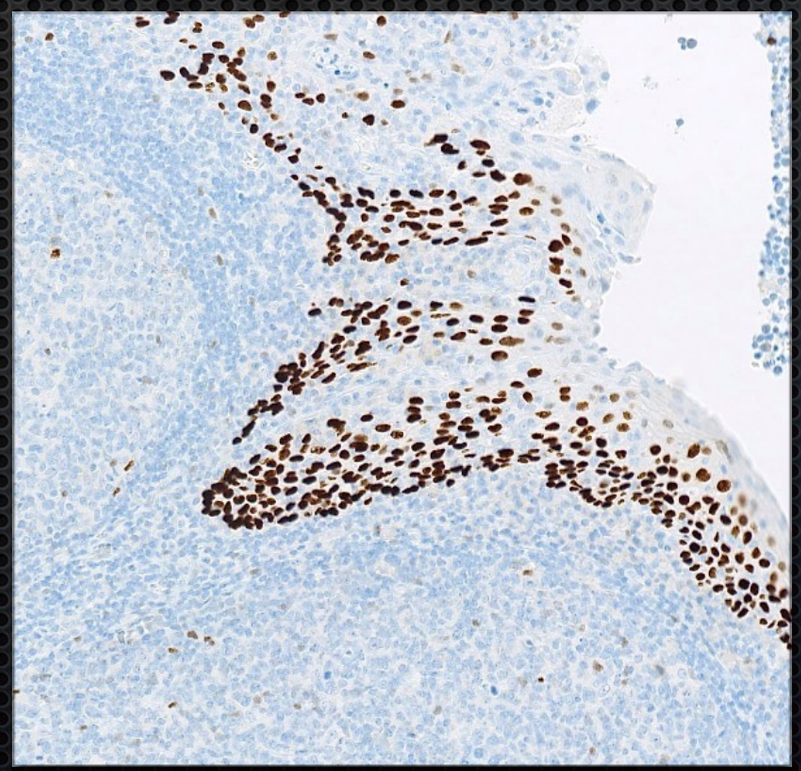


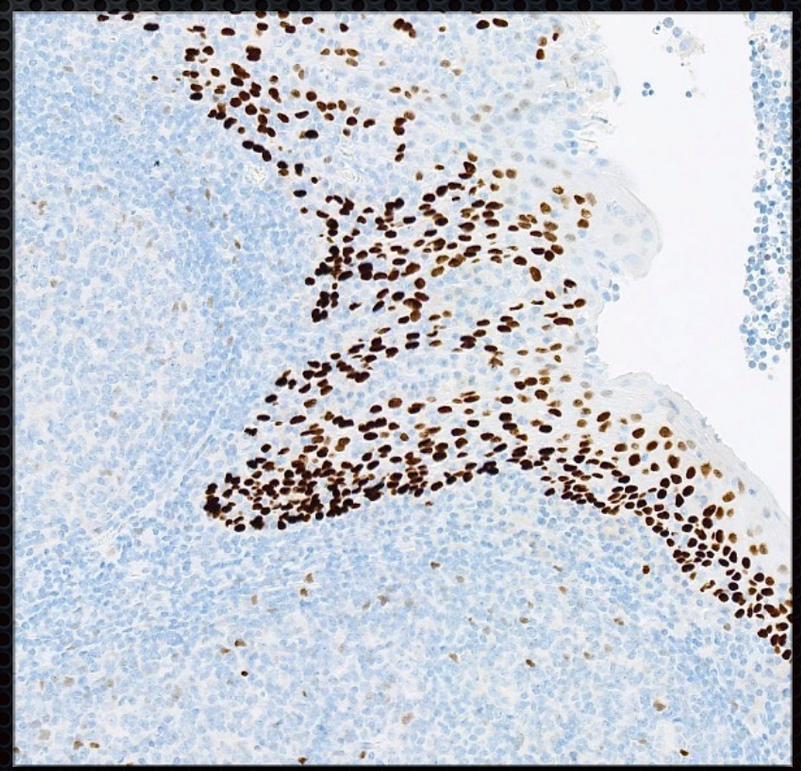
CC1_32_100°C



CC1_48_100°C

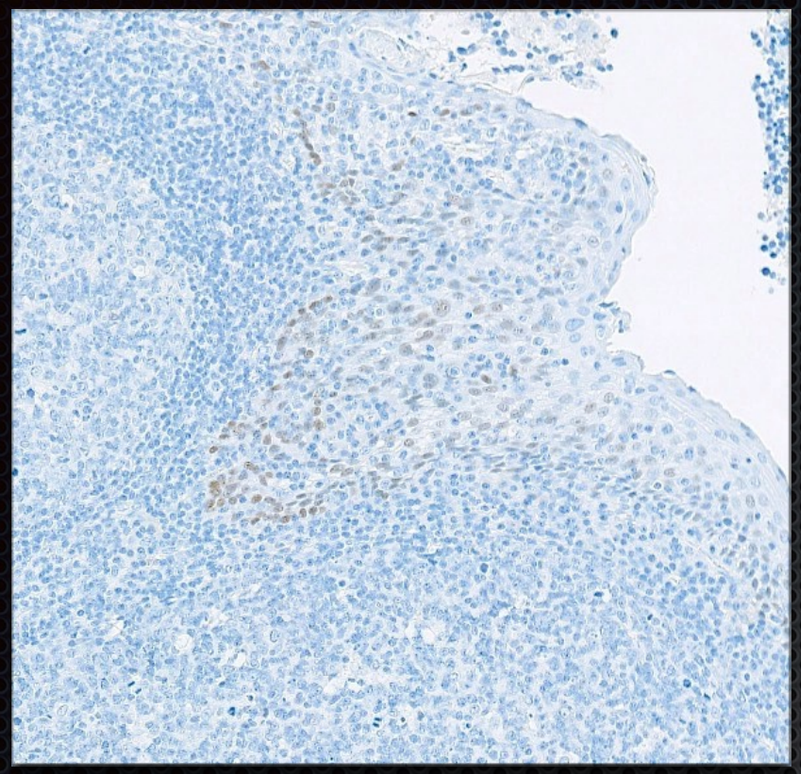


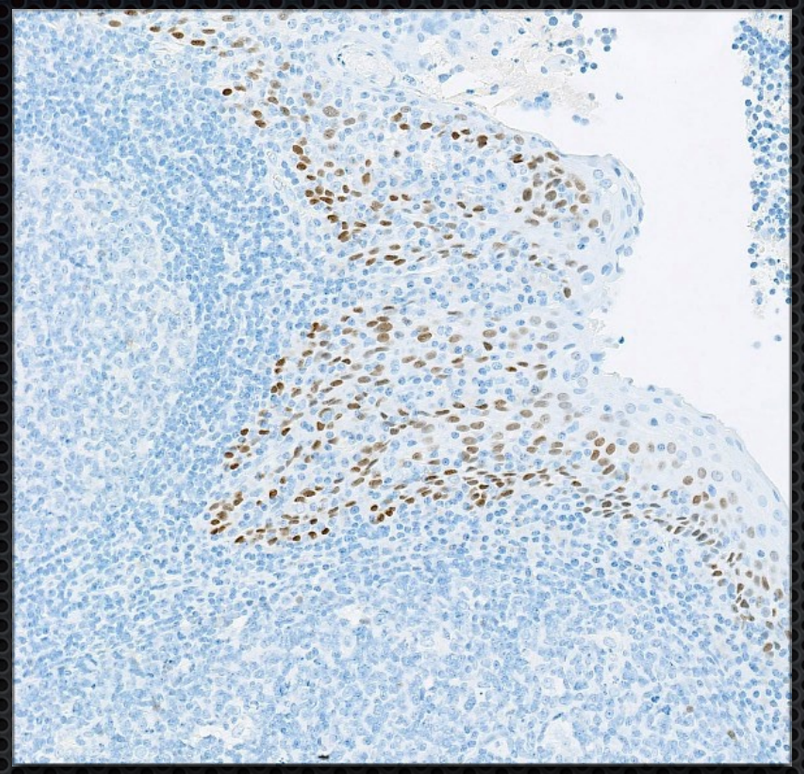
CC1_64_100°C

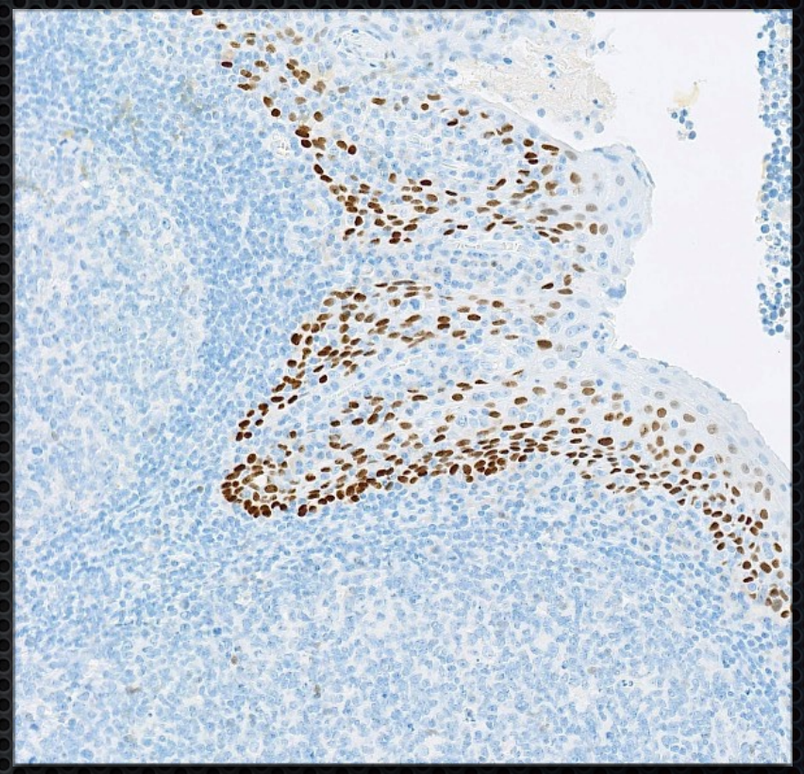


CC1_92_100°C

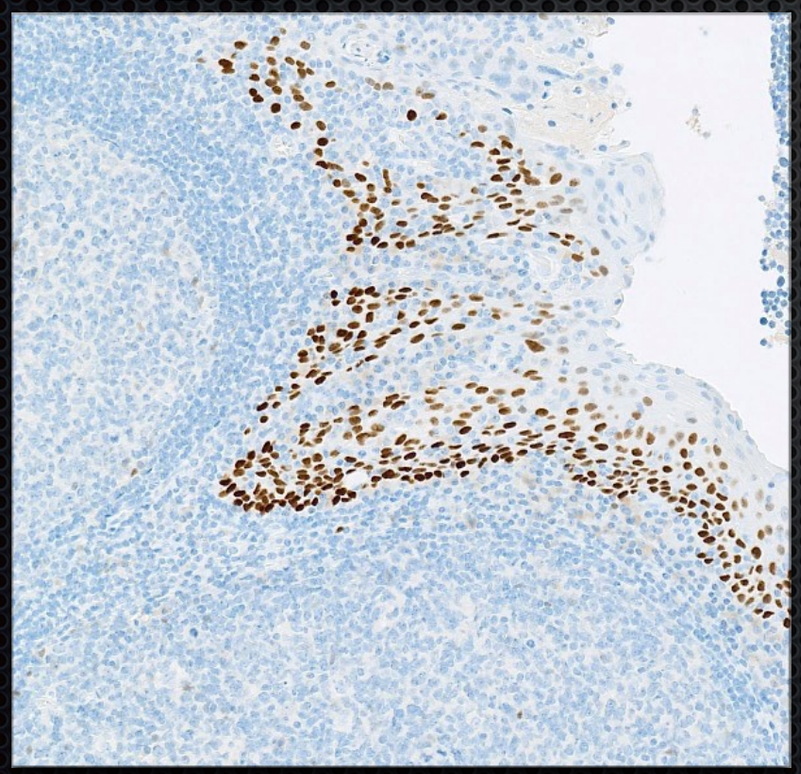
p63, 4A4 - UltraView (2-step) - Various HIER time



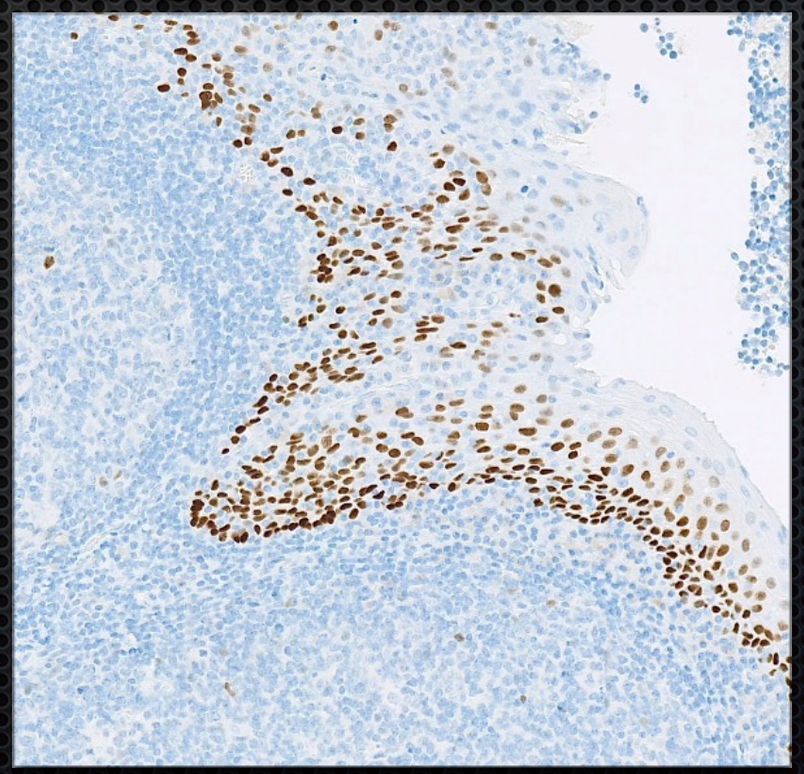




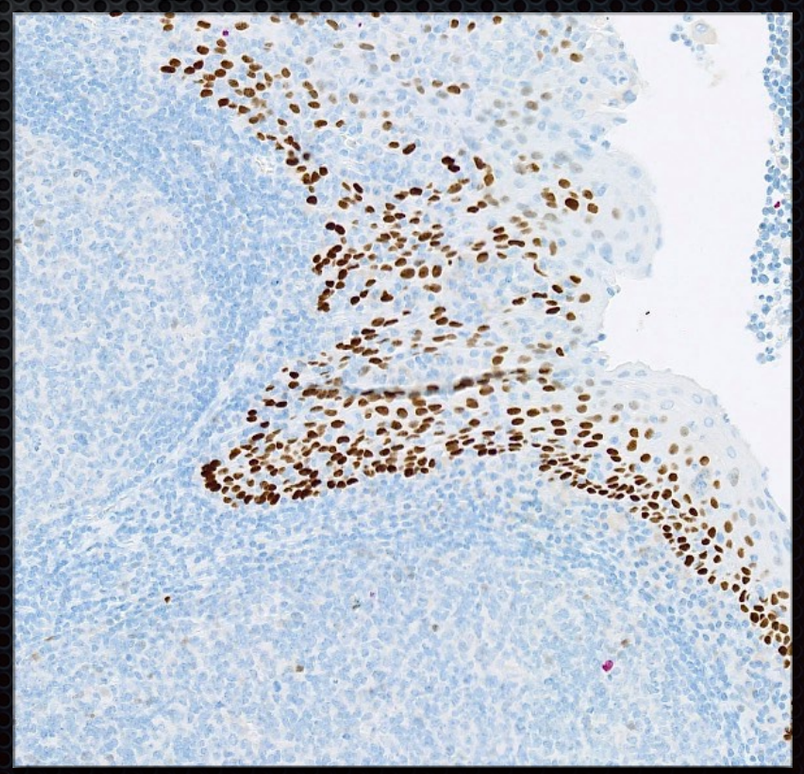
CC1_8_100°C



CC1_20_100°C



CC1_36_100°C

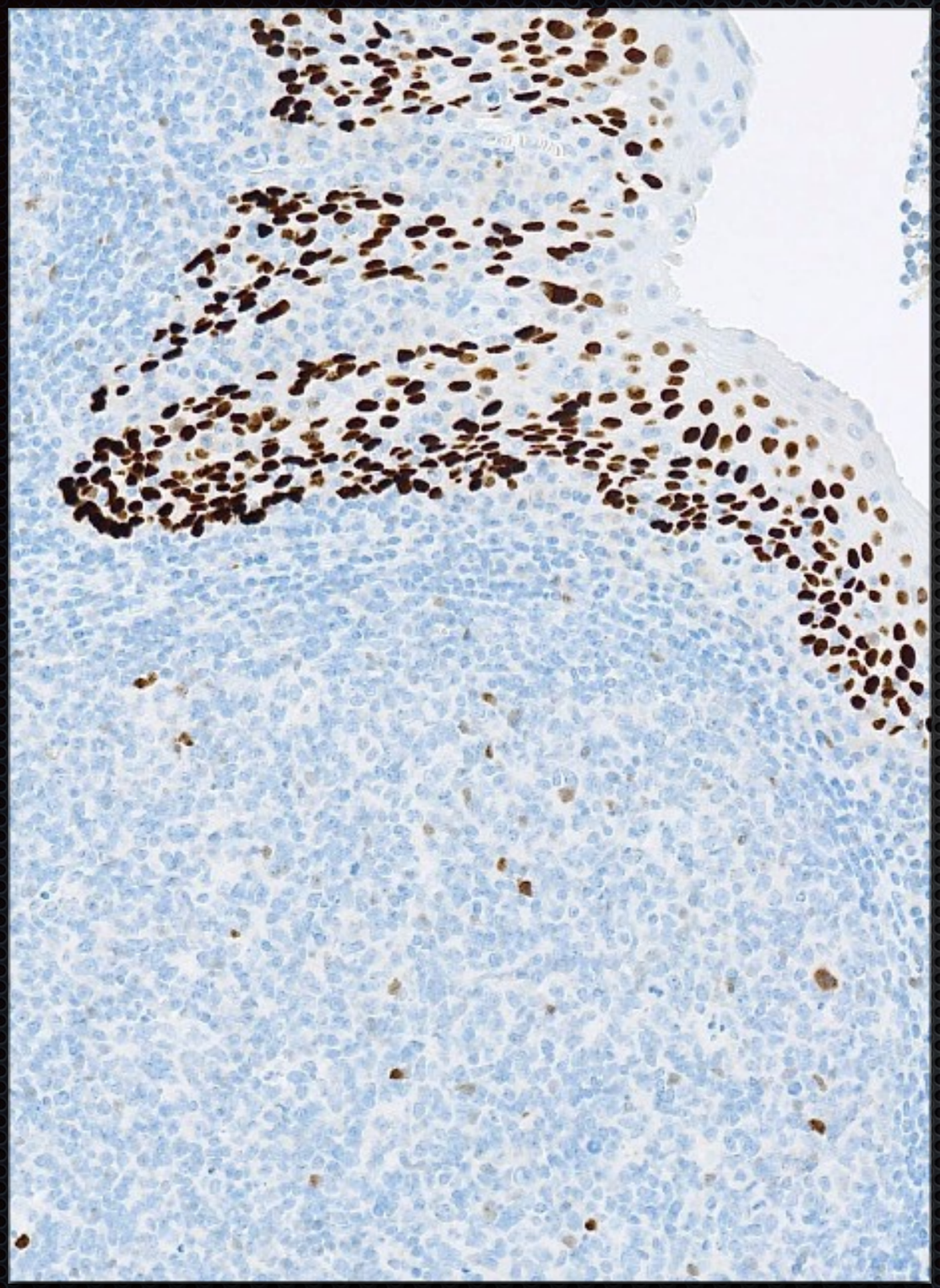


CC1_52_100°C

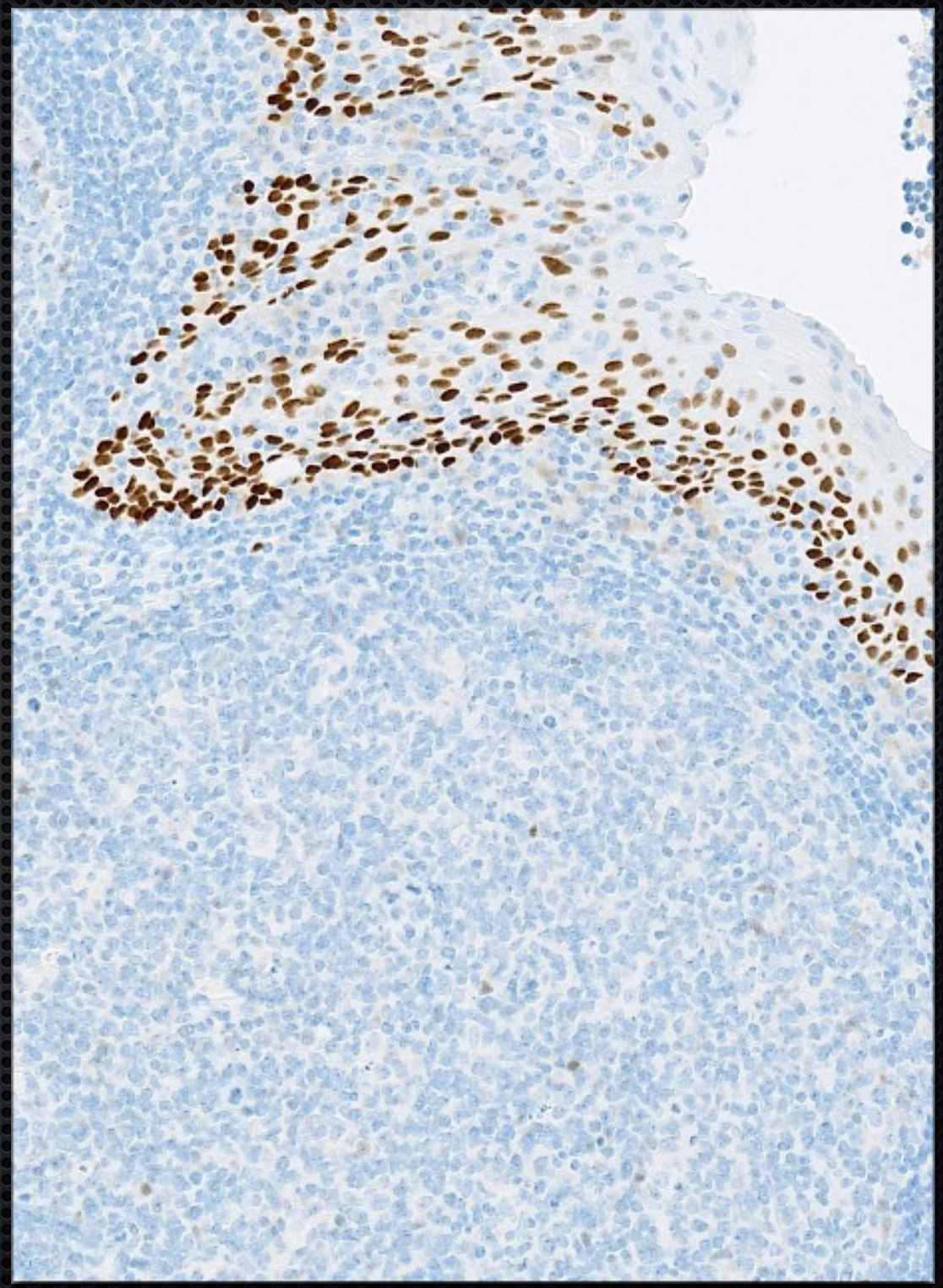
CC1_64_100°C

CC1_92_100°C

p63, 4A4 OptiView (3-step) vs UltraView (2-step)



OptiView - HIER CC1_48_100



UltraView - HIER CC1_52_100

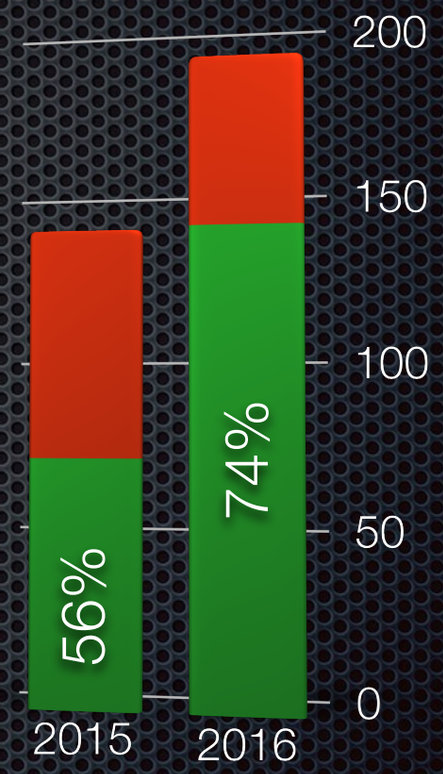
Lung tumours: Antibodies, protocols and controls

p40 / RUN 48 2016

Pass: 74 %

Table 1. **Antibodies and assessment marks for p40, run 48**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BC28**	77 6 2 2 1	Biocare Zytomed Menarini abcam Nordic Biosite	52	24	10	2	86%	89%
rmAb clone **ZR8**	12 1 1	Immunologic Zeta Corporation BioSB	1	6	2	5	50%	67%
pAb **AC13030**	8	Biocare	0	2	6	0	-	-
pAb **RP163**	5	Diagnostic Biosystems	0	1	1	3	-	-
pAb **PC373**	4	Calbiochem, Merck	0	1	0	3	-	-
pAb **RBK054**	3	Zytomed	0	0	1	2	-	-
pAb **PI049**	1	DCS	0	1	0	0	-	-
pAb **PP123**	1	Pathnsitu	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **BC28 API/IPI/AVI 3066**	13	Biocare	5	8	0	0	100%	100%
mAb clone **BC28 790-4950**	39	Ventana	19	15	5	0	87%	94%
mAb clone **BC28 MSG097**	1	Zytomed	1	0	0	0	-	-
mAb clone **ZR8 MAD-000686QD**	3	Master Diagnostica	0	2	1	0	-	-
pAb **API 3030**	6	Biocare	0	0	4	2	-	-
pAb **RAB-066**	1	Maixin	0	1	0	0	-	-
pAb **A00112**	1	Loxo GmbH	0	0	1	0	-	-
Total	188		78	61	32	17	-	
Proportion			42%	32%	17%	9%	74%	



Lung tumours: Antibodies, protocols and controls

p40 / RUN 48 2016

Recommend-able clones	Retrieval	Titre	Detection	RTU	Detection
mAb BC28	HIER, High pH	1:20 - 1:100	3-step	Ventana	3-step
mAb ZR8	HIER, High pH	1:200	3-step + amp	-	-

Table 3. **Proportion of optimal results for p40 for the BC28 antibody as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako OMNIS**		**Ventana BenchMark GX / XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	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 **BC28**	7/20** (35%)	1/1	6/8 (75%)	1/1	30/42 (71%)	-	3/6 (50%)	0/1

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

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

Lung tumours: Antibodies, protocols and controls

p40 / RUN 44 2015

Controls / iCAPC

Positive: Placenta (LLOD)

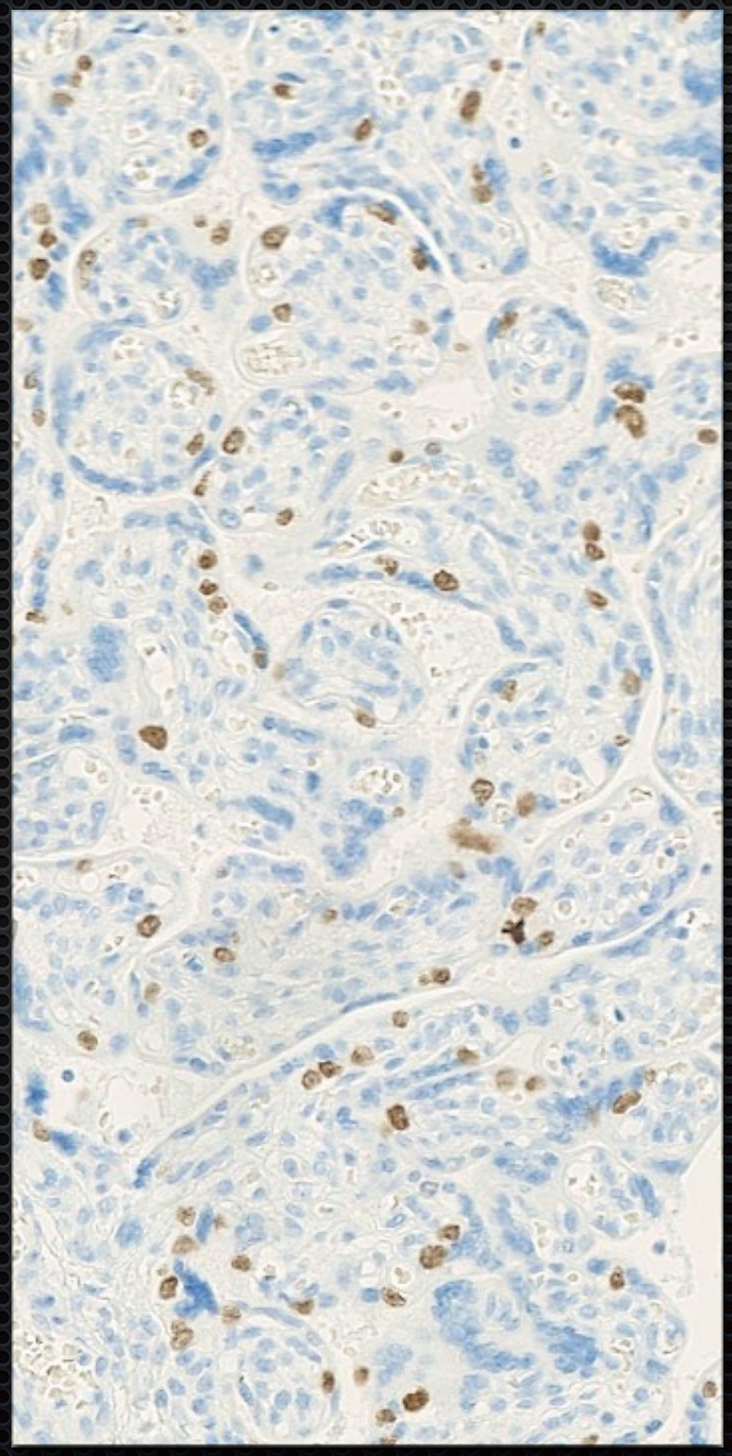
* Cytotrophoblasts must show an at least weak to moderate, distinct nuclear staining reaction.

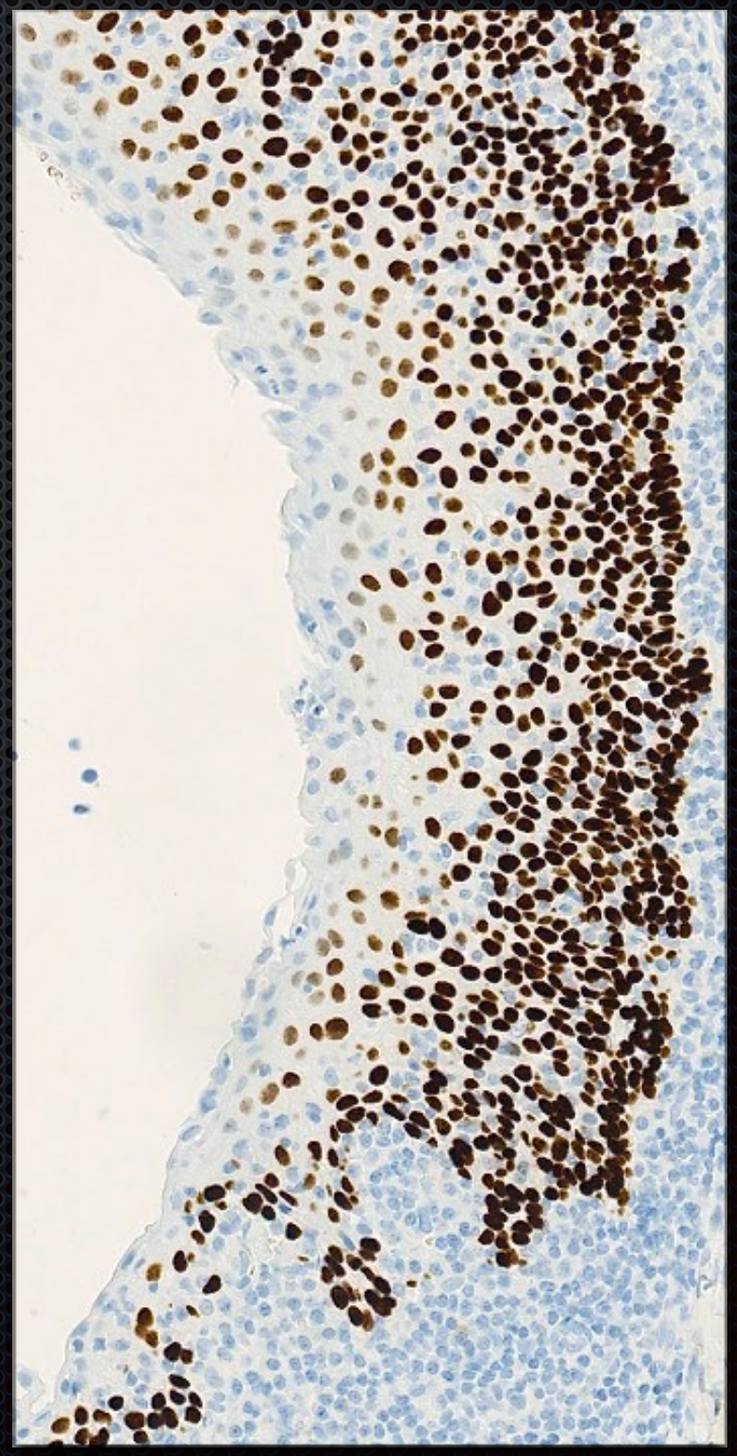
Positive: Tonsil

* Virtually all squamous epithelial cells must show a moderate to strong, distinct nuclear staining reaction.

Negative: Tonsil

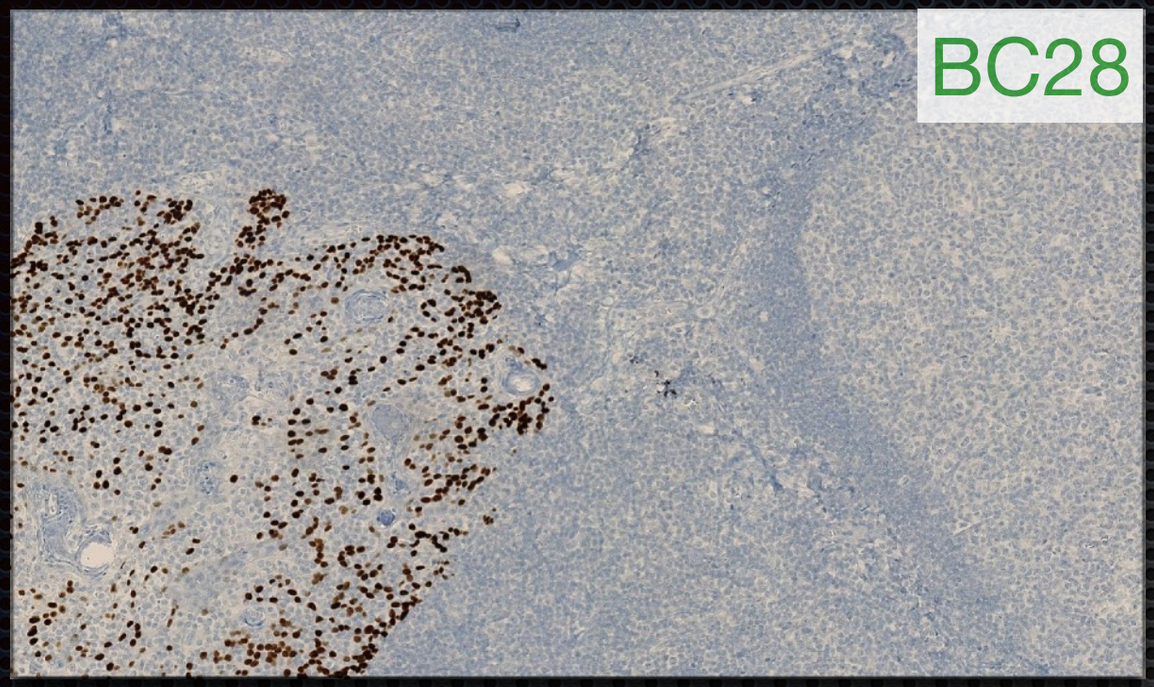
* Lymphocyttes must be negative.

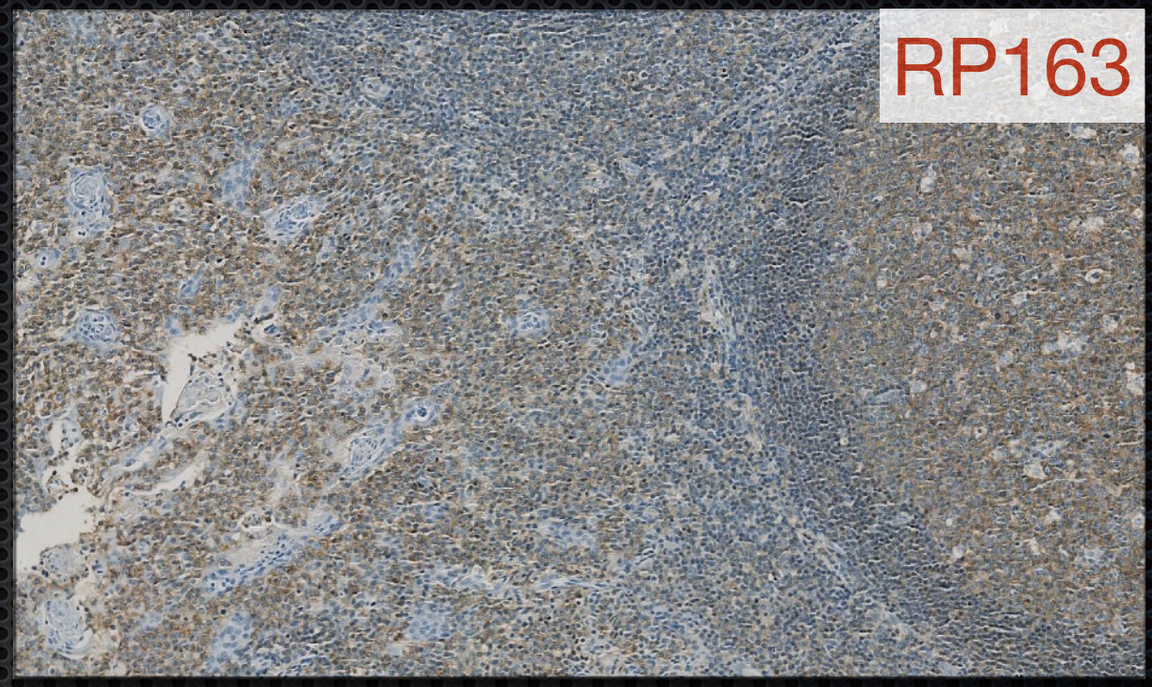


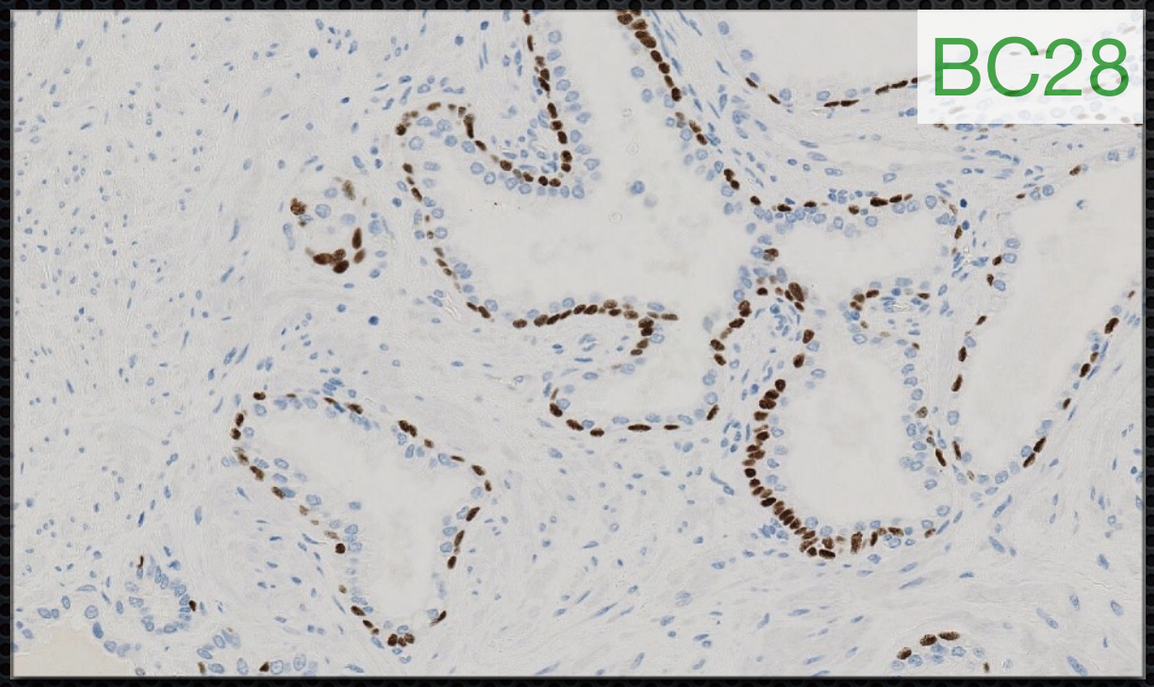


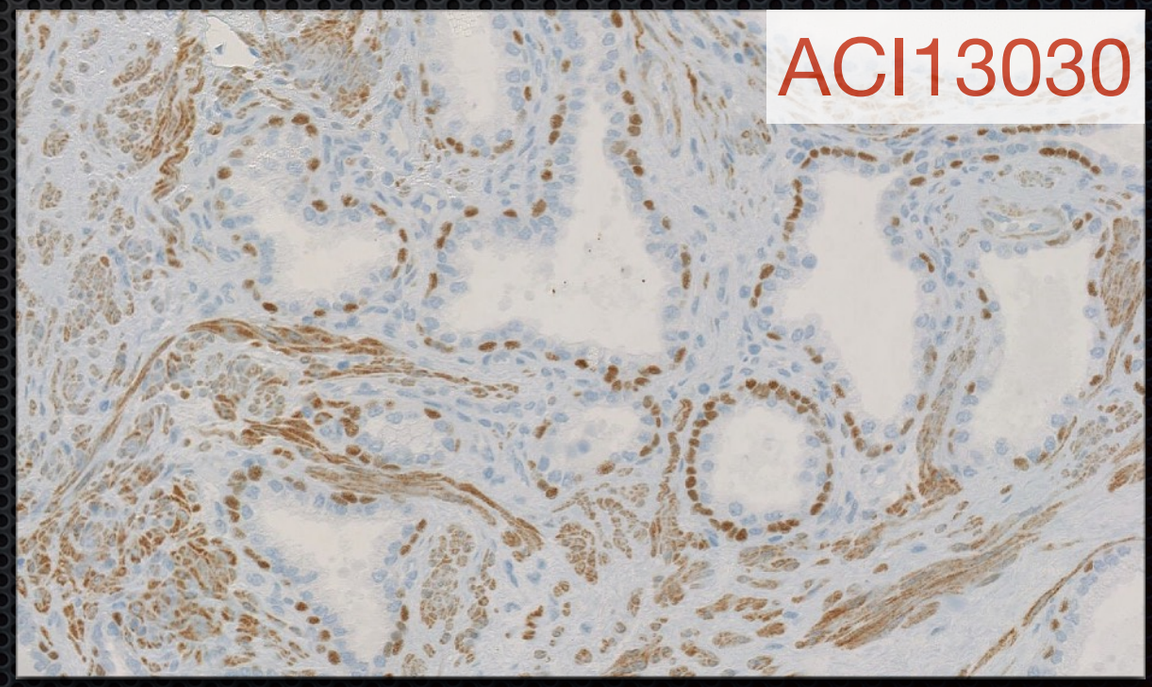
Lung tumours: Antibodies, protocols and controls

p40 / RUN 48 2016







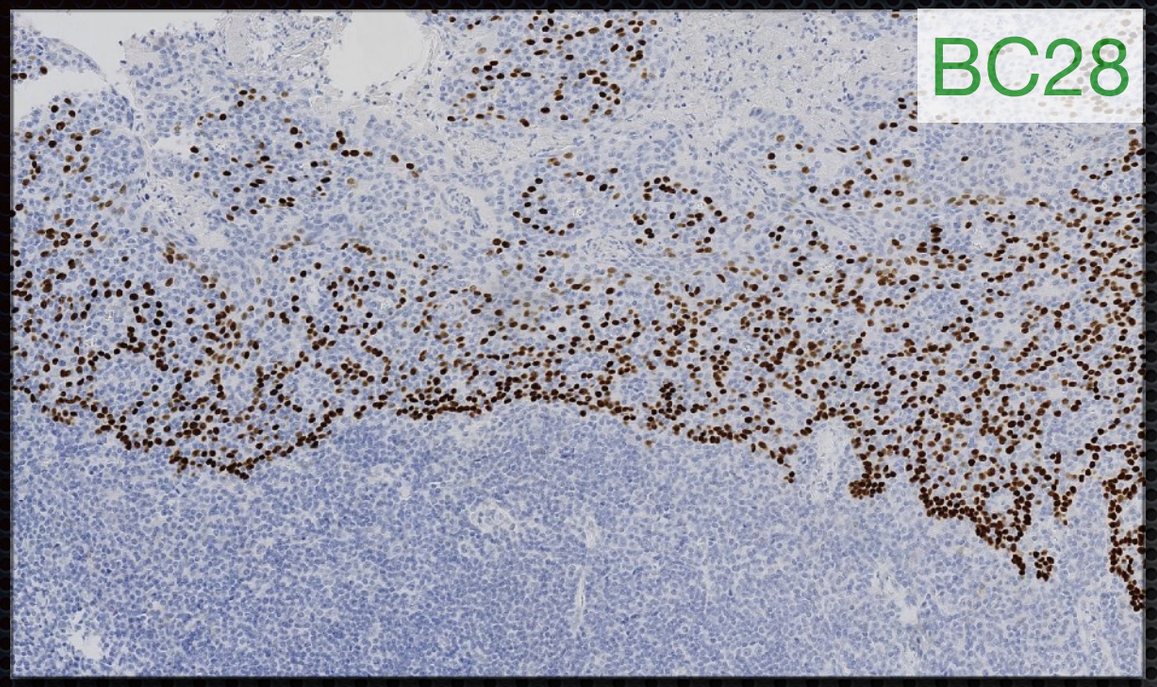


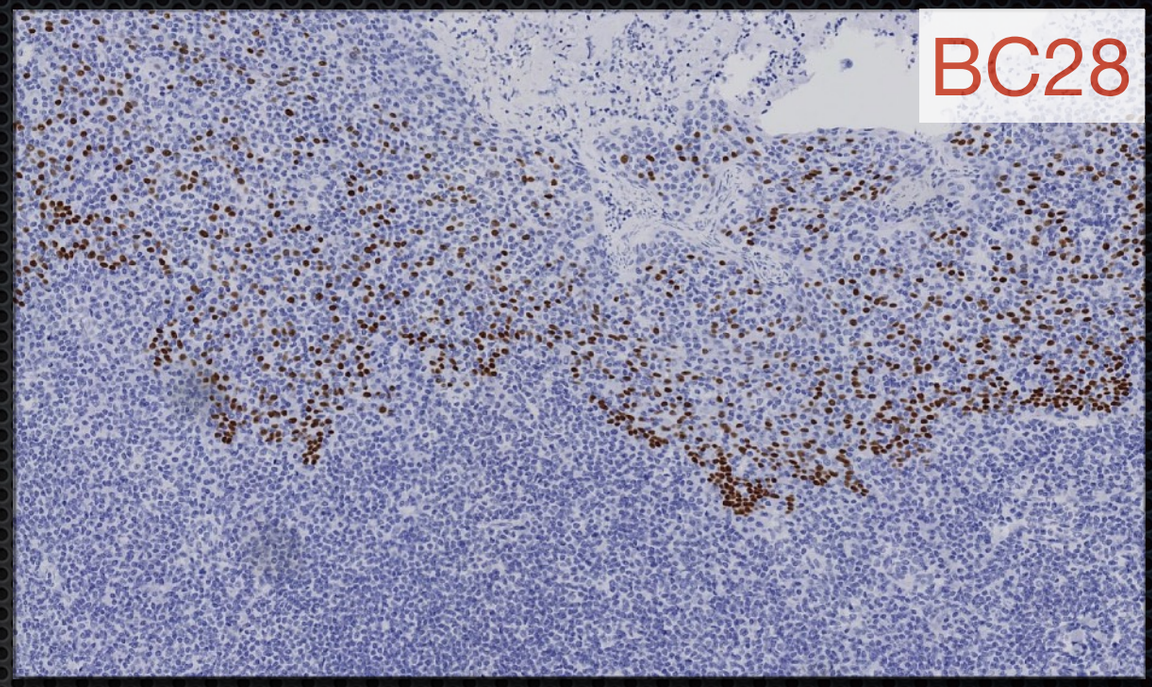
mAb clone BC28 optimally calibrated in a sensitive 3-step polymer system.

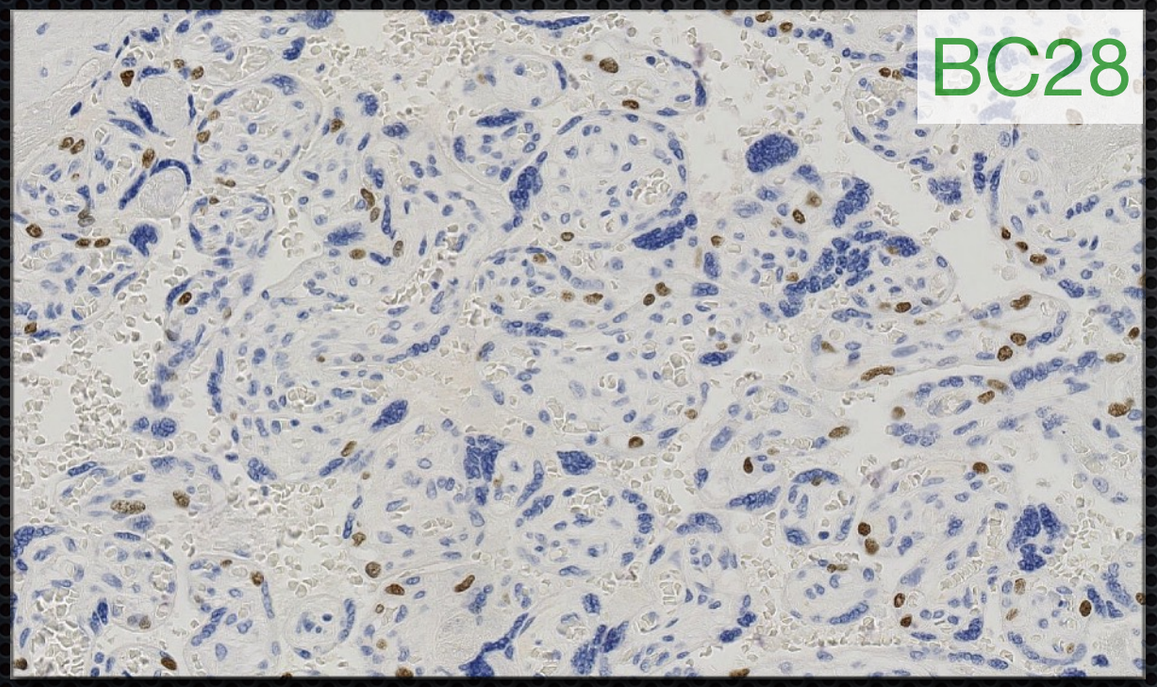
Poor signal to noise ratio using various pAb

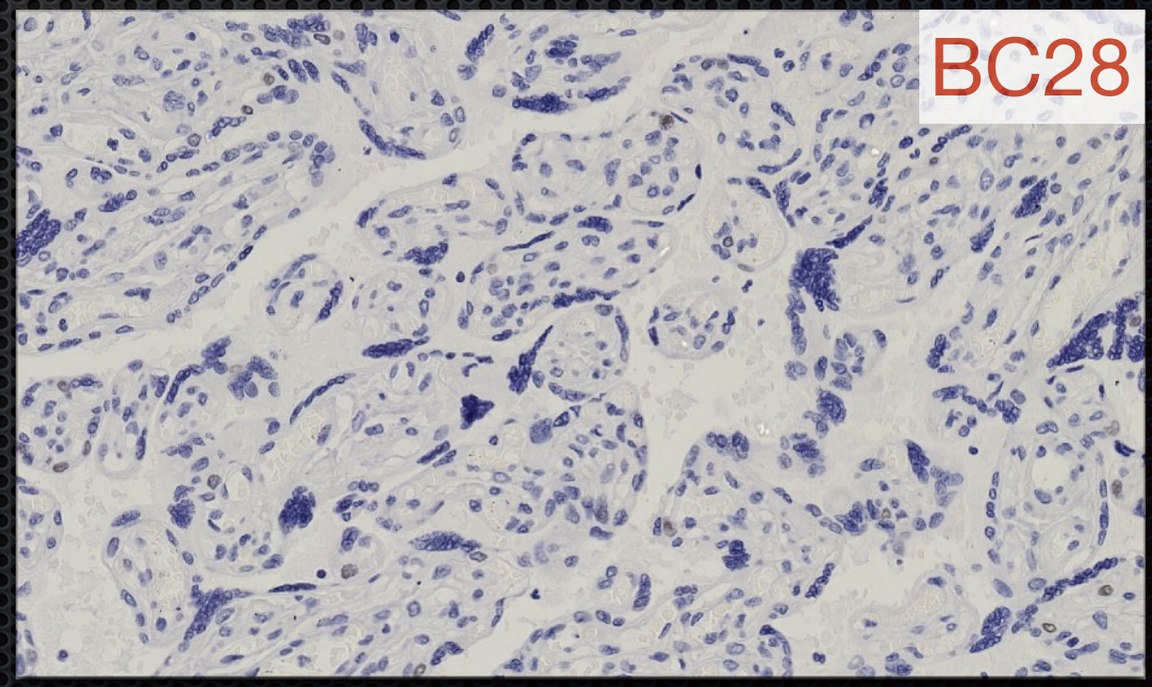
Lung tumours: Antibodies, protocols and controls

p40 / RUN 48 2016







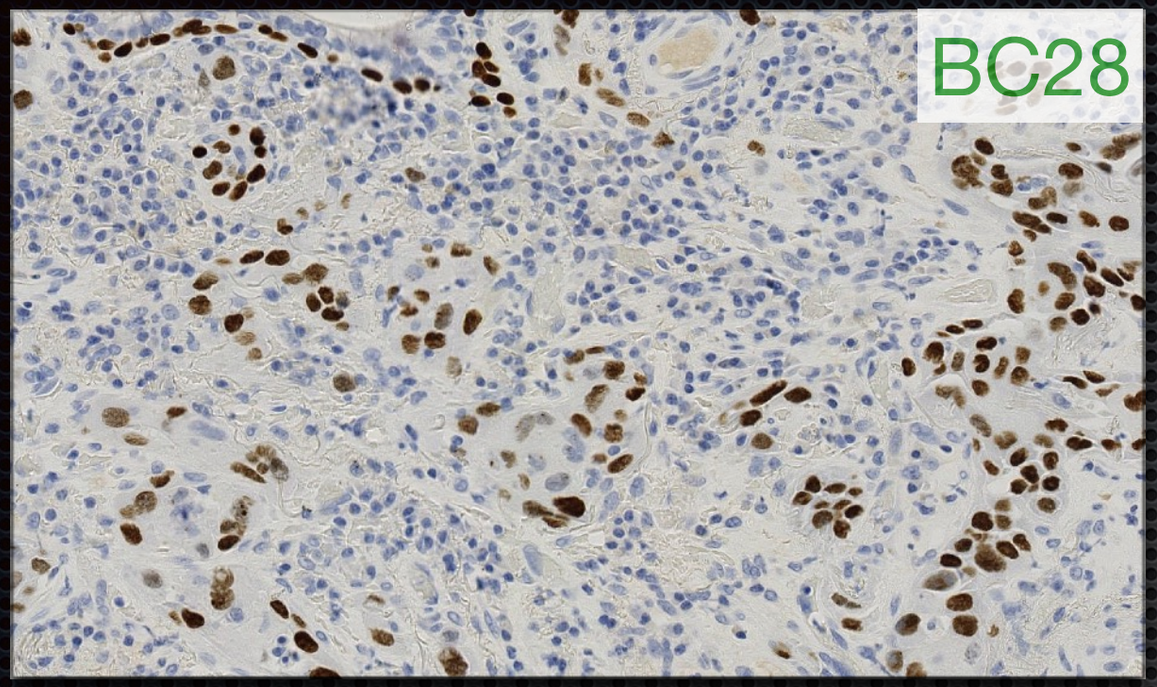


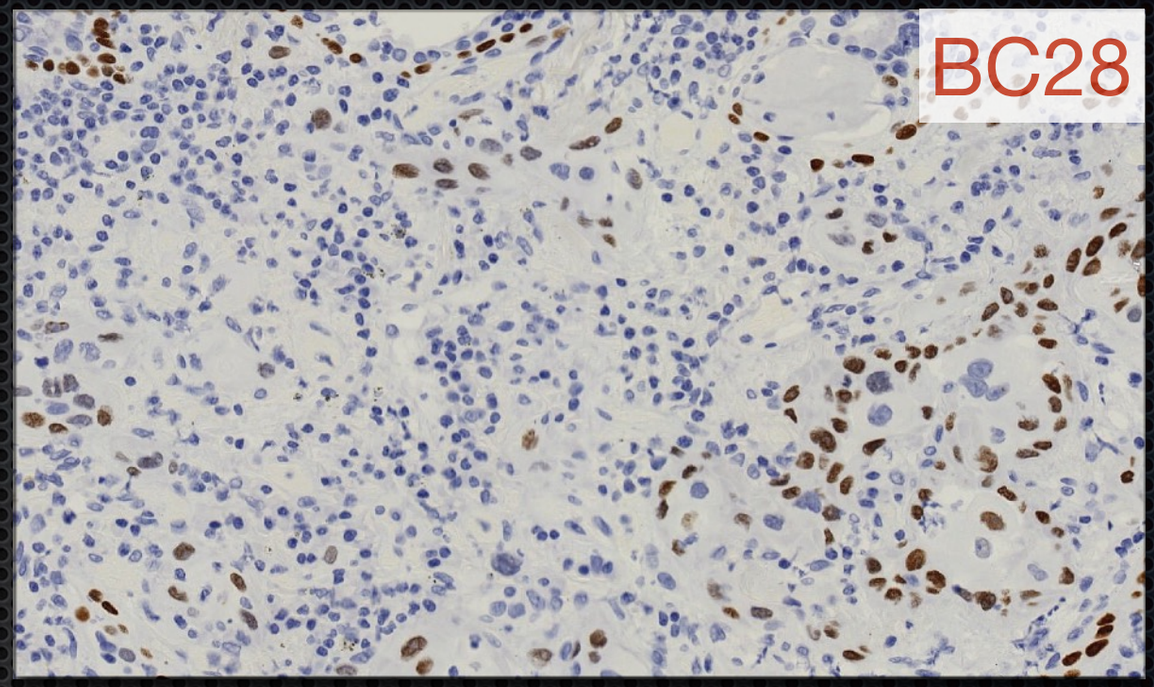
mAb clone BC28 optimally calibrated in a sensitive 3-step polymer system.

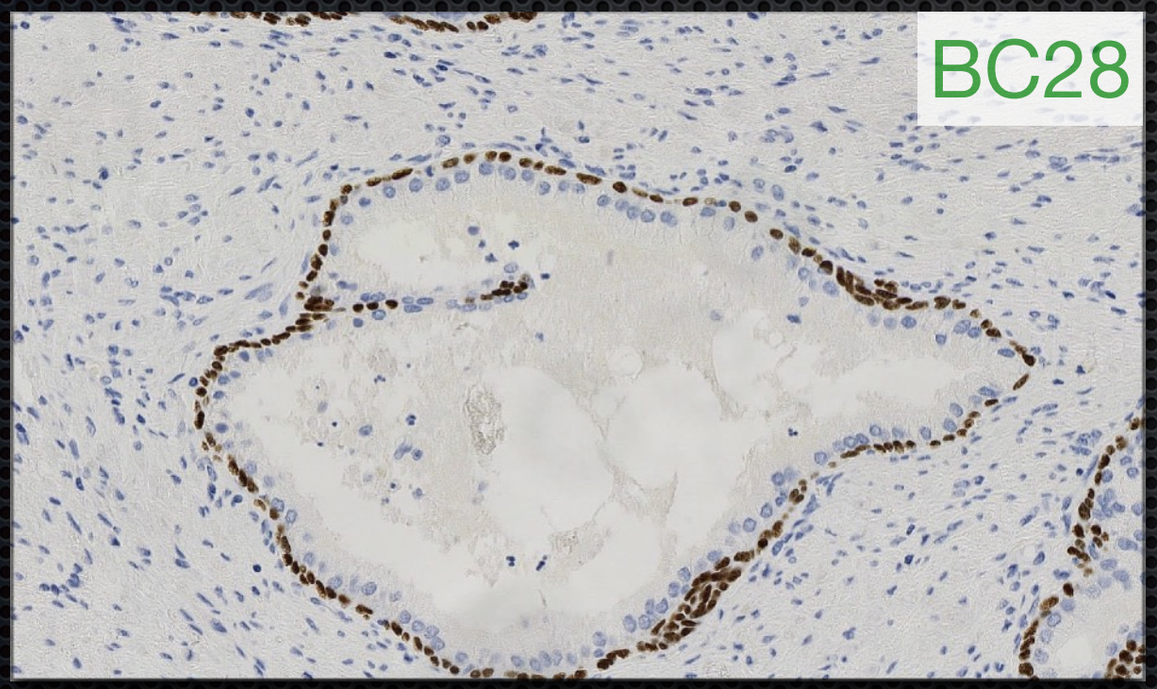
The use of a less sensitive 2-step polymer based detection system.

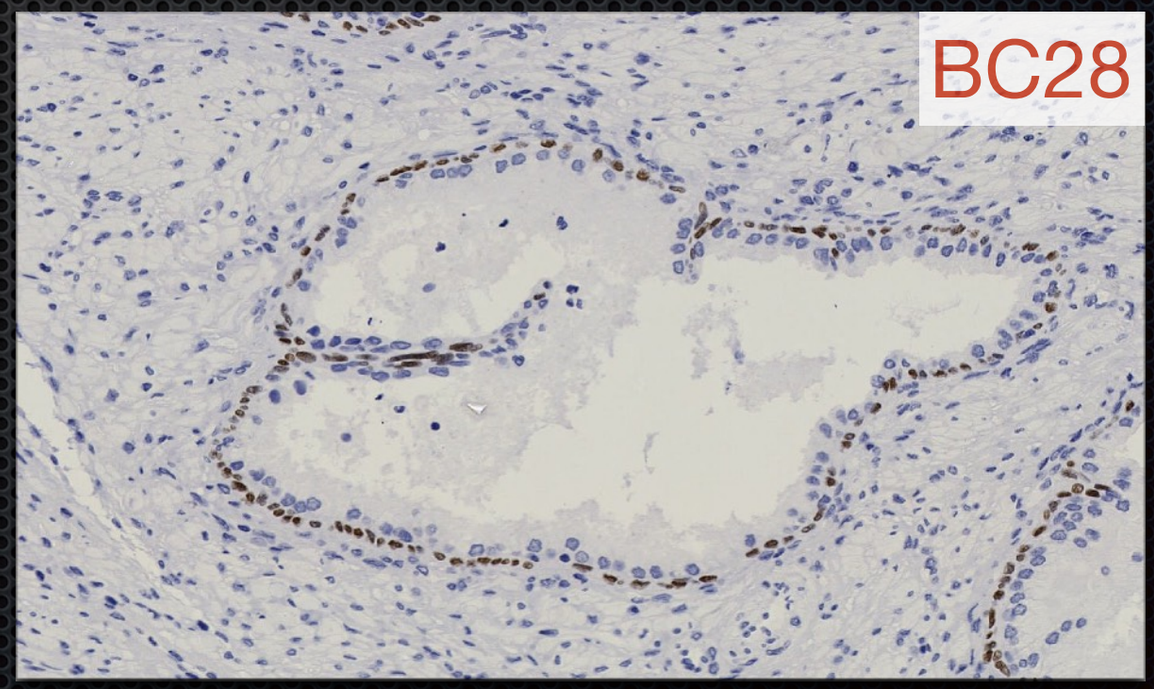
Lung tumours: Antibodies, protocols and controls

p40 / RUN 48 2016









mAb clone BC28 optimally calibrated in a sensitive 3-step polymer system.

The use of a less sensitive 2-step polymer based detection system.

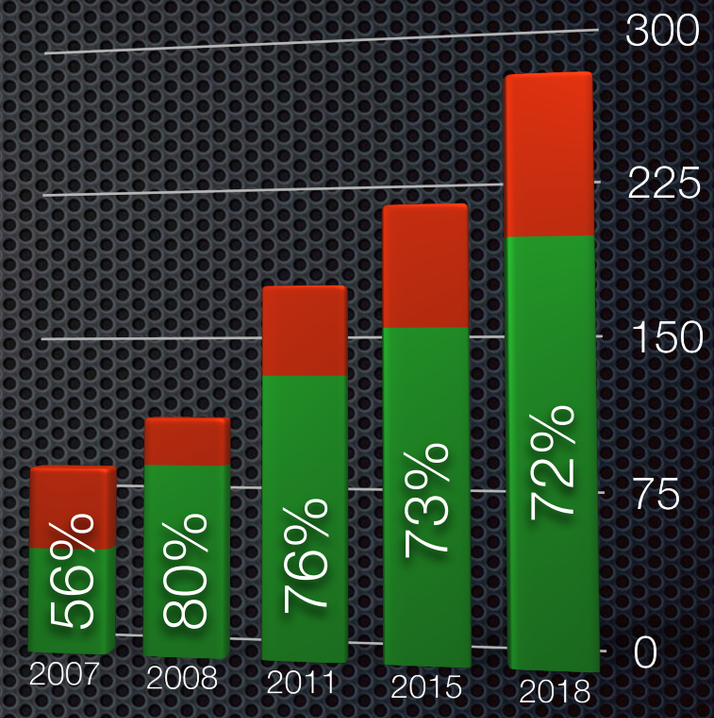
Lung tumours: Antibodies, protocols and controls

Calretinin / RUN 52 2018

Pass: 72 %

Table 1. **Antibodies and assessment marks for CR, run 52**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **2E7**	1	Immunologic	1	0	0	0	-	-
mAb clone **5A5**	3 1	Leica/Novocastra Monosan	1	1	2	0	-	-
mAb clone **CAL6**	7	Leica/Novocastra	1	3	0	3	57%	-
mAb clone **DAK-Calret 1**	34	Dako/Agilent	9	8	8	9	50%	81%
rmAb clone **BSR235**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **SP13**	3 2 2 2	Cell Marque Immunologic Spring Bioscience Thermo Scientific	1	3	4	1	44%	-
pAb **18-0211**	12	Invitrogen/Thermo	3	3	4	2	50%	100%
pAb, **232A**	2	Cell Marque	0	0	2	0	-	-
pAb **61-0006**	1	Genemed	0	0	1	0	-	-
pAb, **CP092C**	1	Biocare	0	0	1	0	-	-
pAb **RBK003**	1	Zytomed Systems	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **CAL6 PA0346**	14	Leica/Novocastra	1	11	2	0	86%	92%
mAb clone **CAL6 PA0346**[3]	1	Leica/Novocastra	1	0	0	0	-	-
mAb clone **DAK-Calret 1 IS/IR627**	35	Dako/Agilent	14	19	2	0	94%	97%
mAb clone **DAK-Calret 1 IS/IR627**[4]	20	Dako/Agilent	0	4	11	5	20%	-
mAb clone **MX027 MAB-0716**	1	Maixin	1	0	0	0	-	-
rmAb **SP13 232R**	1	Cell Marque	0	0	1	0	-	-
rmAb **SP13 MAD-000315QD**	1	Master Diagnostica	0	0	1	0	-	-
rmAb **SP13 RMPD010**	1	Diagnostic Biosystems	0	1	0	0	-	-
rmAb clone **SP65 790-4467**	118	Ventana/Roche	86	20	10	2	90%	96%
pAb **232A-78**	2	Cell Marque	0	0	2	0	-	-
pAb **8223-C010**	1	Sakura Finetek	0	1	0	0	-	-
Unknown RTU Ab	1		0	0	1	0	-	-
Total	269		120	74	52	23	-	
Proportion			45%	27%	19%	9%	72%	



The mAb clone DAK-Calret 1 based RTU system developed for the Autostainer - but used on the Dako Omnis platform

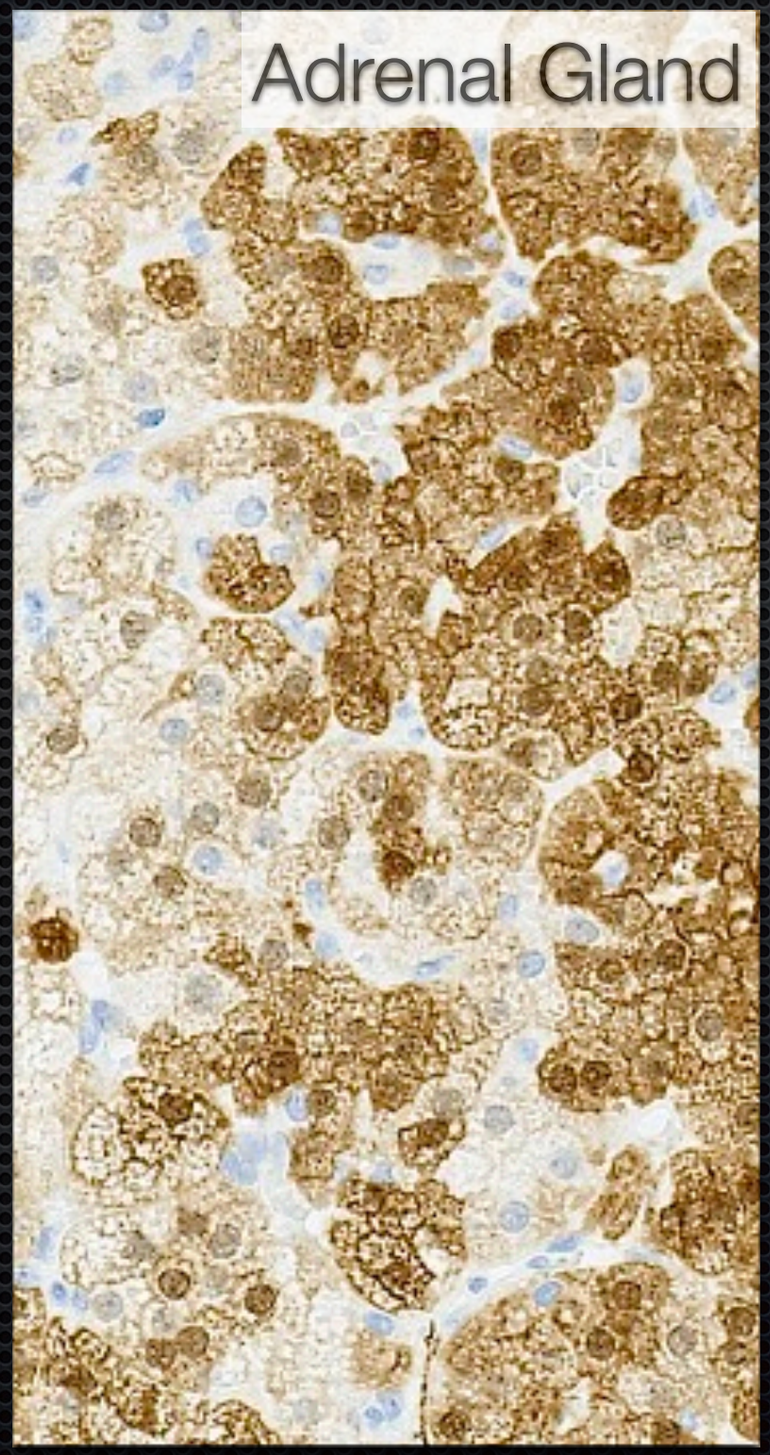
Lung tumours: Antibodies, protocols and controls

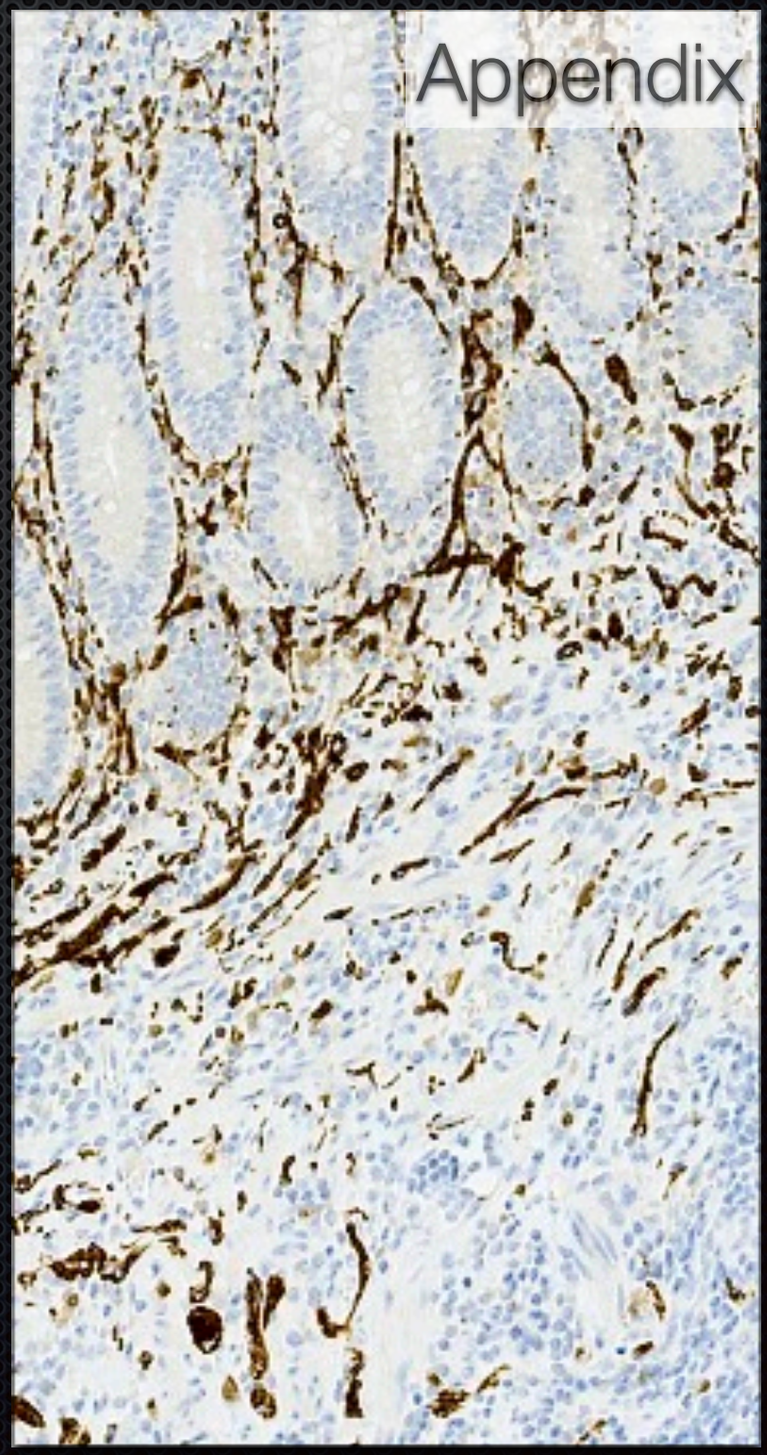
Calretinin / RUN 52 2018

Controls / iCAPC

Adrenal gland will serve as a "low-level expressor" (LE) positive tissue control, in which an at least weak to moderate, distinct cytoplasmic and nuclear staining of the majority of the cortical epithelial cells must be seen.

Appendix serves both as negative tissue and "high-level expressor" (HE) positive tissue control. Columnar epithelial cells and smooth muscle cells should be negative, while strong, distinct cytoplasmic and nuclear staining of the peripheral nerves (ganglion cells and axons) and macrophages should be seen. Furthermore, fat cells in the submucosa of the appendix could serve as an additional LE positive tissue control.





Lung tumours: Antibodies, protocols and controls

Calretinin / RUN 52 2018

Recommend-able clones	Retrieval	Titre	Detection	RTU	Detection
mAb DAK-Calret1	HIER, High pH	1:20 - 1:100	3-step	Dako	2 or 3-step
mAb 5A5	HIER, High pH	1:100	3-step	-	-
mAb CAL6	HIER, High pH	1:15	3-step	Leica	3-step
pAb 18-0211	HIER, High pH	1:50 - 1:150	3-step	-	-
rmAb SP65	HIER, High pH	-	-	Ventana	2 or 3-step

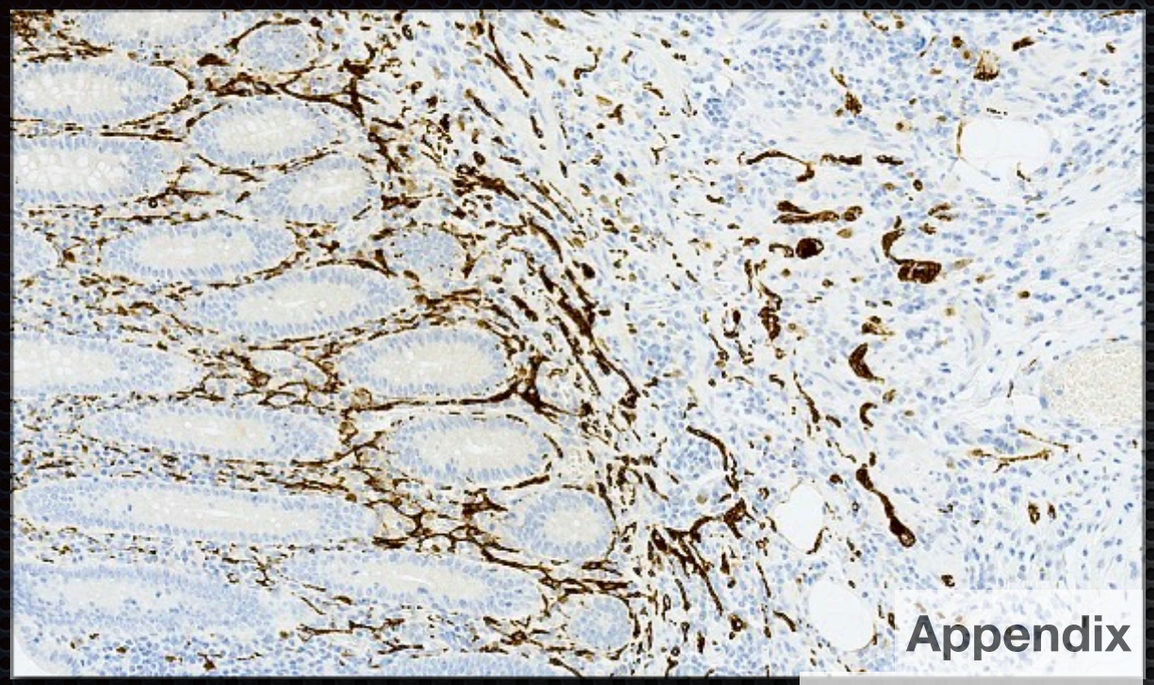
Table 3. **Proportion of optimal results for CR for the most commonly used antibodies as concentrates on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark GX / XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	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 **CAL6**	-	-	1/2 **	-	0/1	-	0/2	0/1
mAb clone **DAK-Calret 1**	3/10 (30%)	-	0/6	-	0/6	-	5/7 (71%)	0/1
rmAb clone **SP13**	-	-	-	-	0/4	-	-	-
pAb **18-0211**	1/2	1/1	-	-	0/6	-	0/1	1/1

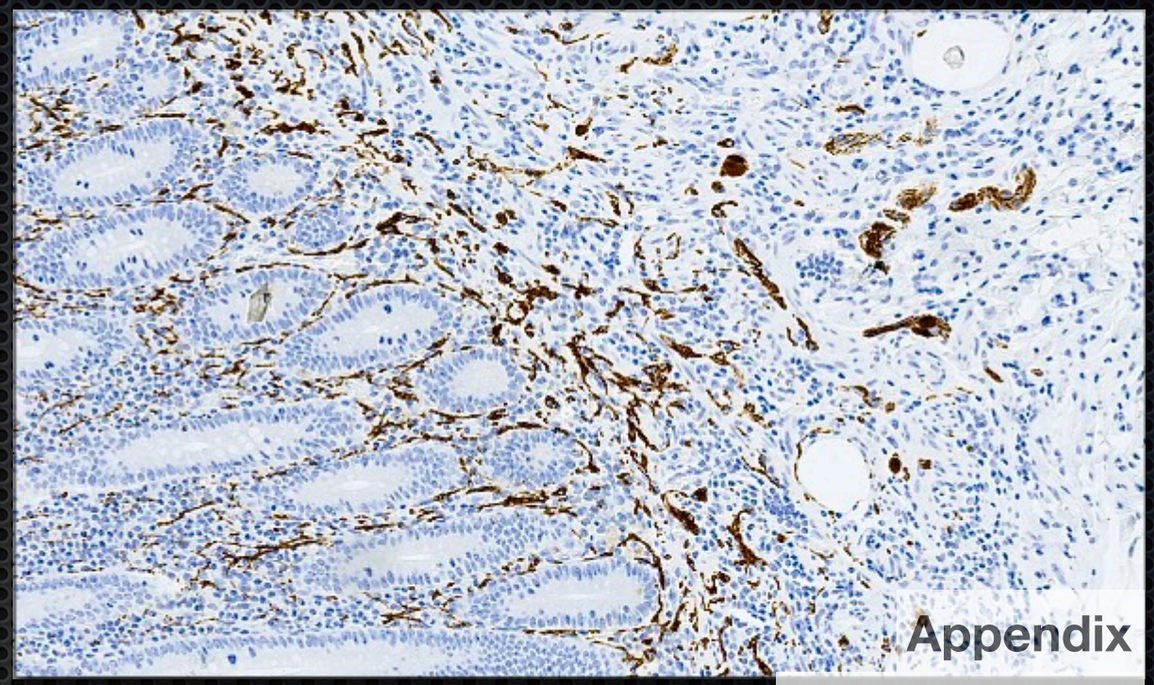
Lung tumours: Antibodies, protocols and controls

Calretinin / RUN 52 2018

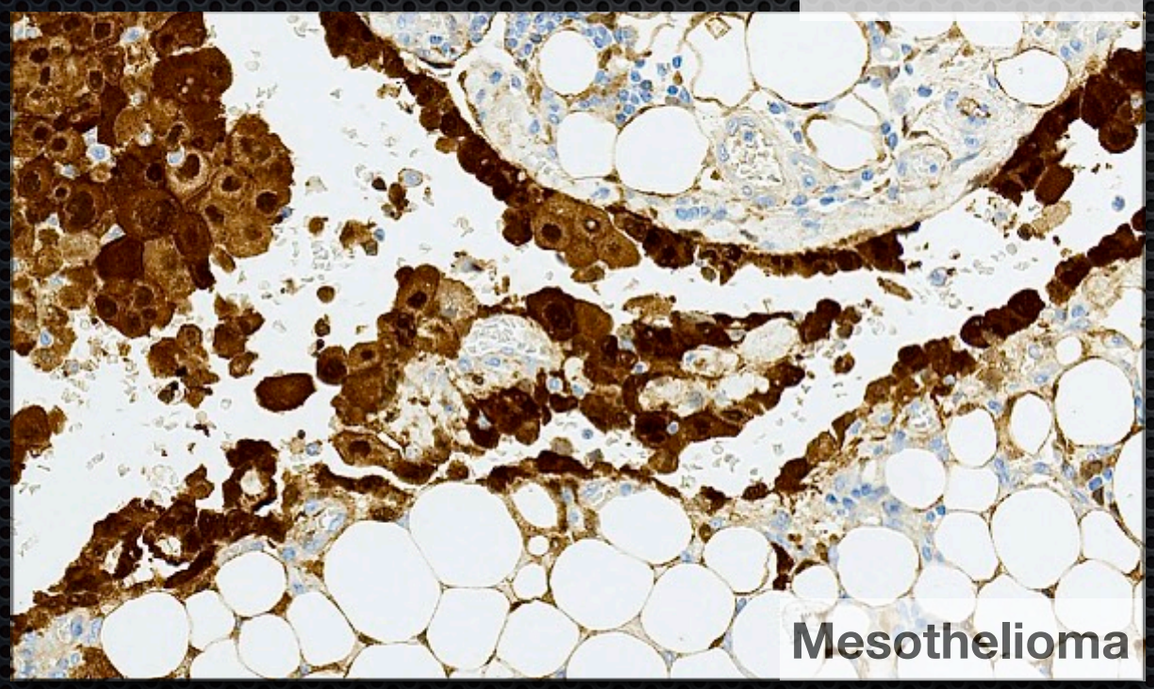
NordiQC

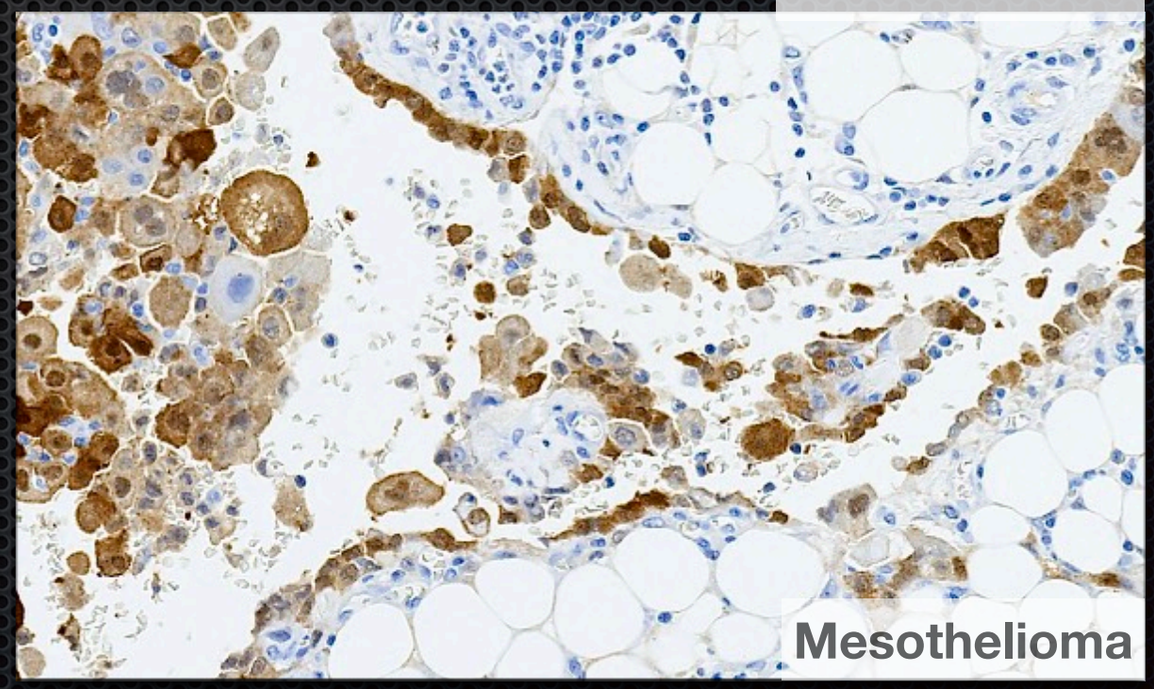


Autostainer



Dako Omnis





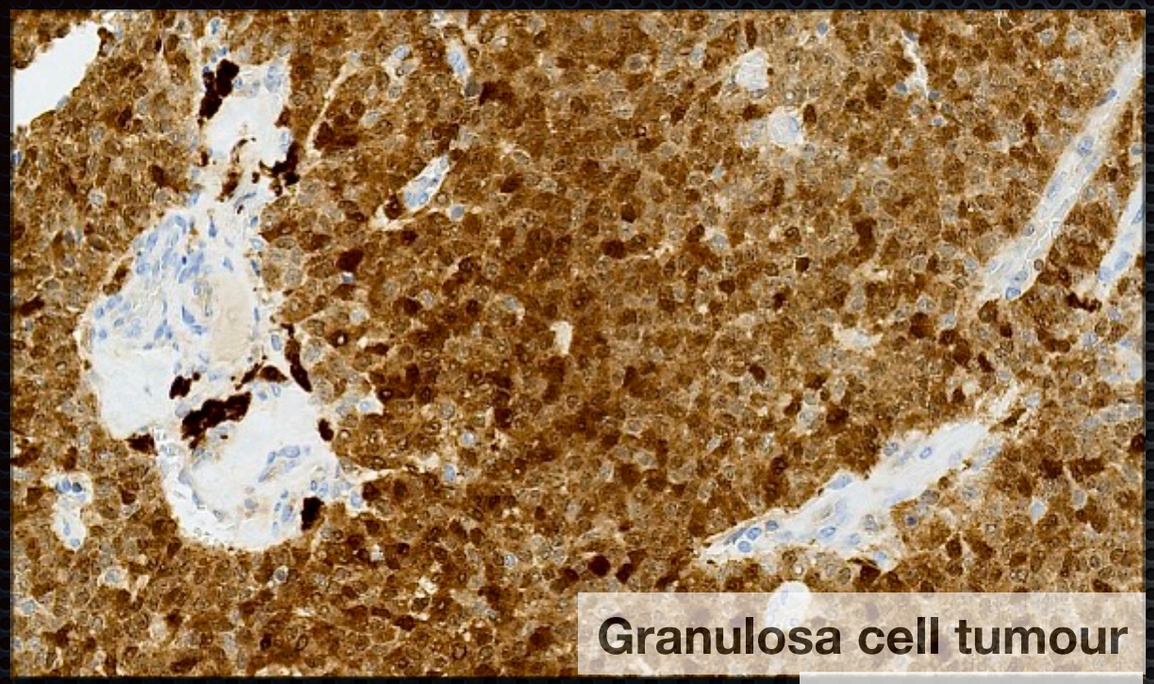
The mAb clone DAK-Calret 1 based RTU system developed for the Autostainer.

The mAb clone DAK-Calret 1 based RTU system developed for the Autostainer - but used on the Dako Omnis platform.

Lung tumours: Antibodies, protocols and controls

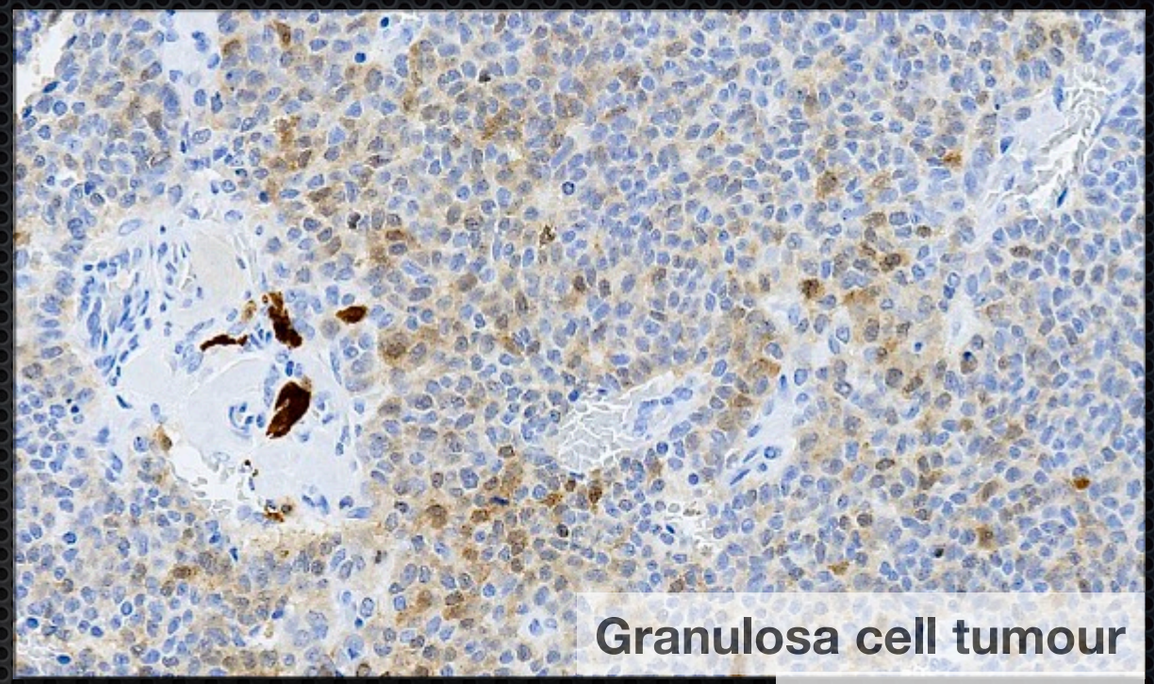
Calretinin / RUN 52 2018

NordiQC



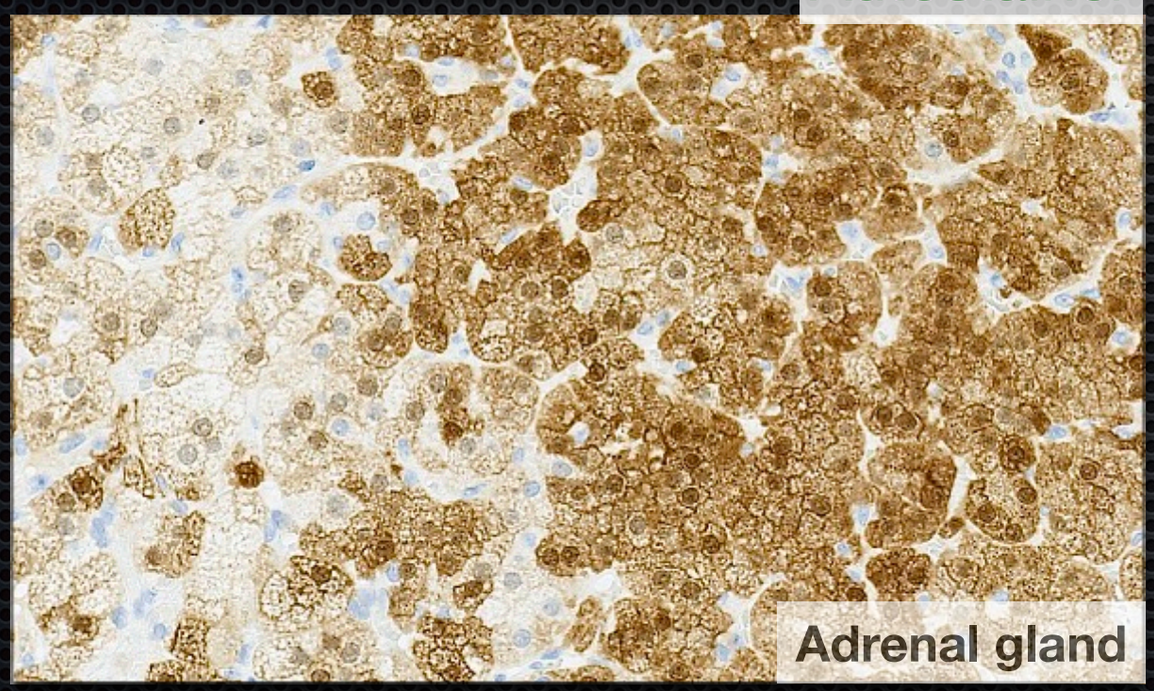
Granulosa cell tumour

Autostainer

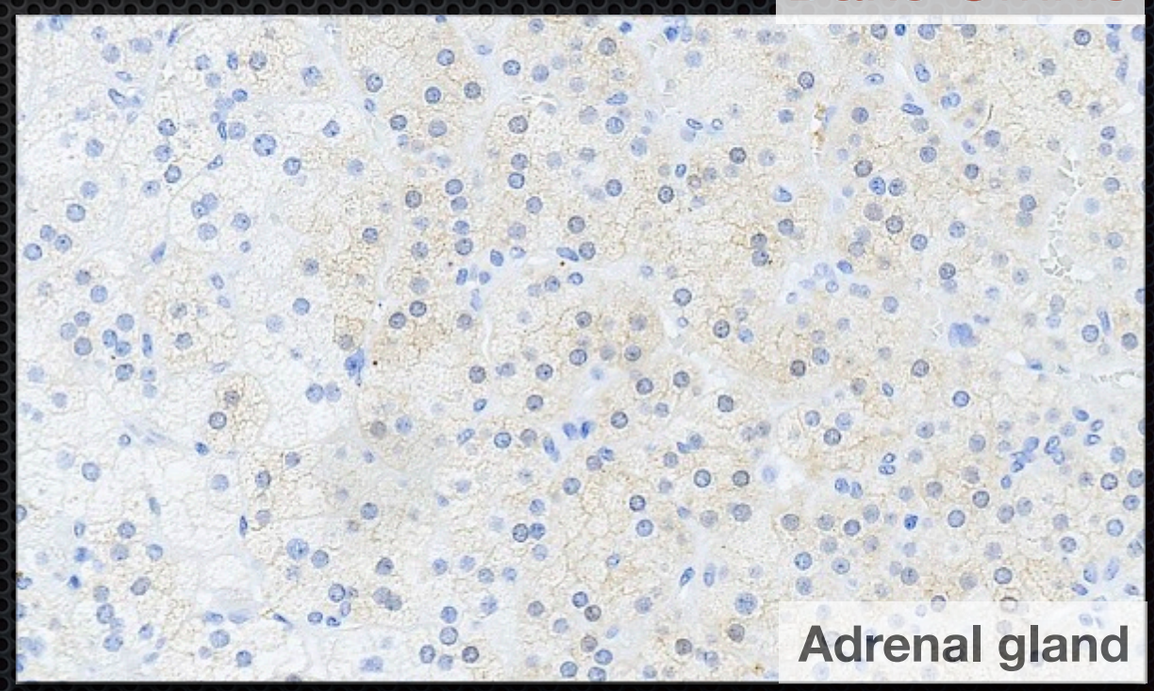


Granulosa cell tumour

Dako Omnis



Adrenal gland



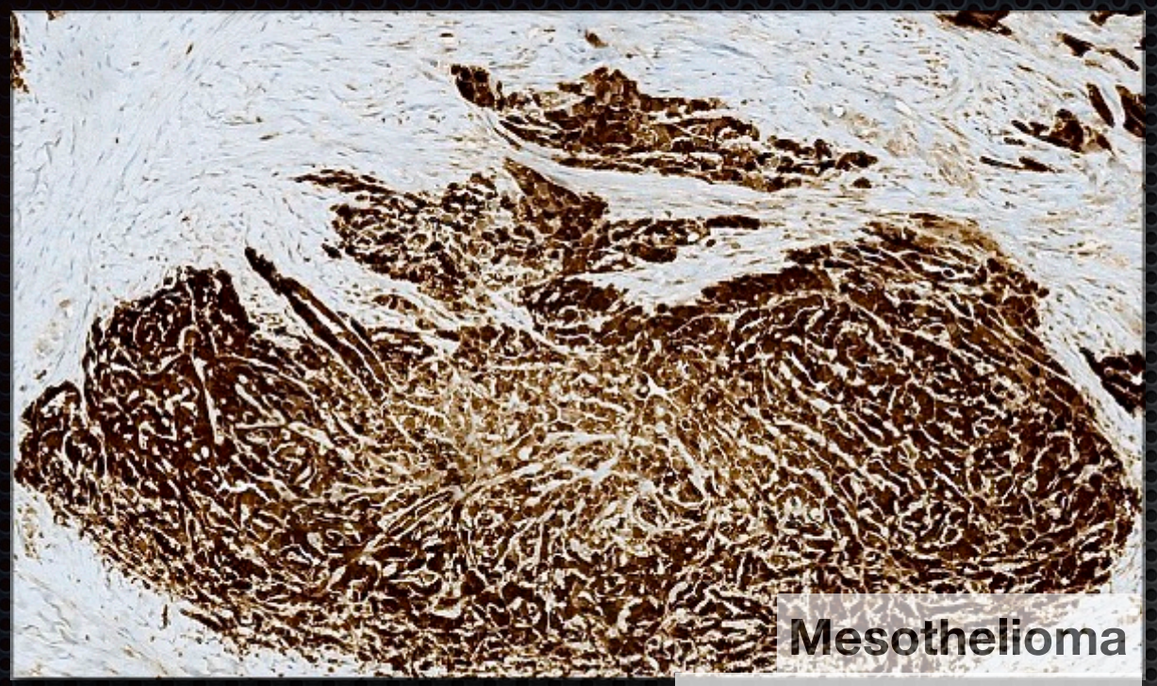
Adrenal gland

The mAb clone DAK-Calret 1 based RTU system developed for the Autostainer.

The mAb clone DAK-Calret 1 based RTU system developed for the Autostainer - but used on the Dako Omnis platform

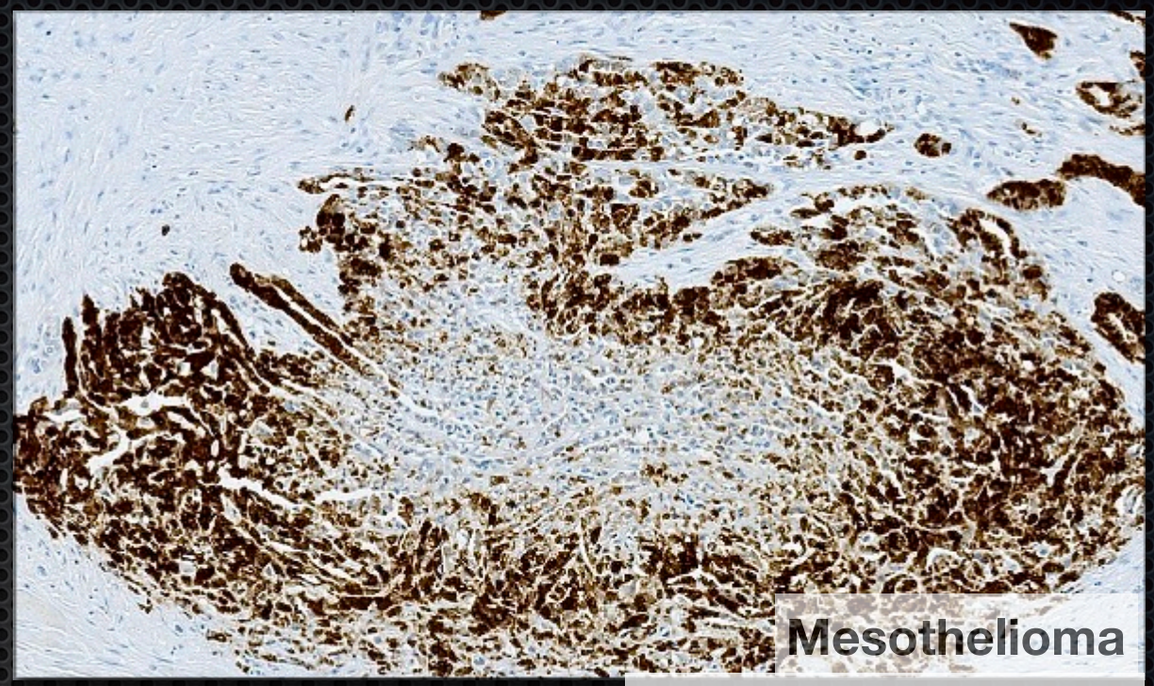
Lung tumours: Antibodies, protocols and controls

Calretinin / RUN 52 2018



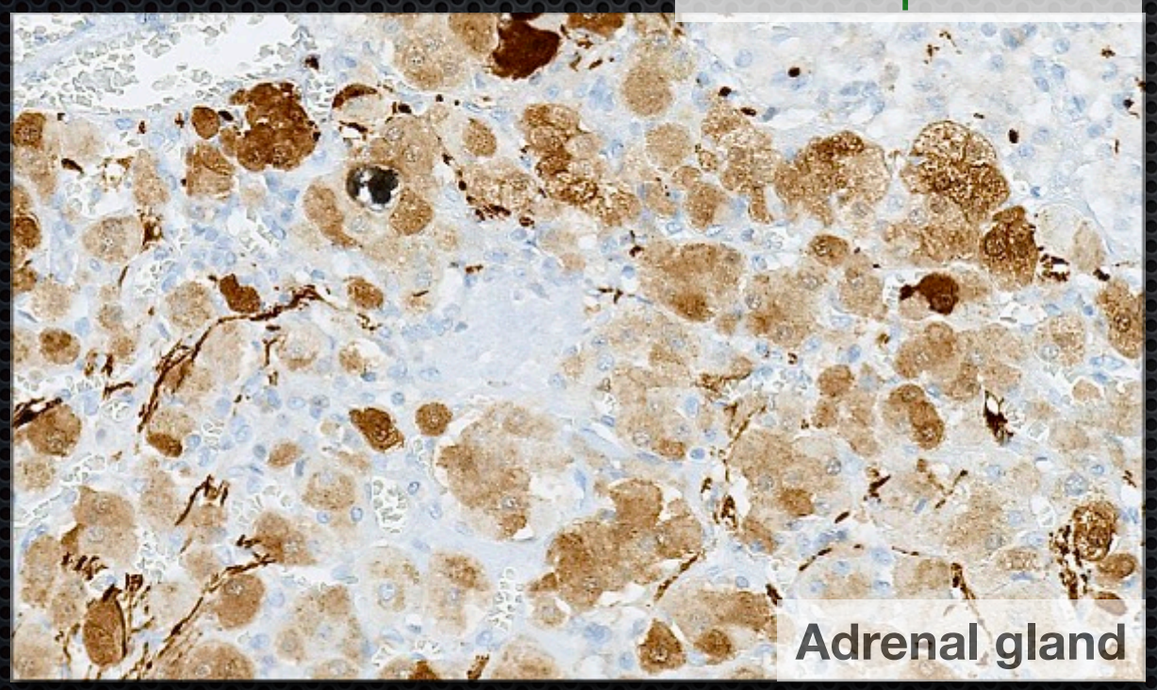
Mesothelioma

Official protocol

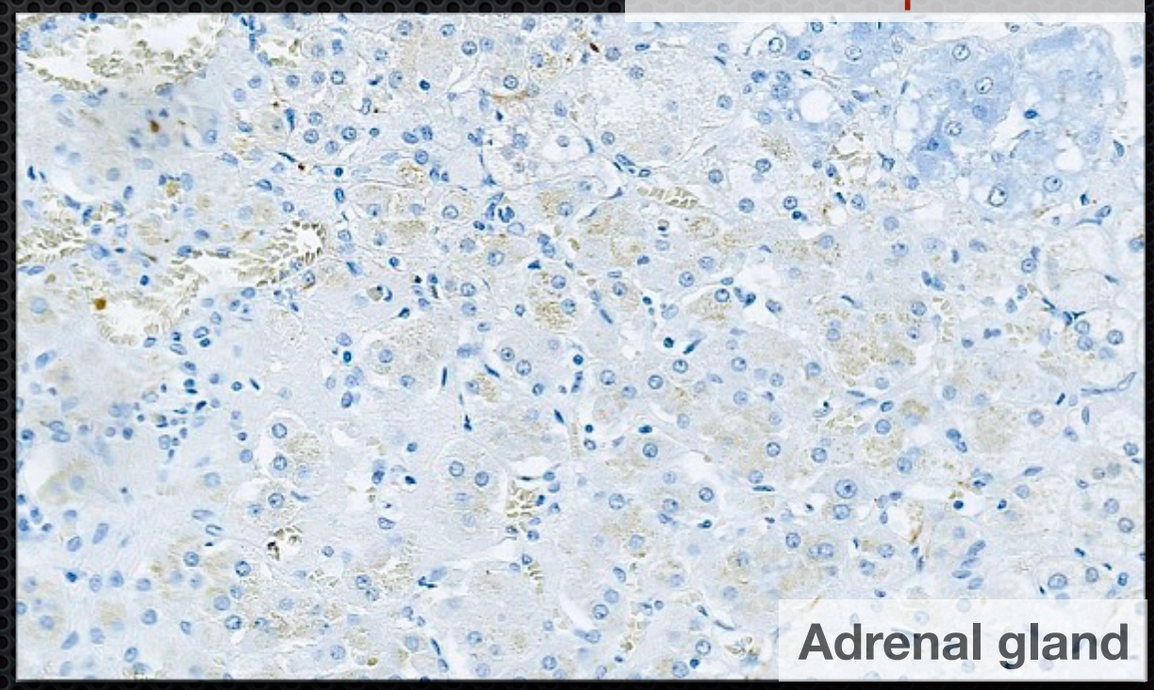


Mesothelioma

Modified protocol



Adrenal gland



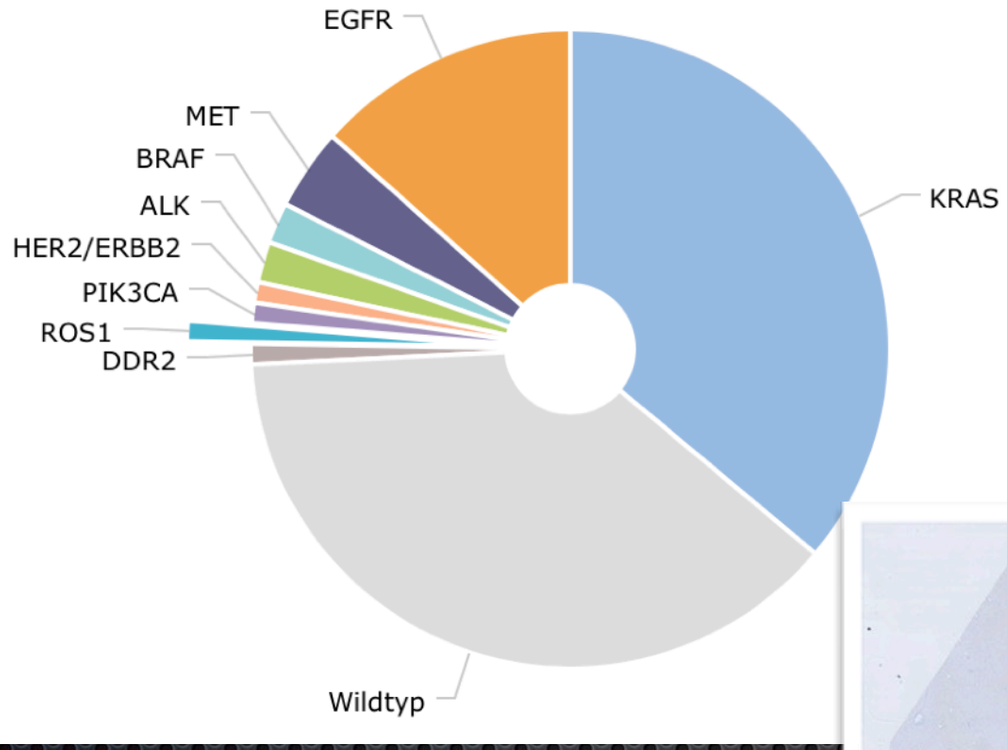
Adrenal gland

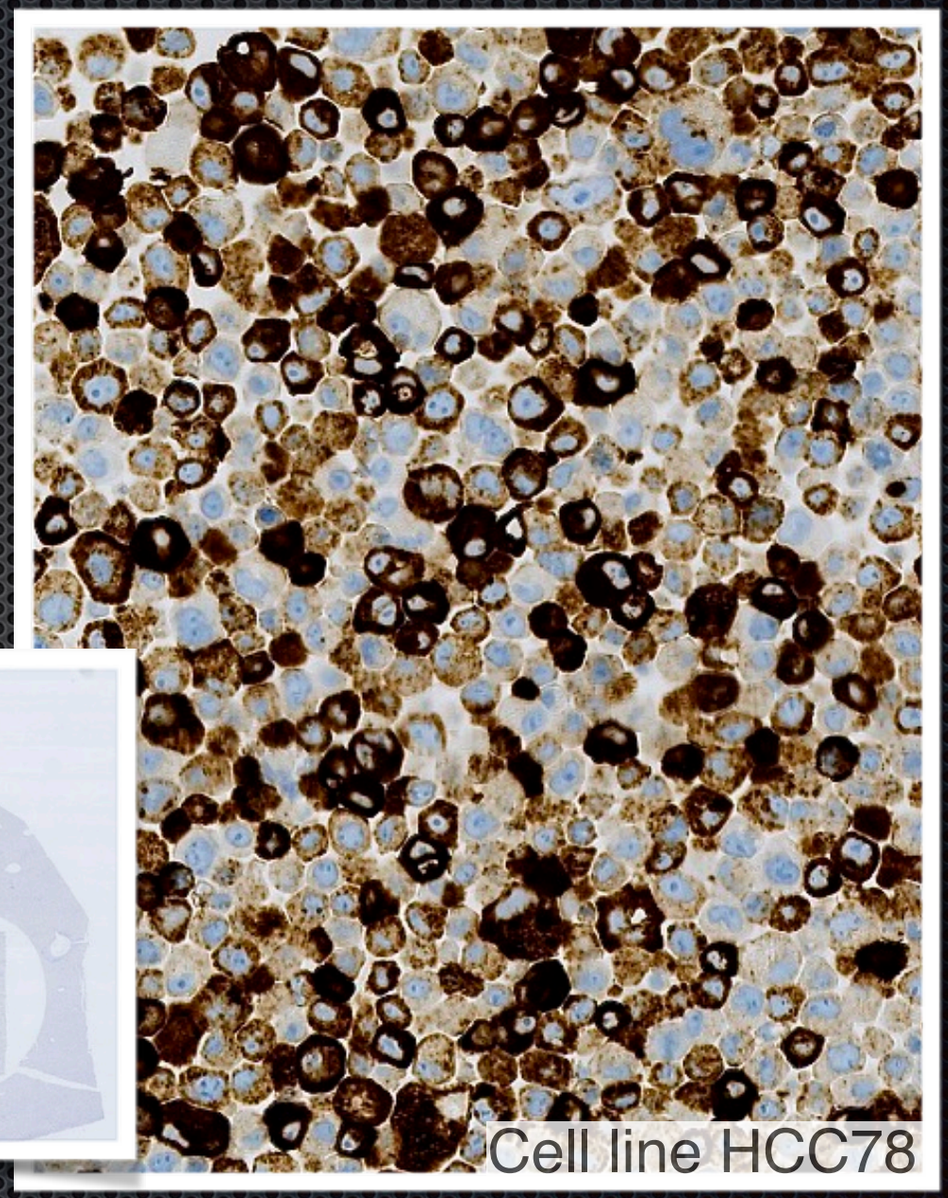
The rmAb clone SP65 based RTU system for the Ventana BenchMark platform. Official protocol: CC1 32 min / Ab 16 / Optiview

The rmAb clone SP65 based RTU system for the Ventana BenchMark platform. Modified protocol: CC1 **4** min / Ab 16 / Optiview**+Amp**

Driver mutations in lungcancer / ROS1

Non-squamous NSCLC





Clone	Company	Retrieval	Titre	Detection
rmAb D4D6	Cell Signaling	High pH*	1:50 - 1:300*	3-step polymer / multimer detection*

** Viola et al. Journal of Thoracic Oncology Vol. 11 No. 7 (2016): 1029-1039*

Coffee…..