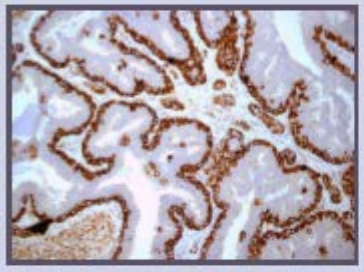

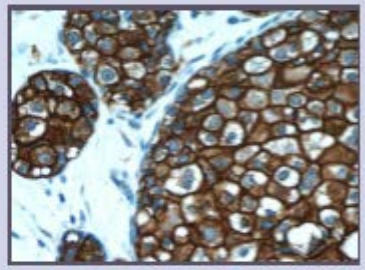

International Symposium on Immunohistochemistry

January 4th - 7th, 2018

QA of IHC in Endocrine, Renal, Prostate and Germ cell pathology

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Global Pathology Manager
Agilent Technologies

(Former Scheme Manager, NordiQC)

IHC – Protocols and controls – End, Ren, Prost...

• CGA	Neuroendocrine
• SYP	
• CD56	
• PSA	Prostate
• NKX3.1	
• SALL4	Germ cell
• OCT3/4	
• *SOX10*	*Melanoma*
• *UP II*	*Bladder*

IHC – Protocols and controls – End, Ren, Prost…

	Recommendable clones (conc.)	Less successful clones (conc.)	RTU ”plug and play” giving optimal result
CGA	mAb LK2H10 pAb 0430	mAb DAK-A3 mAb 5H7	.....!!!!
SYP	mAb 27G112 mAb DAK-SYNAP rmAb MRQ-40 rmAb SP11	mAb SY38	Dako: mAb DAK-SYNAP Leica: mAb 27G12 VMS: rmAb MRQ-40
CD56	mAb 123C3* mAb 123C3.D5* mAb 1B6 mAb CD564 rmAb MRQ-42		Dako: mAb 123C3 Leica: mAb CD564 VMS: rmAb MRQ-42

* Inferior performance on VMS stainer platform

IHC – Protocols and controls – End, Ren, Prost…

	Positive tissue control HE	**Positive tissue control LE**	**Negative tissue control NE**
CGA	Appendix: Endocrine cells	Appendix: Nerves – ganglion cells and axons	Appendix: Epithelial cells
SYP	Appendix: Nerves – ganglion cells and axons	*Appendix: Goblet cells*	Appendix: Epithelial cells
CD56	Appendix: Nerves – ganglion cells and axons	Tonsil: NK-cells and CD4/CD8 double hit pos. T-cells	Appendix: Epithelial cells

NordiQC

Assessment Run 46 2016
Chromogranin A (CGA)

Material
The slide to be stained for CGA comprised:
1. Appendix, 2. Pancreas, 3. Colon adenocarcinoma, 4. Small cell lung carcinoma,
5. Pancreatic neuroendocrine tumour, 6. Thyroid medullary carcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CGA staining as optimal included:

- A strong and distinct cytoplasmic staining reaction of neuroendocrine cells in the appendiceal mucosa and islets of pancreas.
- An at least weak to moderate, distinct granular cytoplasmic staining reaction of normal ganglion cells and axons in the nerve plexus of appendix.
- At least a moderate, distinct cytoplasmic reaction of virtually all neoplastic cells in the pancreatic neuroendocrine carcinoma and the medullary thyroid carcinoma.
- An at least weak, distinct granular cytoplasmic staining reaction of the vast majority of neoplastic cells in the small cell lung carcinoma.
- No staining reaction of the appendiceal columnar epithelial cells, pancreatic exocrine cells and neoplastic cells in the colon adenocarcinoma.

Participation

Number of laboratories registered for CGA, run 46	262
Number of laboratories returning slides	242 (92%)

Results
242 laboratories participated in this assessment. 162 (67%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks given (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful primary antibody
- Too low concentration of the primary antibody
- Omission of HIER
- Insufficient HIER – too short efficient heating time

Performance history
This was the sixth NordiQC assessment of CGA. The pass rate decreased slightly compared to the previous run as shown in table 2.

Table 2. **Proportion of sufficient results for CGA in the six NordiQC runs performed**

	Run 9 2003	Run 13 2005	Run 18 2006	Run 22 2008	Run 31 2011	Run 46 2016
Participants, n=	74	88	94	117	170	262
Sufficient results	39%	64%	70%	61%	75%	67%

The reduced pass rate in this run may in part be explained by a large proportion of new participants and new and more challenging tissue material circulated. However, also increased use of less successful Abs in this assessment seemed to have an impact. In run 31, 2011 12% of the laboratories used mAb clones 5H7 or DAK-A3, compared to 19% in this run. As shown in table 2, these two Abs provided an inferior performance compared to e.g mAb LK2H10.

Conclusion
The mAb clone **LK2H10** was the most successful Ab for the demonstration of CGA. As concentrated format within a laboratory developed assay, optimal results were obtained on all three main IHC platforms (Dako, Leica and Ventana) and a high pass rate was observed in general. The widely used mAb clone DAK-A3 provided a low pass rate, concordant to the results observed in previous CGA assessments.
HIER was mandatory for an optimal result. In this context it has to be stressed that the data sheets for mAb clone LK2H10 from the vast majority of vendors still provide misleading information for this clone recommending a protocol omitting HIER.

End, Ren, Prost…

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

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **5H7**	4	Leica/Novocastra	0	0	3	1	-	-
mAb clone **DAK-A3**	36	Dako/Agilent	0	2	17	17	6%	-
mAb clone **LK2H10**	22 18 6 3 2 2 1 1 1 1 1 1	Thermo/Neomarkers Cell Marque Immulologic Biogenex Millipore Zytomed Abcam A.Menarini Diagnostic Biosystems Europroxima Monosan Unknown	24	31	0	4	93%	98%
mAb clone **PHE5**	1	Unknown	0	0	1	0	-	-
mAb clones **LK2H10+PHE5**	6 5	Thermo/Neomarkers Biocare	3	8	0	0	100%	100%
rmAb clone **EP38**	1	Epitomics	0	1	0	0	-	-
rmAb clone **SP12**	1 1	Master Diagnostica Thermo/NeoMarkers	0	0	0	2	-	-
pAb **A0430***	38	Dako/Agilent	8	17	8	5	66%	-
pAb **NB120-17064**	1	Novus Biologicals	0	1	0	0	-	-
pAb **RB-9003**	1	Thermo/NeoMarkers	0	1	0	0		
Ready-To-Use antibodies								
mAb clone **5H7 PA0430**	6	Leica/Novocastra	0	0	2	4	-	-
mAb clone **LK2H10 760-2519**	69	Ventana/Roche	27	28	6	8	80%	96%
mAb clone **LK2H10 E001**	3	Linaris	0	3	0	0	-	-
mAb **LK2H10 AM126-5M**	1	Biogenex	0	0	1	0	-	-
mAb **LK2H10 238M-90**	1	Cell Marque	1	0	0	0	-	-
mAb clone **LK2H10 MAD-000616QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clones **LK2H10+PHE5 PM010**	2	Biocare	1	1	0	0	-	-
mAb clones **LK2H10+PHE5 BSB5345**	1	Bio SB	0	1	0	0		
mAb clones **LK2H10+PHE5 MAB-0202**	1	Maixin	1	0	0	0		
pAb **IR502***	2	Dako	0	1	1	0		
Total	242		66	96	39	41	-	
Proportion			27%	40%	16%	17%	67%	

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

Clone / pAb
HIER high pH

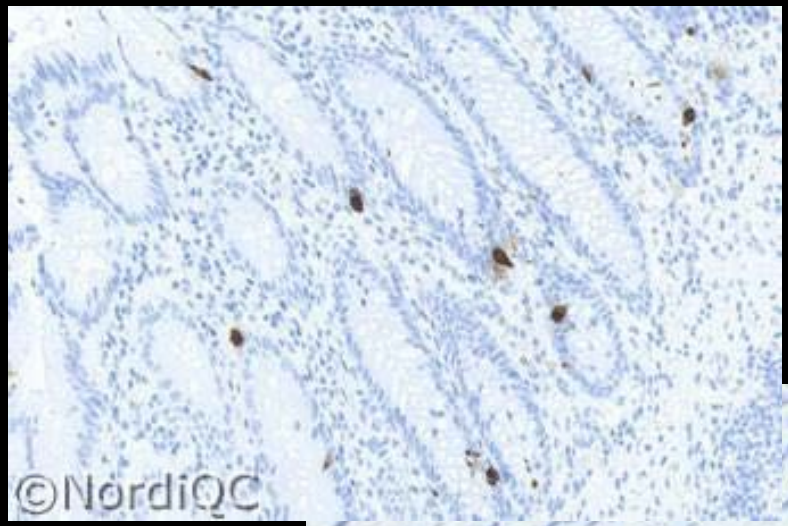
3-step detection

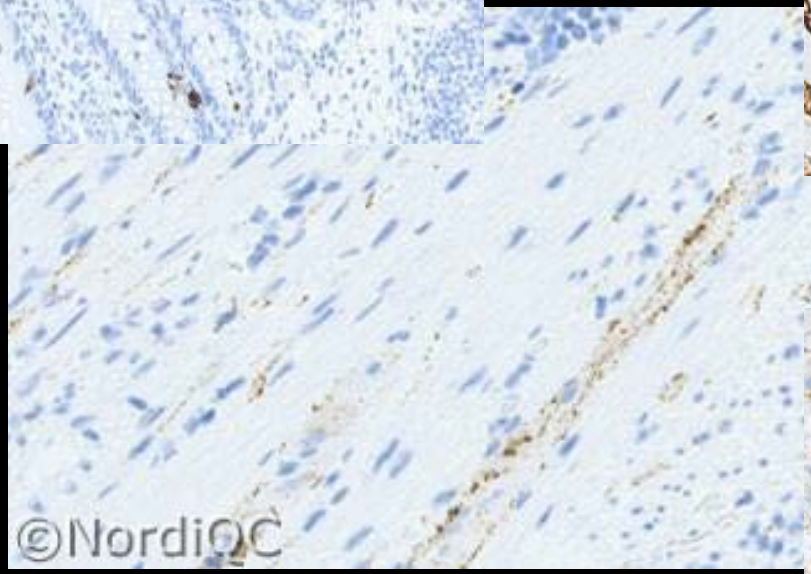
Calibration……

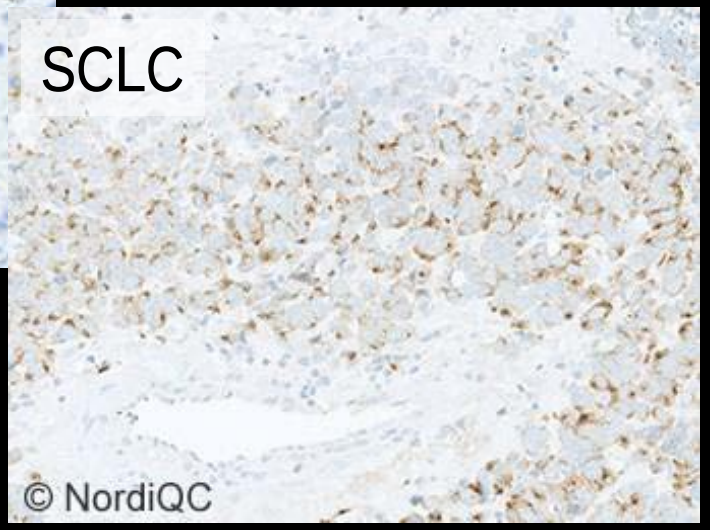
Table 2. **Proportion of sufficient results for CGA in the six NordiQC runs performed**

	Run 9 2003	Run 13 2005	Run 18 2006	Run 22 2008	Run 31 2011	Run 46 2016
Participants, n=	74	88	94	117	170	262
Sufficient results	39%	64%	70%	61%	75%	67%

IHC – Protocols and controls – End, Ren, Prost…

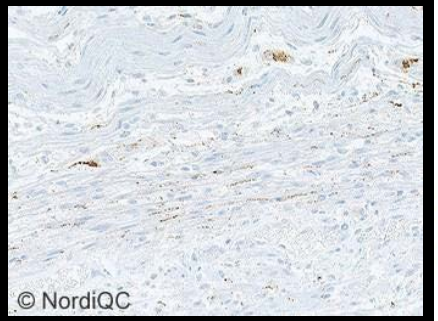






Level of target analyte must be same in controls as clinical samples

IHC – Proto … Prost…



NERVES!!

Pancreas cannot be used as pos tissue cont.

!!!!!!!!!!!!!!!

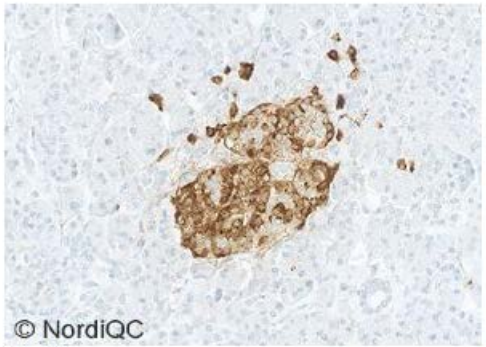


Fig. 1a
Optimal CGA staining of the pancreas using the mAb clone LK2H10 as Ready-To-Use format 760-2519, Ventana, by a laboratory modified protocol using HIER in CC1 and a 3-step multimer based detection system (OptiView).
The vast majority of endocrine islet cells show a moderate to strong and distinct cytoplasmic staining reaction and a high signal-to-noise ratio is observed.

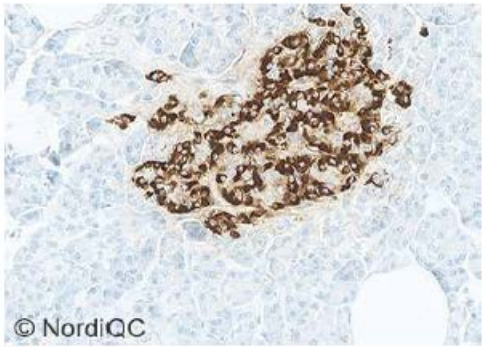
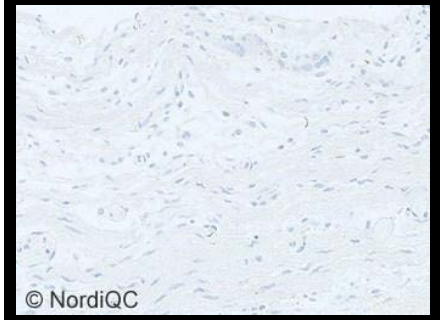


Fig. 1b
CGA staining of the pancreas using an insufficient protocol giving a too low sensitivity.
The protocol was based on the same mAb clone LK2H10, Ready-To-Use format 760-2519, Ventana omitting HIER as recommended in the package insert for the product. OptiView was used as detection system.
Also compare with Figs. 2b - 4b – same protocol.



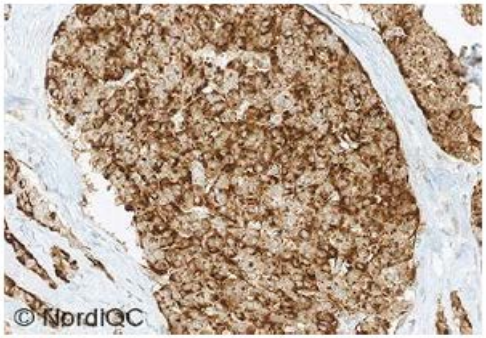


Fig. 3a
Optimal CGA staining of the pancreatic neuroendocrine carcinoma using same protocol as in Figs. 1a. and 2a. Virtually all the neoplastic cells show a strong and distinct staining reaction. No background staining is

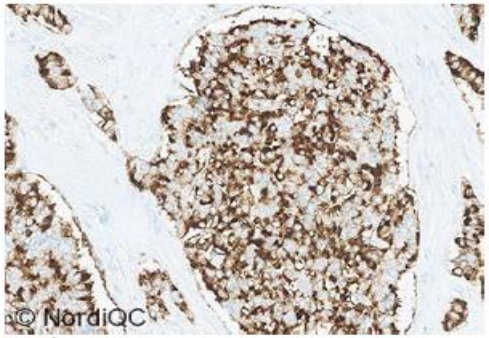


Fig. 3b
Staining for CGA of the pancreatic neuroendocrine carcinoma using same insufficient protocol as in Figs. 1b and 2b - same field as in Fig. 3a. The vast majority of the neoplastic cells are demonstrated. However also

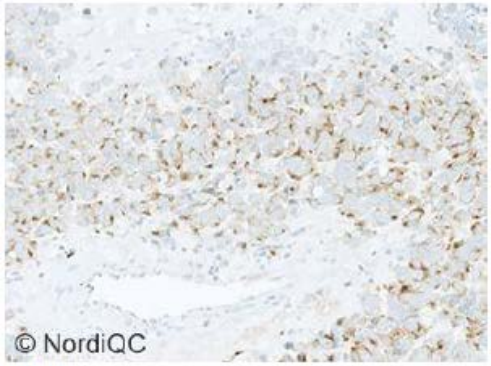


Fig. 4a
Optimal CGA staining of the SCLC using same protocol as in Figs. 1a – 3a.
Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining reaction with a dot-like accentuation. No background staining is seen.

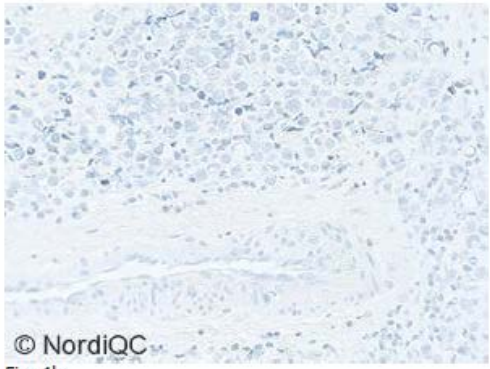
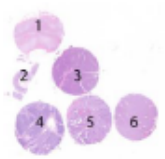


Fig. 4b
Insufficient CGA staining of the SCLC using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
Only scattered neoplastic cells show a weak and diffuse cytoplasmic staining reaction.

NordiQC

Assessment Run 43 2015
Synaptophysin (SYP)

Material
The slide to be stained for SYP comprised:



1. Adrenal gland, 2. Colon, 3. Pancreas, 4. Small cell lung carcinoma, 5. Colon adenocarcinoma, 6. Intestinal neuroendocrine tumour

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing SYP staining as optimal included:

- A strong, distinct cytoplasmic staining reaction of virtually all endocrine islet cells in the pancreas.
- A moderate to strong, distinct cytoplasmic staining reaction of neuroendocrine cells, ganglion cells and axons of the nerve plexus in the colon
- A moderate to strong, distinct cytoplasmic, dot-like staining reaction of the majority of cortical epithelial cells of the adrenal gland.
- A weak to moderate staining of the majority of goblet cells in the colon mucosa
- An at least moderate, distinct, cytoplasmic staining reaction of the majority of neoplastic cells of the small cell lung carcinoma, and the intestinal neuroendocrine tumour.
- No staining of neoplastic cells in the colon adenocarcinoma.

A weak cytoplasmic staining reaction of the exocrine pancreatic epithelial cells was accepted.

Participation

Number of laboratories registered for SYP, run 43	258
Number of laboratories returning slides	243 (94%)

Results
243 laboratories participated in this assessment. 200 (82%) of these achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:
- HIER in a non-alkaline buffer
- Too low concentration of the primary antibody
- Use of less sensitive and specific detection systems

Performance history
This was the fifth NordiQC assessment of SYP. A major improvement of the pass rate was seen compared to previous runs (see table 2).

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

	Run 18 2006	Run 22 2008	Run 29 2010	Run 37 2013	Run 43 2015
Participants, n=	94	112	151	214	243
Sufficient results	68%	58%	55%	58%	82%

Conclusion
The mAb clones **27G12**, **BS15**, **DAK-SYNAP** and **Snp88** and the rmAb clones **MRQ-40** and **SP11** could all be used to obtain an optimal staining reaction for SYP. Irrespective of clone, HIER in an alkaline buffer is mandatory to give an optimal staining reaction, and concentration of the primary Ab must be carefully calibrated.
3-step polymer / multimer based detection systems provided a higher proportion of optimal results compared to 2-step and biotin-based detection systems.
mAb clone **27G12**, was the most commonly used antibody within a laboratory developed assay and provided an optimal result on all three main IHC platforms (Dako, Leica and Ventana).
Colon is at present the most recommendable positive tissue control for SYP. Nerves must show a strong staining reaction, while an at least weak but distinct cytoplasmic staining reaction must be seen in the majority of goblet cells.

, Ren, Prost…

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

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **27G12**	69 2 1 1	Leica/Novocastra Biocare Monosan Genetech	28	35	6	4	86%	88%
mAb clone **BS15**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **DAK-SYNAP**	12	Dako	7	3	2	0	83%	100%
mAb clone **SNP88**	7	Biogenex	2	5	0	0	100%	100%
mAb clone **SY38***	3	Dako	0	2	1	0	-	-
rmAb clone **MRQ-40**	5 1	Cell Marque Monosan	3	2	1	0	83%	100%
rmAb clone **SP11**	10 2 1 1	Thermo/Neomarkers Spring Bioscience Abcam Immunologic	7	4	3	0	79%	83%
pAb **180130**	1	Immuno Diagnostics	0	0	1	0	-	-
pAb **RB-1461**	1	Thermo/Neomarkers	0	0	1	0	-	-
pAb **RBK011**	1	Zytomed	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **27G12 PA0299**	9	Leica/Novocastra	3	3	2	1	67%	100%
mAb clone **27G12 PM371**	1	Biocare	0	1	0	0	-	-
mAb clone **DAK-SYNAP IR660**	38	Dako	11	23	4	0	89%	90%
mAb clone **SNP88 AM363-5M**	2	Biogenex	0	1	1	0	-	-
mAb clone **SY38 IR/IS776***	5	Dako	0	2	2	1	-	-
rmAb **MRQ-40 760-4595**	31	Ventana/Cell Marque	23	7	1	0	97%	100%
rmAb clone **MRQ-40 336R**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **SP11 790-4407**	33	Ventana	9	14	9	1	70%	81%
rmAb clone **SP11 KIT-0022**	1	Maixin	0	1	0	0	-	-
rmAb clone **SP11 MAD-000313QD**	2	Master Diagnostica	0	1	1	0	-	-
pAb **336A-78**	1	Cell Marque	0	1	0	0	-	-
Total	243		95	105	35	8	-	
Proportion			39%	43%				

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

Clone
HIER high pH

3-step detection

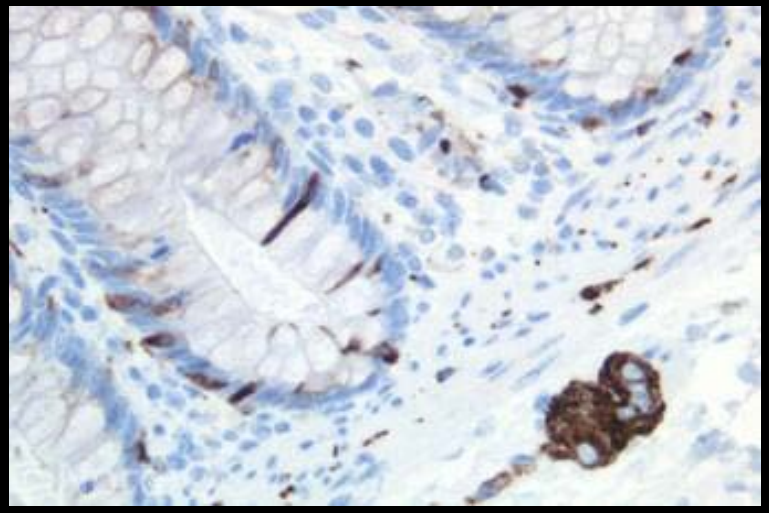
Calibration……

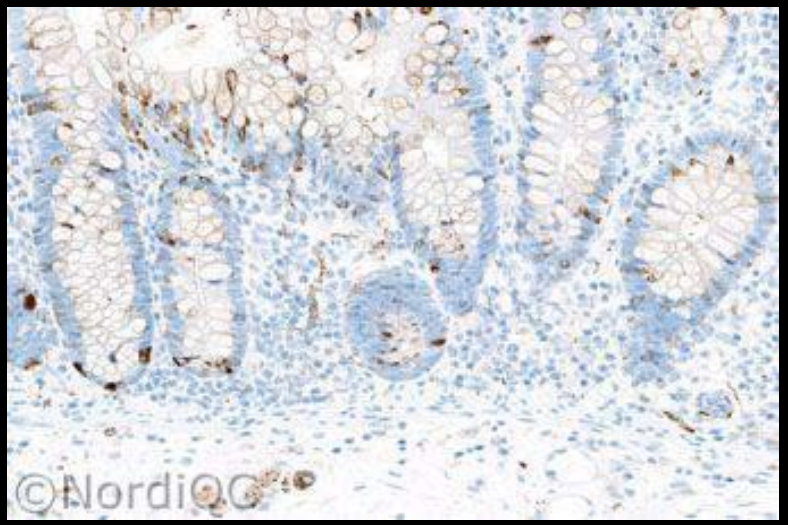
SY38 no-go

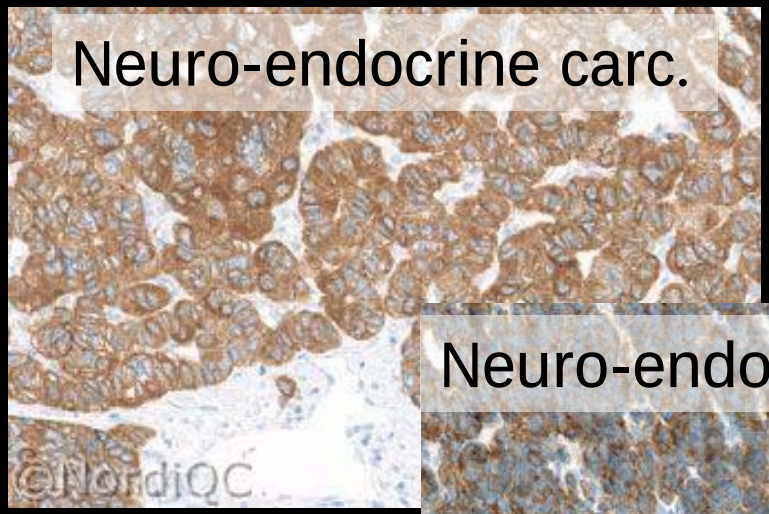
Table 2. **Proportion of sufficient results for SYP in the five NordiQC runs performed**

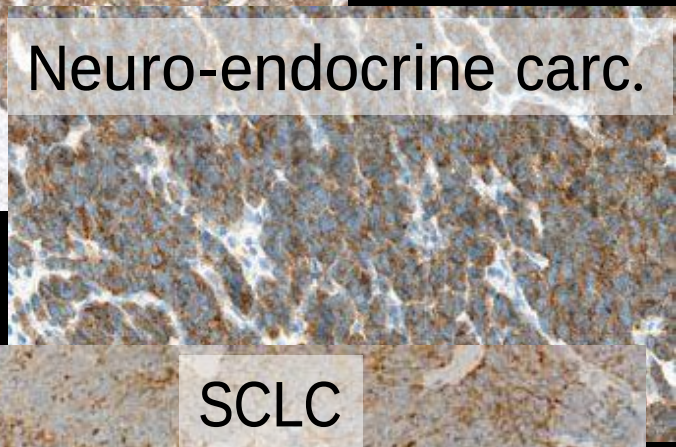
	Run 18 2006	Run 22 2008	Run 29 2010	Run 37 2013	Run 43 2015
Participants, n=	94	112	151	214	243
Sufficient results	68%	58%	55%	58%	82%

IHC – Protocols and controls – End, Ren, Prost…









Level of target analyte must be same in controls as clinical samples

IHC

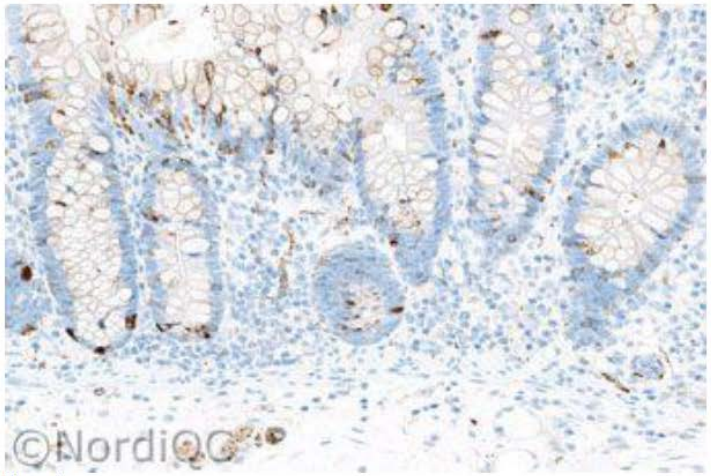


Fig. 1a
Optimal SYP staining of the colon using the rmAb clone MRQ-40, optimally calibrated and with HIER in an alkaline buffer. The peripheral nerves
strong and distinct cyt
smooth muscle cells ar
a weak to moderate cy
Also compare with Figs

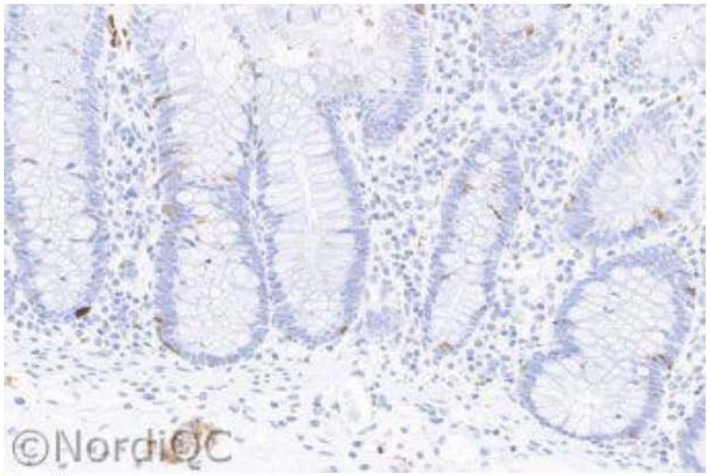


Fig. 1b
Insufficient SYP staining of the colon using the mAb clone Snp88 by protocol settings giving a too low sensitivity (too low
on system) – same
nonstrated, while the
action in the
ıg reaction is seen in
rotocol.

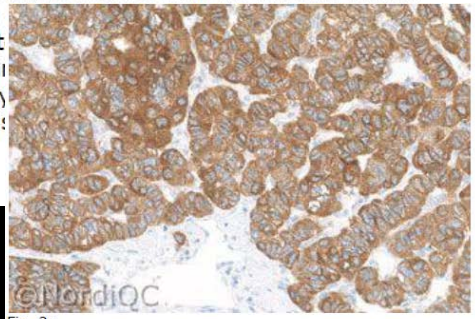


Fig. 2a
Optimal SYP staining of the pancreatic neuroendocrine carcinoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct staining reaction. No background staining is seen.

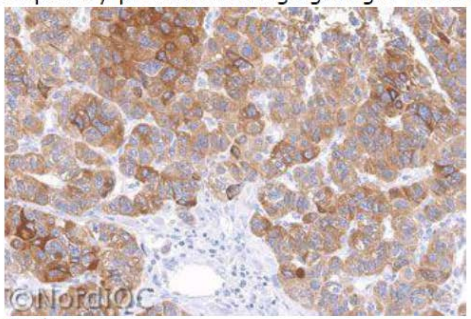


Fig. 2b
Staining for SYP of the pancreatic neuroendocrine carcinoma using same insufficient protocol as in Fig. 1b - same field as in Fig 2a. The vast majority of the neoplastic cells are demonstrated. However also compare with Fig. 3b – same protocol.

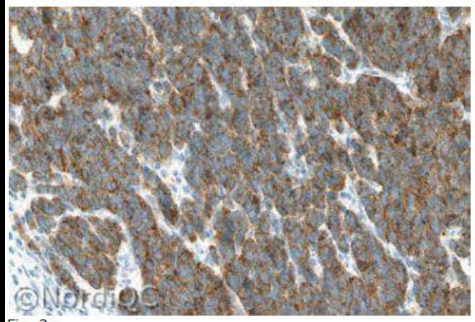


Fig. 3a
Optimal SYP staining of the SCLC using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining reaction with a dot-like accentuation. No background staining is seen.

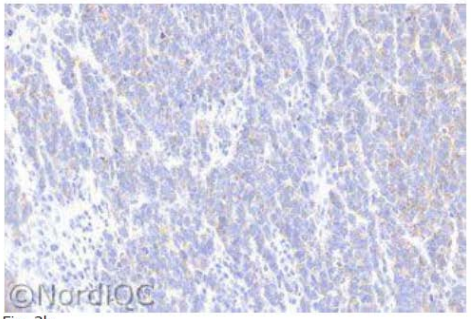


Fig. 3b
Insufficient staining SYP of the SCLC using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
Only scattered neoplastic cells show a weak and diffuse cytoplasmic staining reaction.

Goblet cells
+
Endocrine cells

Nerves

No-go!

Table 1. **Antibodies and assessment marks for CD56, run 37.**

Concentrated Abs	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1B6**	42 1 1	Novocastra/Leica Linaris Vector Lab.	16	17	10	1	75%	77%
mAb clone **123C3**	18 4 2 1	Dako Monosan Invitrogen Spring Bioscience	10	10	3	2	80%	100%
rmAb clone **MRQ-42**	21 1	Cell Marque Immunologic	21	1	0	0	100%	100%
mAb clone **123C3.D5**	18 1	NeoMarkers/Thermo Immunologic	5	6	5	3	58%	100%
mAb clone **CD564**	8 1	Novocastra/Leica Monosan	5	4	0	0	100%	100%
mAb clone **56C04**	2	NeoMarkers/Thermo	1	1	0	0	-	-
rmAb clone **RCD56**	1	Zytomed System	0	0	1	0	-	-
Ready-To-Use Abs:								
mAb clone **123C3, IR628**	34	Dako	16	13	3	2	85%	88%
rmAb clone **MRQ-42 760-4596**	16	Ventana	14	2	0	0	100%	100%
mAb clone **123C3, 790-4465**	9	Ventana	2	1	6	0	33%	-
mAb, clone **CD564, PA0191**	6	Novocastra/Leica	3	3	0	0	100%	100%
mAb, clone **1B6**	4	Novocastra/Leica	0	2	0	2	-	
mAb, clone **123C3.D5, Mon-RTU1049**	1	Monosan	0	1	0	0	-	
mAb clone **BC56C04, PM164**	2	Biocare	0	2	0	0	-	
rmAb clone **MRQ-42, 156R-97**	1	Cell Marque	1	0	0	0	-	
mAb clone **56C04, MAD-000218QD**	1	Master Diagnostica	1	0	0	0	-	
Total	196		95	63	28	10		
Proportion			49%	32%	14%	5%	81%	

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

, Prost...

NordiQC

Clone to platform

HIER high pH

3-step detection

Calibration.....

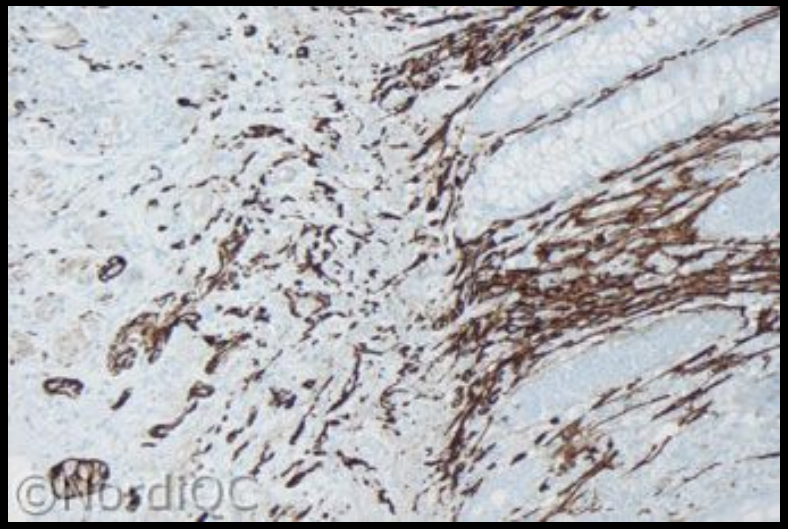
Run 37

VMS users changed Ab >60% to MRQ-42 (LDT)

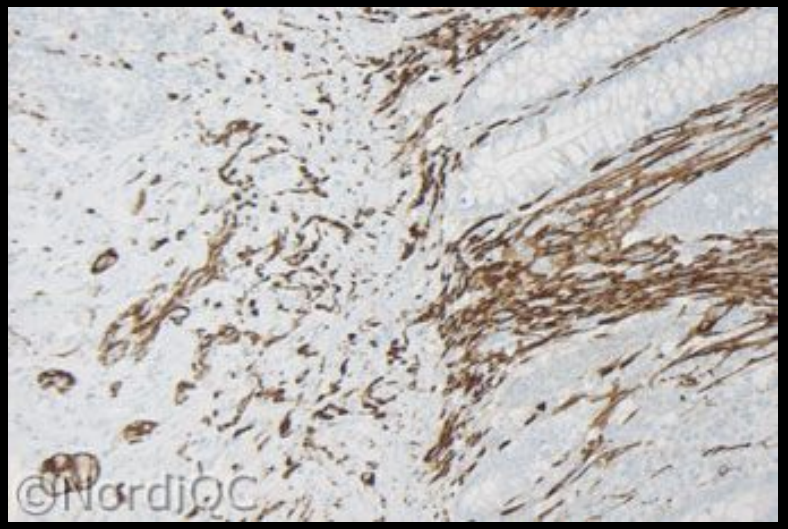
Table 2. **Proportion of sufficient results for CD56 in the two NordiQC runs performed.**

	Run 31 2011	Run 37 2013
Participants, n=	153	196
Sufficient results	48%	81%

NordiQC



CD56: Optimal

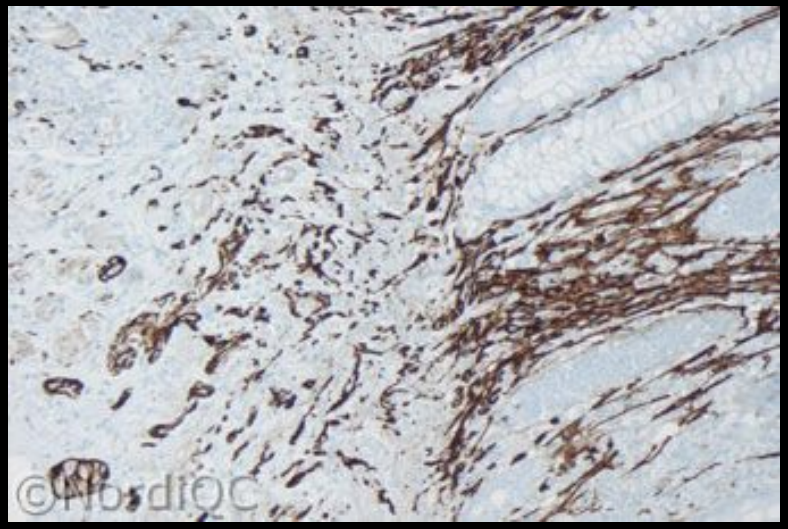


Poor - Insufficient.....

Colon/App. used as external positive and negative control

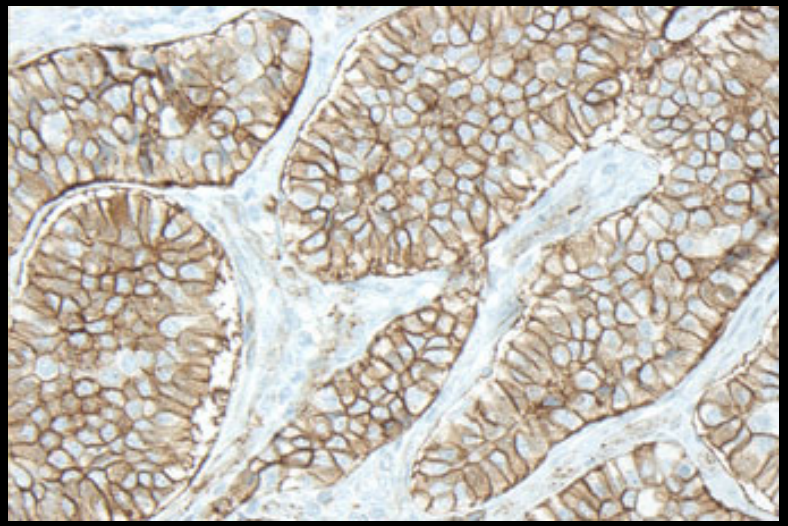
Virtually all nerves strongly positive
The epithelial cells negative

NordiQC

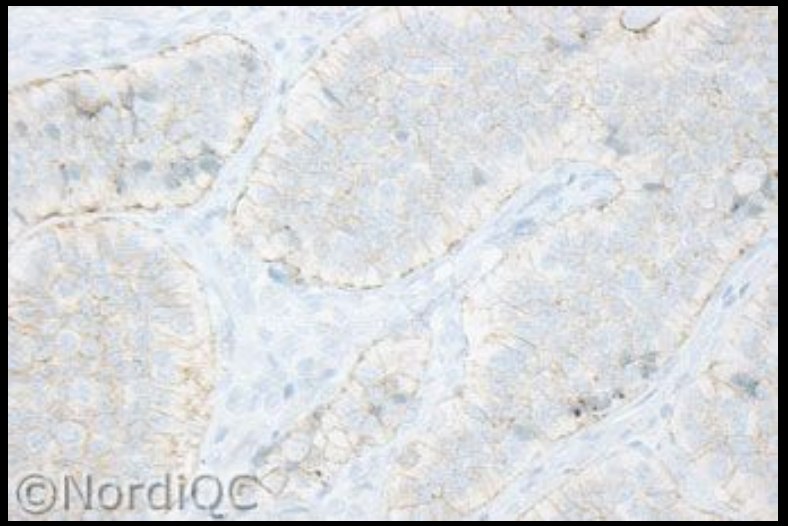


CD56: Optimal

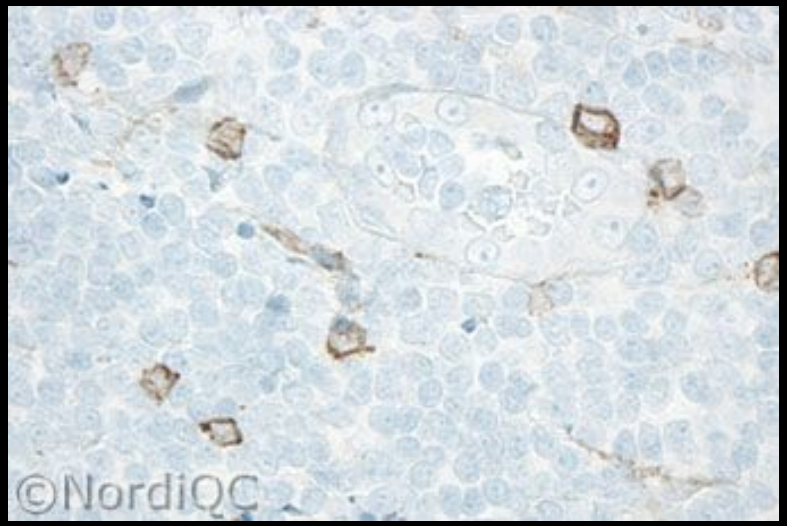
Insufficient.....



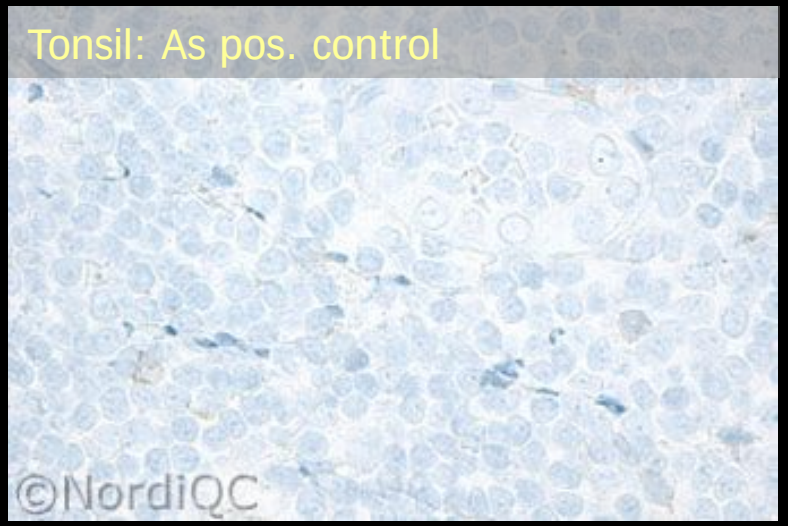
Neuroendocrine carcinoma



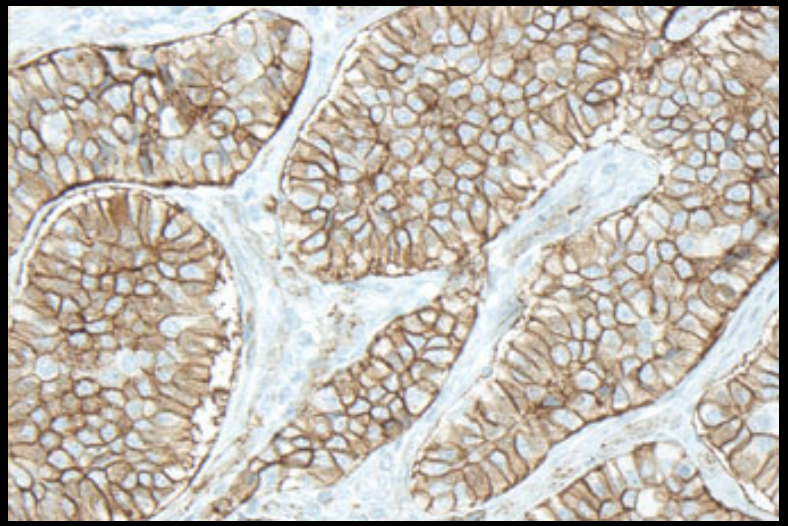
NordiQC



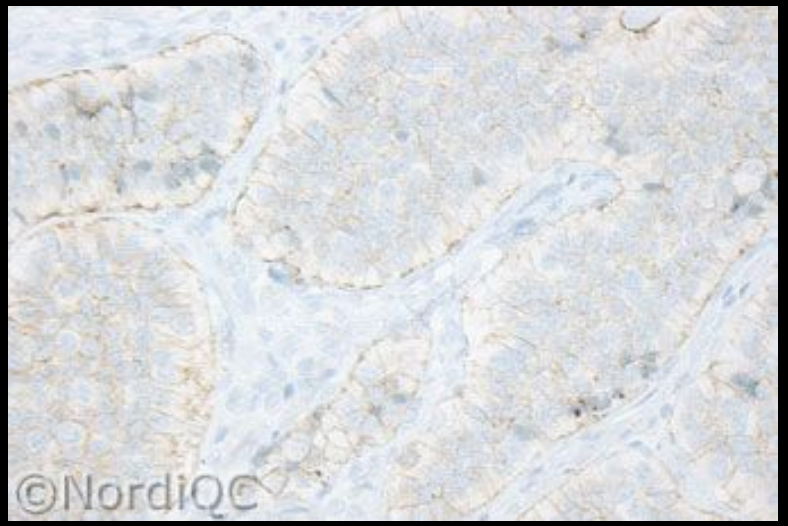
CD56: Optimal



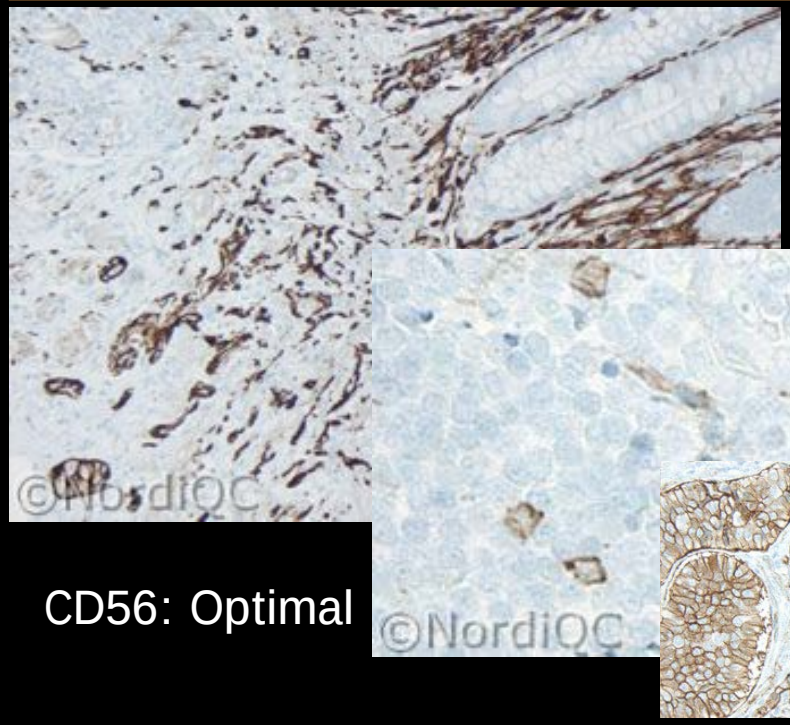
Insufficient



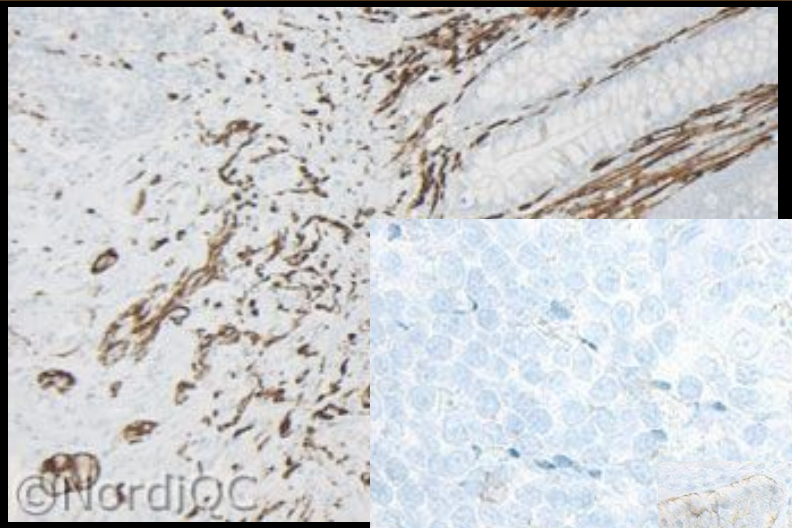
Neuroendocrine carcinoma



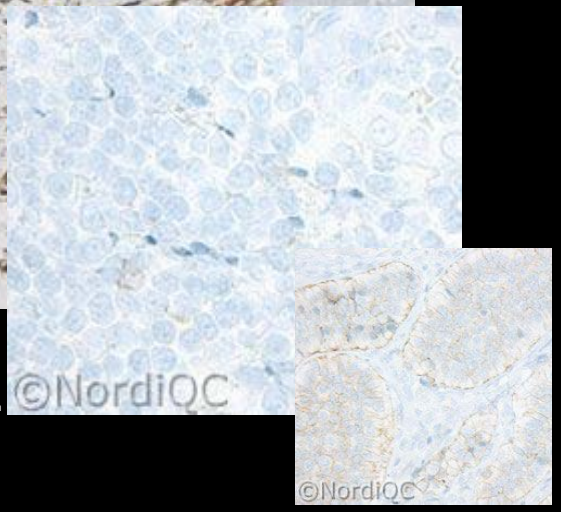
NordiQC



CD56: Optimal



Insufficient....



Tissues/cells with only high expression will not identify:

1. A poorly calibrated IHC assay
2. A reduced sensitivity in an optimally calibrated IHC assay

If an IHC test is used to demonstrate the target antigen being expressed at different levels, the controls must reflect this !

IHC – Protocols and controls – End, Ren, Prost…

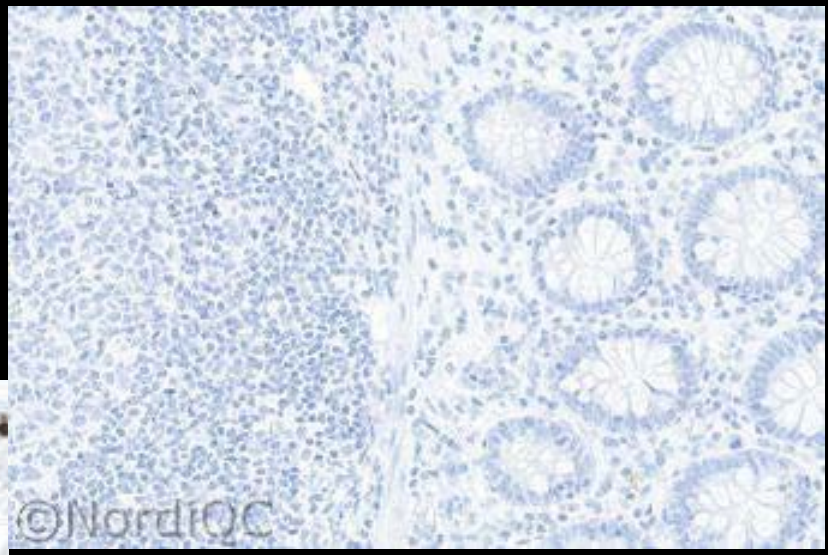
	Recommendable clones (conc.)	Less successful clones (conc.)	RTU ”plug and play” giving optimal result
OCT3/4	mAb C10 mAb MRQ-10 mAb N1NK	pAbs	Leica: mAb N1NK VMS: mAb MRQ-10
SALL4	mAb 6E3		VMS: mAb 6E3

IHC – Protocols and controls – End, Ren, Prost…

	Positive tissue control HE	Positive tissue control LE	Negative tissue control NE
OCT3/4	Germ cell neoplasia in situ: Neoplastic cells	?	Appendix: Epithelial cells
SALL4	Germ cell neoplasia in situ: Neoplastic cells	Testis: Spermatogonia cells	Appendix: Epithelial cells

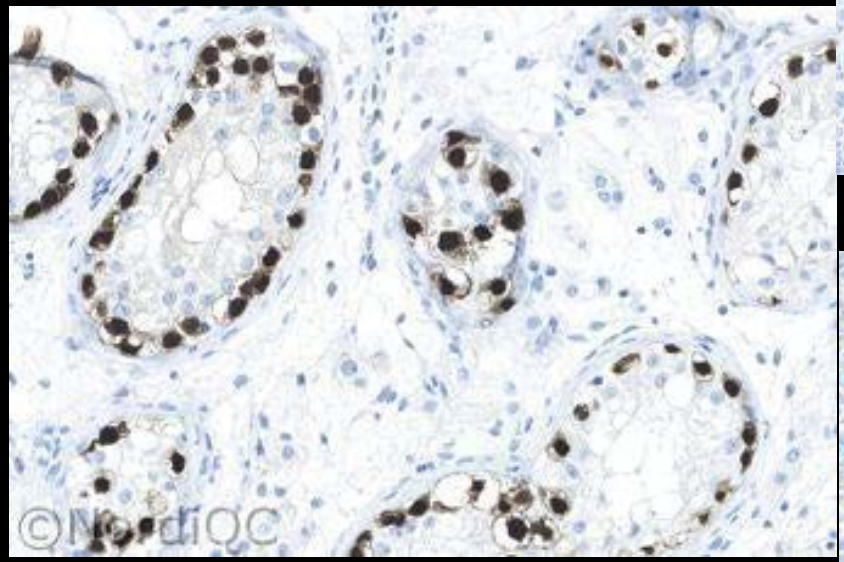
IHC – Protocols and controls – End, Ren, Prost…

mAb
C10
N1NK

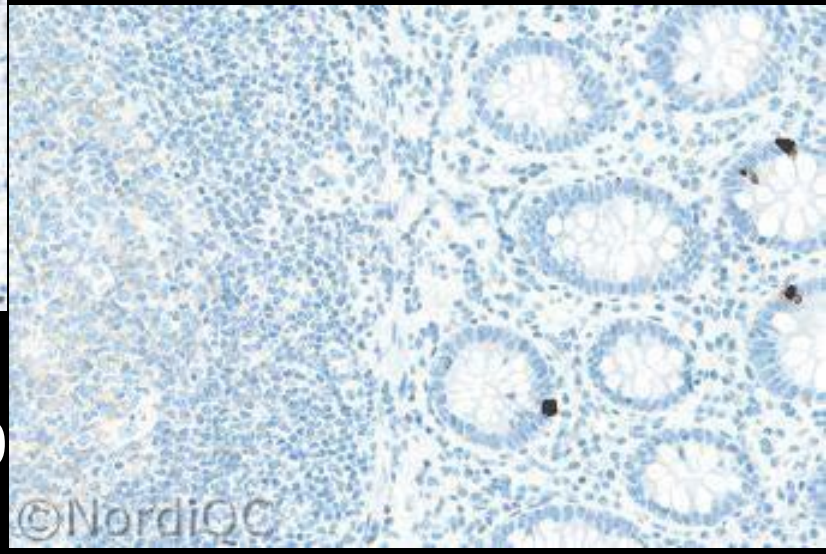


Germ cell neoplasia in situ

mAb
C10
MRQ-10
N1NK



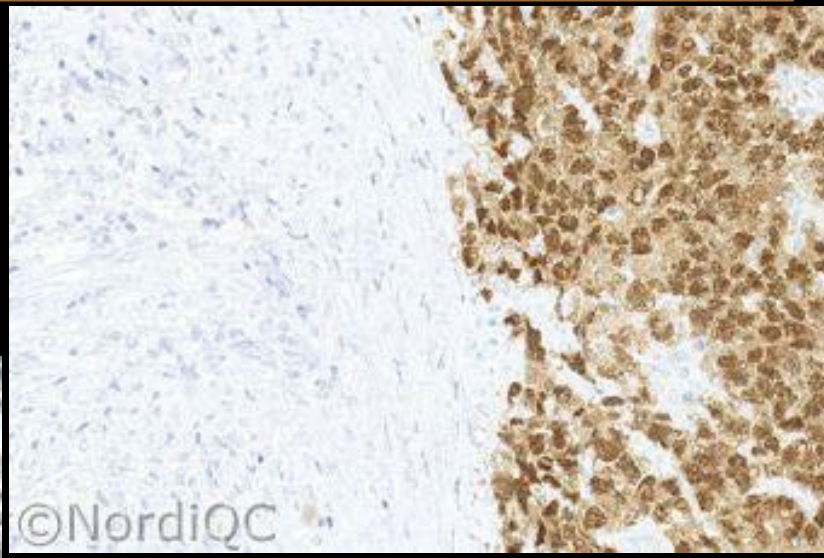
mAb
MRQ-10



Appendix/Colon

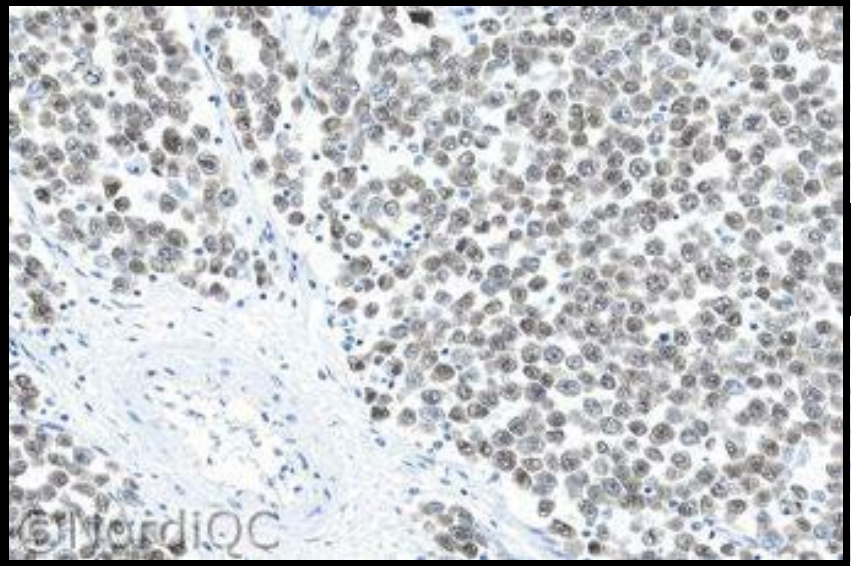
IHC – Protocols and controls – End, Ren, Prost…

mAb
C10
N1NK

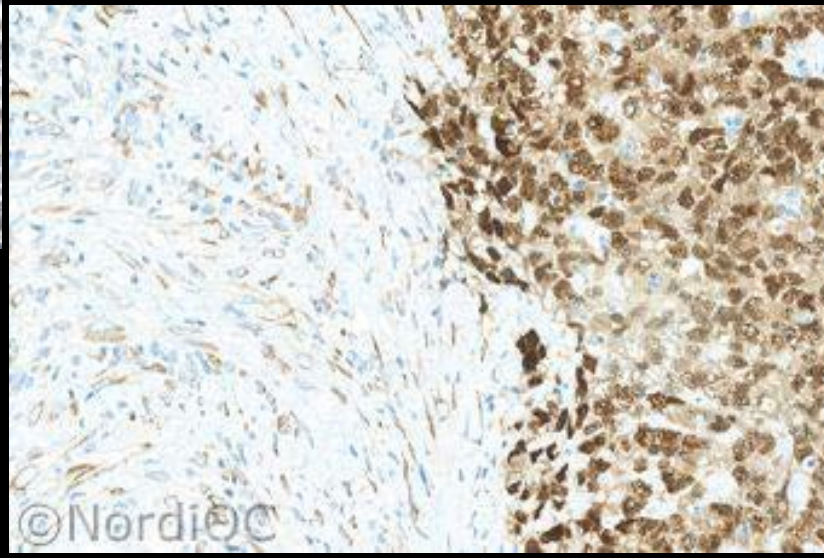


Seminoma

mAb
C10
MRQ-10
N1NK



mAb
MRQ-10



”sensitive to delayed fix”

Embryonal carc (myofibro)

IHC – Protocols and controls – End, Ren, Prost…

Table 1. **Antibodies and assessment marks for SALL4, run 43**

Concentrated antibodies:	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **6E3**	4 6 2 10 1 2 3 1 1 1	Abnova Biocare Biosite Cell Marque Master Diagnostica Novus Biological Sigma Aldrich Abcam Beijingzhongsan Novus Biologicals	22	8	1	0	98%	97%
Ready-To-Use Abs:								
mAb clone **6E3**	8	Ventana/Cell Marque	5	3	0	0	100%	100%
mAb clone **6E3 CM385**	7	Cell Marque	5	2	0	0	100%	100%
mAb clone **6E3 MAD-000572QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **6E3 MAB-0691**	2	Maixin	2	0	0	0	-	-
mAb clone **6E3 PM384**	1	Biocare	1	0	0	0	-	-
Total	51		36	14	1	0	-	
Proportion			71%	27%	2%	0	98%	

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

SALL4 the ”perfect IHC assay”
1 Ab – 1 clone
High affinity – High specificity – Robust
Tissue control with low level expression

IHC – I

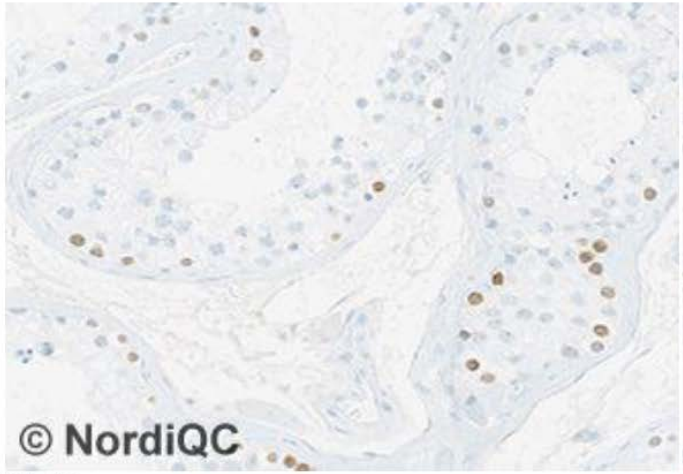


Fig. 1a
Optimal SALL4 staining of normal testis using the mAb clone 6E3 as a concentrate (CM384, Cell Marque) optimally calibrated at a titre of 1:100, HIER in CC1 FOR 48 min. and a 3-step multimer based detection system (OptiView 760-700, Ventana). Spermatogonia at the basement membrane of the tubules show a moderate distinct nuclear staining reaction and no background staining is seen. Also compare with Figs. 2a – 4a, same protocol.

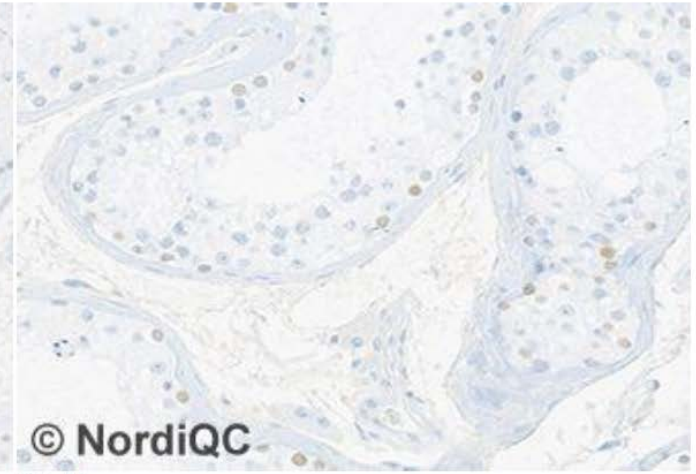


Fig. 1b
Staining for SALL4 of the normal testis assessed as "Good". The intensity of the nuclear staining reaction in the spermatogonia is reduced compared to the result obtained in Fig. 1a – same field. However also compare with Figs. 2b and 3b, same protocol. A fully diagnostic sufficient result overall is obtained. The protocol was based on the same mAb and titre as in Fig. 1a, but used with HIER for 32min. in CC1 and a 2-step multimer based system (UltraView 760-500, Ventana).

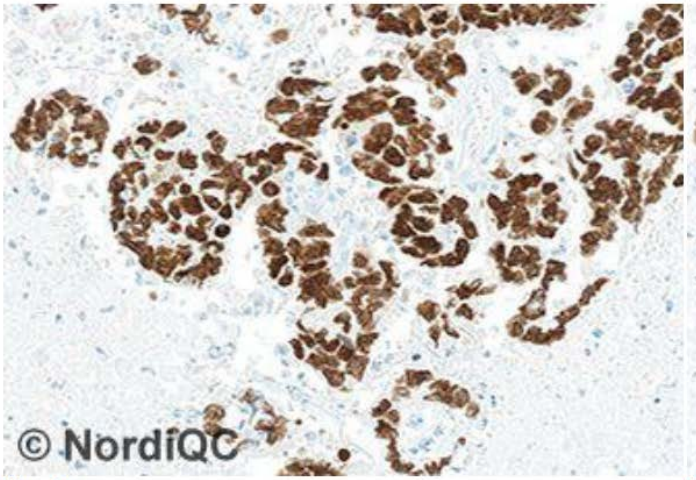


Fig. 2a
Optimal staining for SALL4 of the embryonal carcinoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct nuclear staining reaction. No background staining is seen.

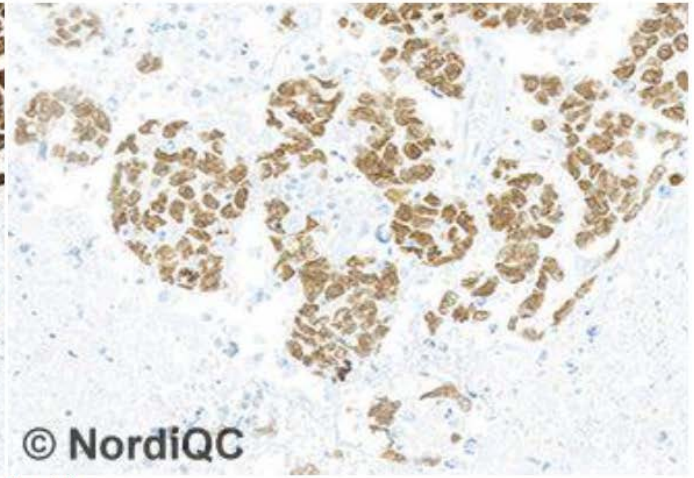


Fig. 2b
Staining for SALL4 of the embryonal carcinoma assessed as "Good" using same protocol as in Fig. 1b - same field as in Fig. 2a. The neoplastic cells are demonstrated, but the intensity is reduced.

IHC – Protocols and controls – End, Ren, Prost…

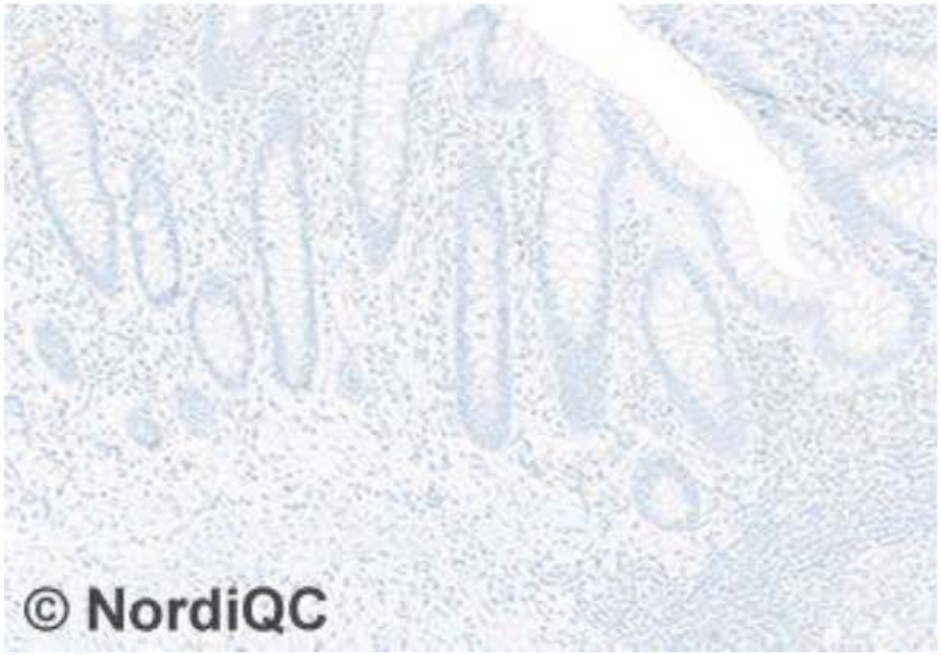


Fig. 4a
Optimal staining for SALL4 of the appendix using same protocol as in Figs. 1a - 3a. No staining reaction is seen.

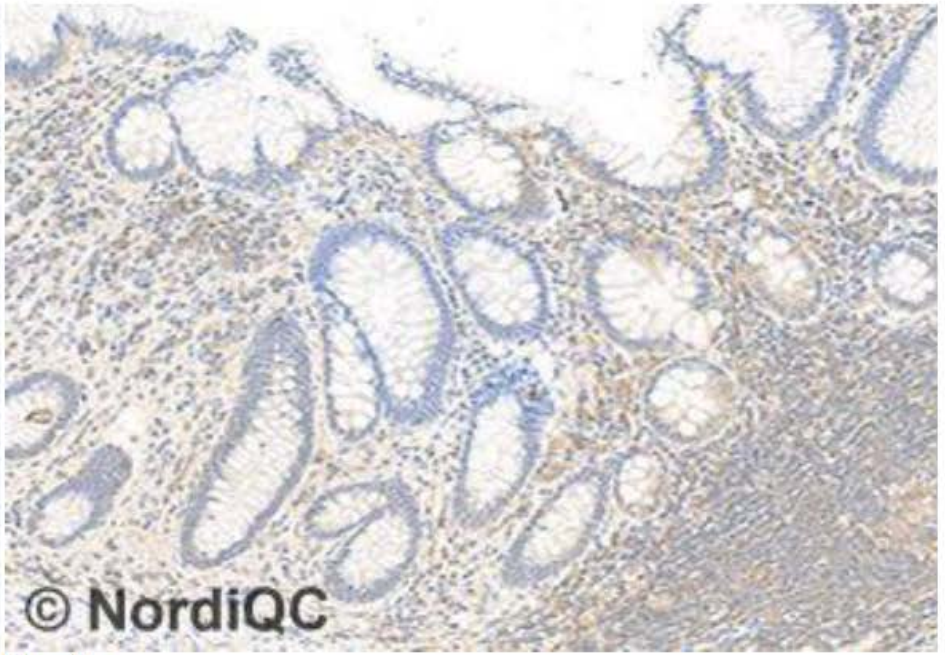


Fig. 4b
Insufficient staining reaction for SALL4 of the appendix. A diffuse background staining and aberrant cytoplasmic staining reaction of stromal cells, lymphocytes etc. complicates the interpretation. The result was assessed as "Borderline" and most likely caused by a too high concentration of the primary mAb clone 6E3.

IHC – Protocols and controls – End, Ren, Prost…

	Recommendable clones (conc.)	Less successful clones (conc.)	RTU ”plug and play” giving optimal result
PSA	mAb 35H9 mAb ER-PR8 rmAb EP109 pAb 0562		Dako: pAb Leica: mAb 35H9 VMS: pAb
NKX3.1	mAb UMAB196 rmAb EP356 pAb CP422		

IHC – Protocols and controls – End, Ren, Prost…

	Positive tissue control HE	Positive tissue control LE	Negative tissue control NE
PSA	Prostate: Luminal epithelial cells	.....	Appendix: Epithelial cells
NKX3.1	Prostate: Luminal epithelial cells.	*Prostate: Basal cells* Testis: Germ cells	Appendix: Epithelial cells

Table 1. **Antibodies and assessment marks for PSA, run 49**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **35H9**	21 1 1 1 1	Leica/Novocastra Monosan Abnova Diagnostic Biosystem Gene Tech	21	3	1	0	96%	95 %
mAb clone **ER-PR8**	31 3 1 1	Dako/Agilent Cell Marque Zeta Zytomed Systems	19	11	6	0	82%	82%
mAb clone **ER-PR8+A67-B/E3***	1	Biocare Medical	1	0	0	0	-	-
mAb clone **28A4***	1	Leica/Novocastra	0	0	1	0	-	-
rmAb clone **EP109**	5 1	Biocare Medical Cell Marque	6	0	0	0	100%	100%
pAb **0562**	62	Dako/Agilent	33	16	12	1	79%	85%
Ready-To-Use antibodies								
mAb clone **35H9 PA0431**	11	Leica Biosystems	6	5	0	0	100%	100%
mAb clone **35H9 PDM087**	1	Diagnostic biosystems	1	0	0	0	-	-
mAb clone **ER-PR8 760-4271**	18	Ventane/Roche	8	8	2	0	89%	87%
mAb clone **ER-PR8 760-4930**	3	Cell Marque	1	2	0	0	-	-
mAb clone **ER-PR8 324M-17/18**	2	Cell Marque	1	1	0	0	-	-
mAb clone **ER-PR8 AM014-10M**	2	Biogenex	2	0	0	0	-	-
mAb clone **ER-PR8 MAD-000532QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **ER-PR8 MAB-0146**	1	Maixin	1	0	0	0	-	-
rmAb clone **EP109 PME390**	1	Biocare medical	1	0	0	0	-	-
pAb **760-2506**	51	Ventana/Roche	34	11	5	1	88%	93%
pAb **IS/IR514**	33	Dako/Agilent	31	2	0	0	100%	100%
pAb **IS/IR514**[3]	5	Dako/Agilent	4	0	1	0	-	-
pAb **GA514**	20	Dako/Agilent	20	0	0	0	100%	100%
pAb **GA514**[4]	3	Dako/Agilent	2	1	0	0	-	-
Total	284		193	61	28	2	-	
Proportion			68%	21%	10%	1%	89%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only (see below).
3) RTU system developed for the Dako/Agilent´s semi-automated systems (Autostainer Link/+) but used by laboratories on different platforms (e.g. Ventana Benchmark).
4) RTU system developed for the Dako/Agilent´s full-automated systems (Omnis) but used by laboratories on different platforms (e.g. Ventana Benchmark).
* Discontinued by the vendor

nd, Ren, Prost...

NordiQC

pAb slightly inferior (LDT)

Best performance:
mAb 35H9
rmAb EP109

<u>HIER</u>
2 & 3-step methods

IHC – Protocols and controls – End, Ren, Prost…

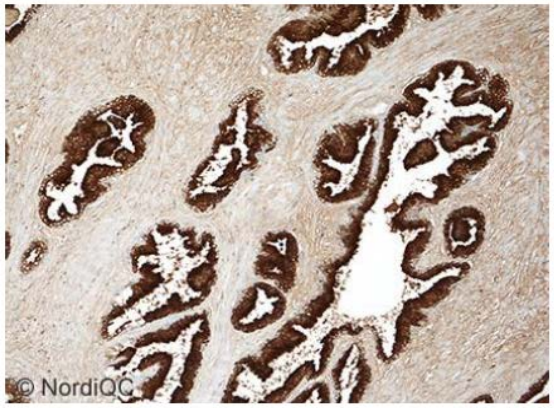


Fig. 1a (x100)
Optimal staining for PSA of the prostate hyperplasia using the pAb 760-2506 (RTU format , Ventana), HIER in an alkaline buffer (CC1) and a multimer based detection system (UltraView, Ventana) - same RTU format used in Figs. 2a - 5a.
The prostate glands show a strong distinct cytoplasmic staining reaction. A weak to moderate stromal reaction is seen (due to leakage of the antigen), which has to be accepted for optimal performance.

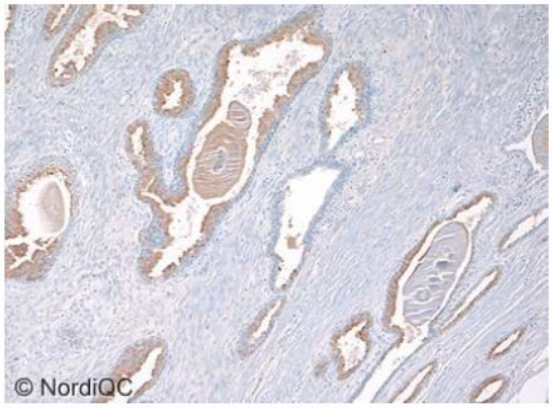


Fig. 1b (x100)
Insufficient staining for PSA of the prostate hyperplasia using the pAb 760-2506 (RTU format , Ventana no pre-treatment and UltraView (Ventana) as the detection system - same protocol used in Figs. 2b – 3b.
The intensity of the staining reaction is significantly reduced and stromal reactivity is absent - compare with Fig. 1a (same field).

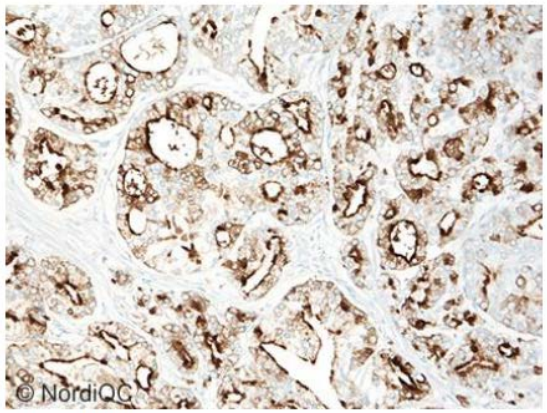


Fig. 3a (x200)
Optimal staining for PSA in the prostate adenocarcinoma, core 4, using same protocol as in Figs. 1a and 2a.
The majority of the neoplastic cells shows a weak to moderate but distinct cytoplasmic staining reaction.

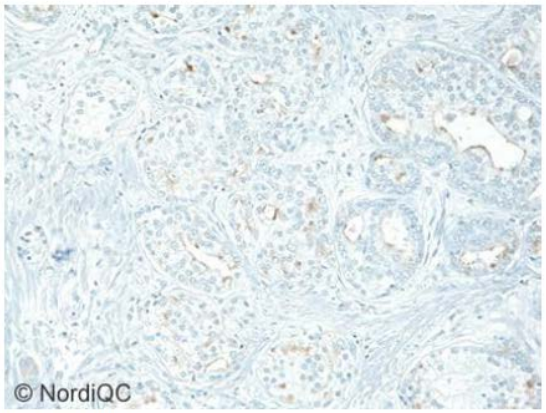


Fig. 3b (x200)
Insufficient staining for PSA in the prostate adenocarcinoma, core 4 using same protocol as in Figs. 1b and 2b.
The intensity of the neoplastic cells is significantly reduced and some glandular structures are completely negative - compare with Fig. 3a.

IHC – Protocols and controls – End, Ren, Prost…

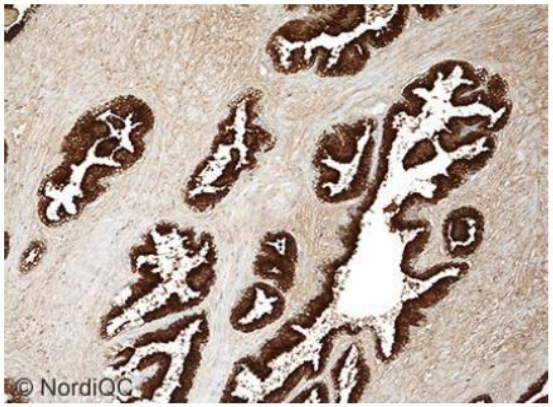


Fig. 1a (x100)
Optimal staining for PSA of the prostate hyperplasia using the pAb 760-2506 (RTU format , Ventana), HIER in an alkaline buffer (CC1) and a multimer based detection system (UltraView, Ventana) - same RTU format used in Figs. 2a - 5a.
The prostate glands show a strong distinct cytoplasmic staining reaction. A weak to moderate stromal reaction is seen (due to leakage of the antigen), which has to be accepted for optimal performance.

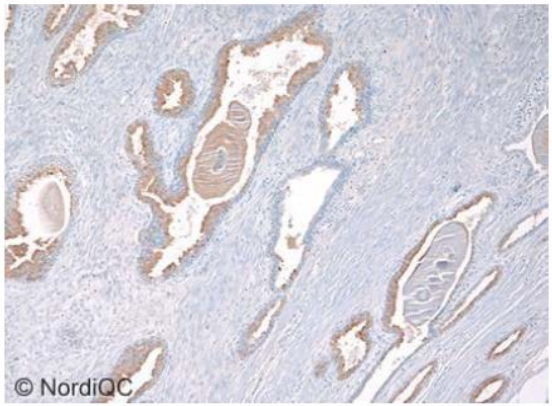


Fig. 1b (x100)
Insufficient staining for PSA of the prostate hyperplasia using the pAb 760-2506 (RTU format , Ventana no pre-treatment and UltraView (Ventana) as the detection system - same protocol used in Figs. 2b – 3b.
The intensity of the staining reaction is significantly reduced and stromal reactivity is absent - compare with Fig. 1a (same field).

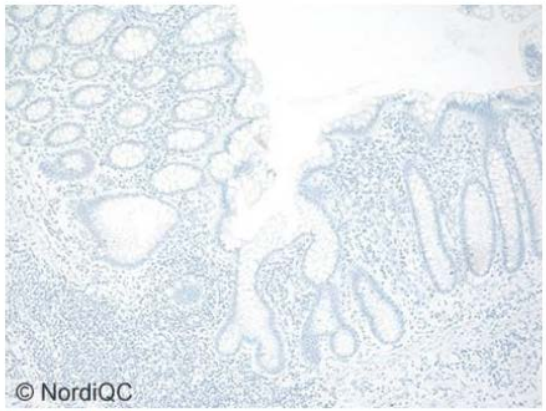
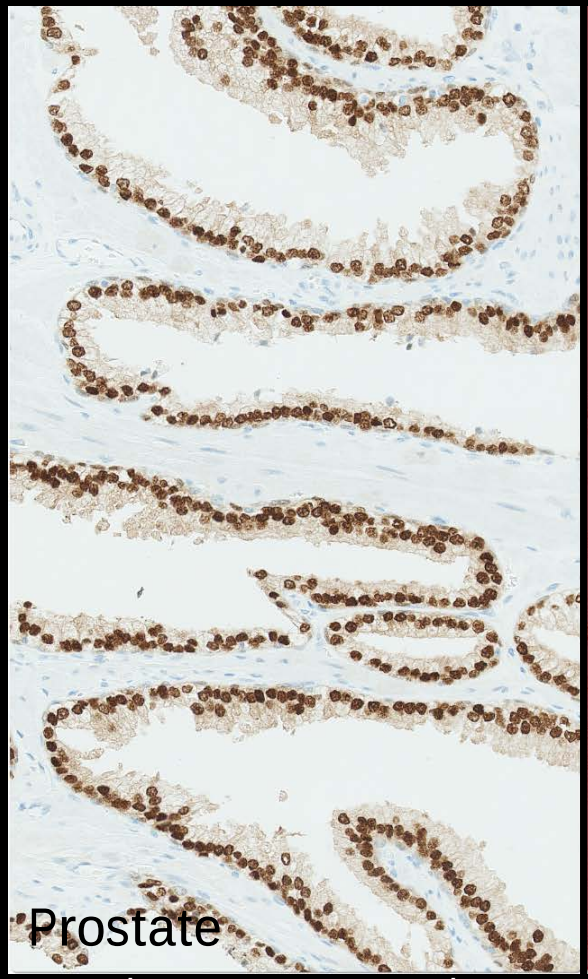


Fig. 4a (x100)
Optimal staining for PSA of the appendix using same protocol as in Figs. 1a - 3a. As expected, no staining reaction is seen of the epithelium and stromal cells.

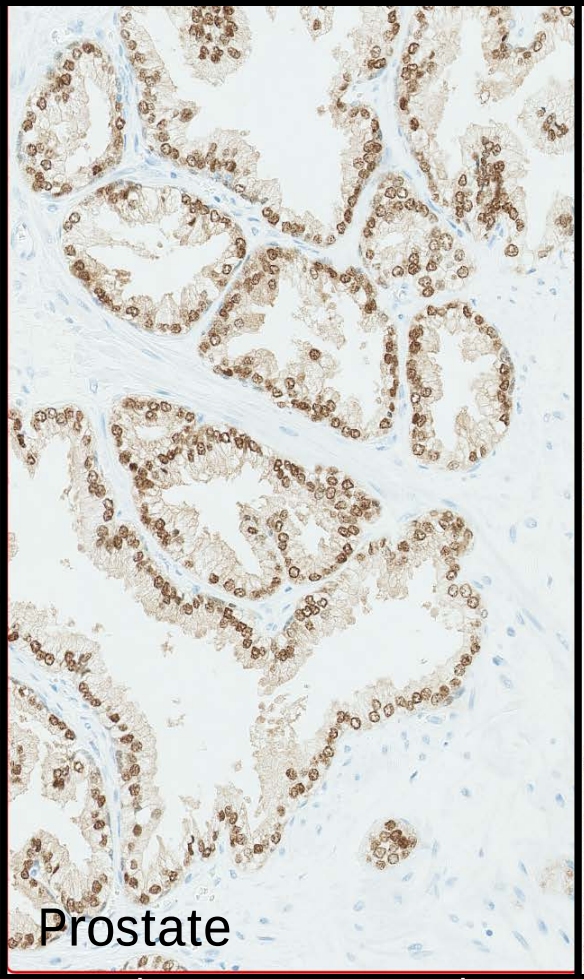
Fig. 4b (x100)
Insufficient staining for PSA of the appendix using the pAb 0562 as concentrate (too high concentration), HIER in alkaline buffer (TRIS-EDTA) and a polymer based detection system (EnVision, Dako) – same protocol used in Fig. 5b.
The epithelial cells and scattered stromal cells are false positive - compare with Fig. 4a.

IHC – Protocols and controls – End, Ren, Prost…

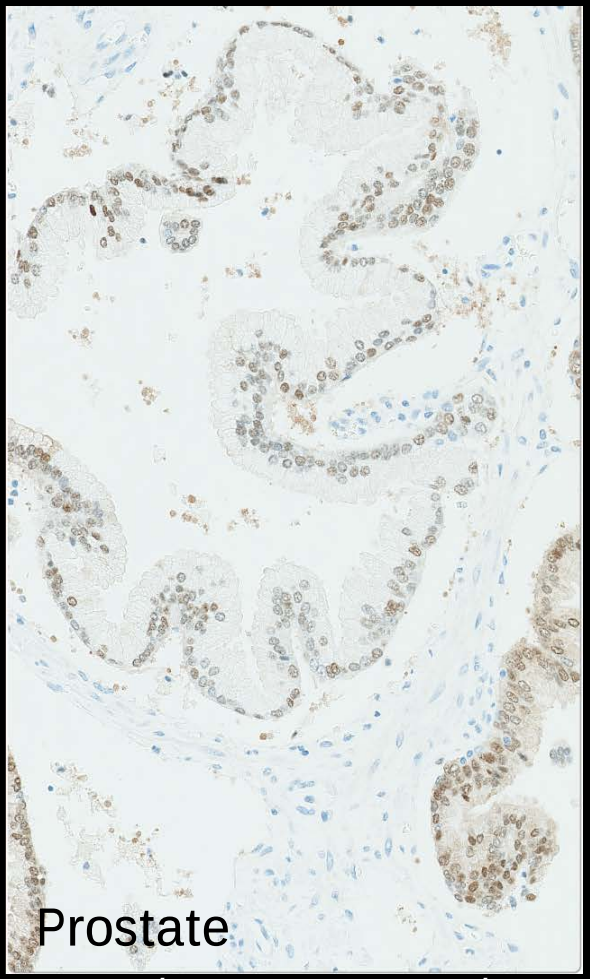
NKX3.1 reaction pattern



A moderate to strong nuclear staining reaction of the vast majority of luminal epithelial cells.



A moderate to strong nuclear staining reaction of the vast majority of luminal epithelial cells.

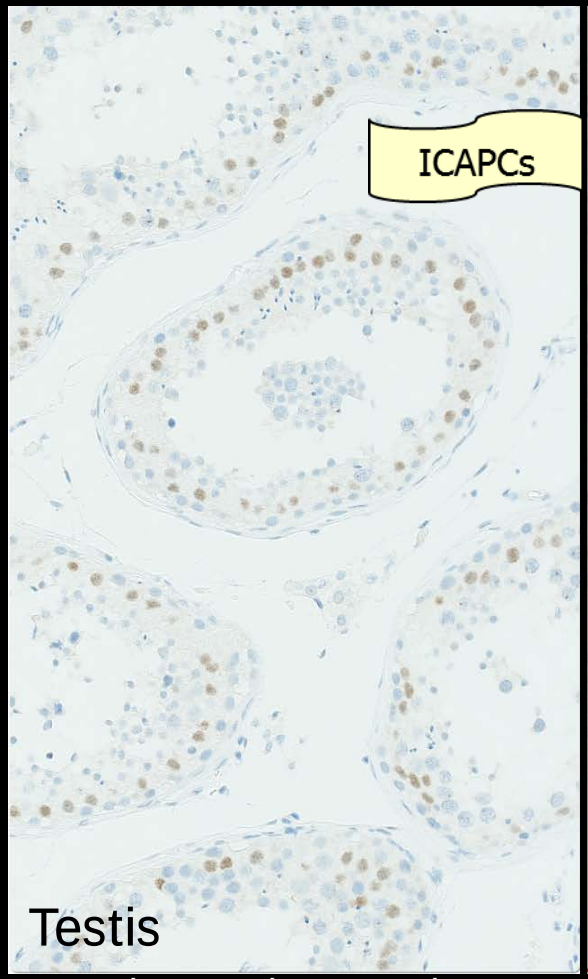


A moderate to strong nuclear staining reaction of the vast majority of luminal epithelial cells.

A certain variation in NKX3.1 expression in different prostate specimens....

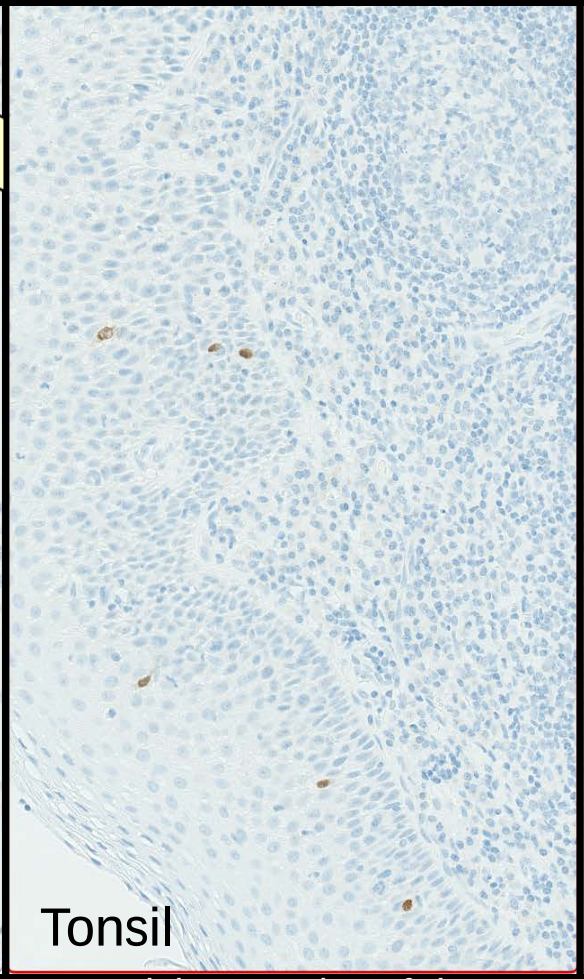
IHC – Protocols and controls – End, Ren, Prost…

NKX3.1 reaction pattern



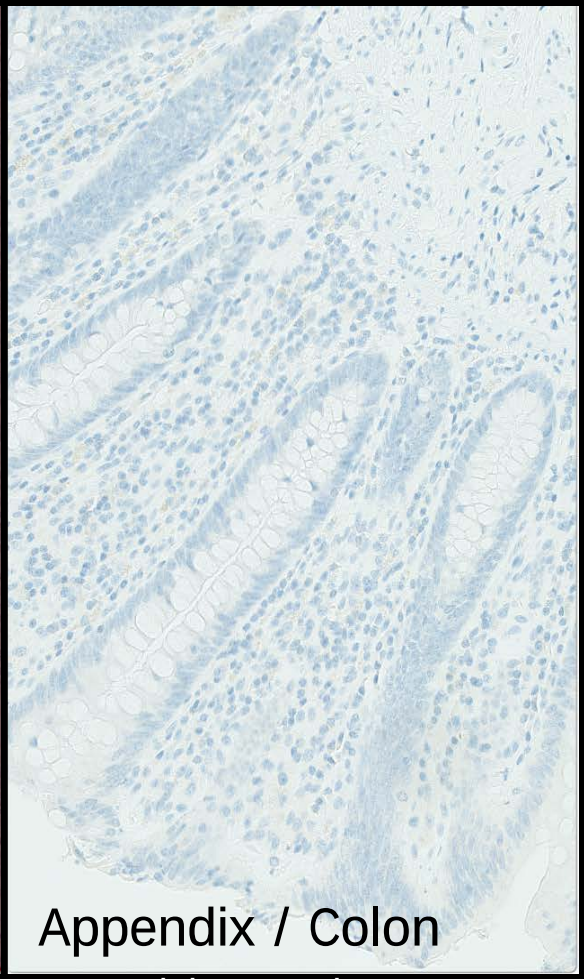
Testis

A weak to moderate nuclear staining reaction of dispersed germ cells.



Tonsil

No staining reaction of the vast majority of cells. Dispersed squamous epithelial cells can be demonstrated.

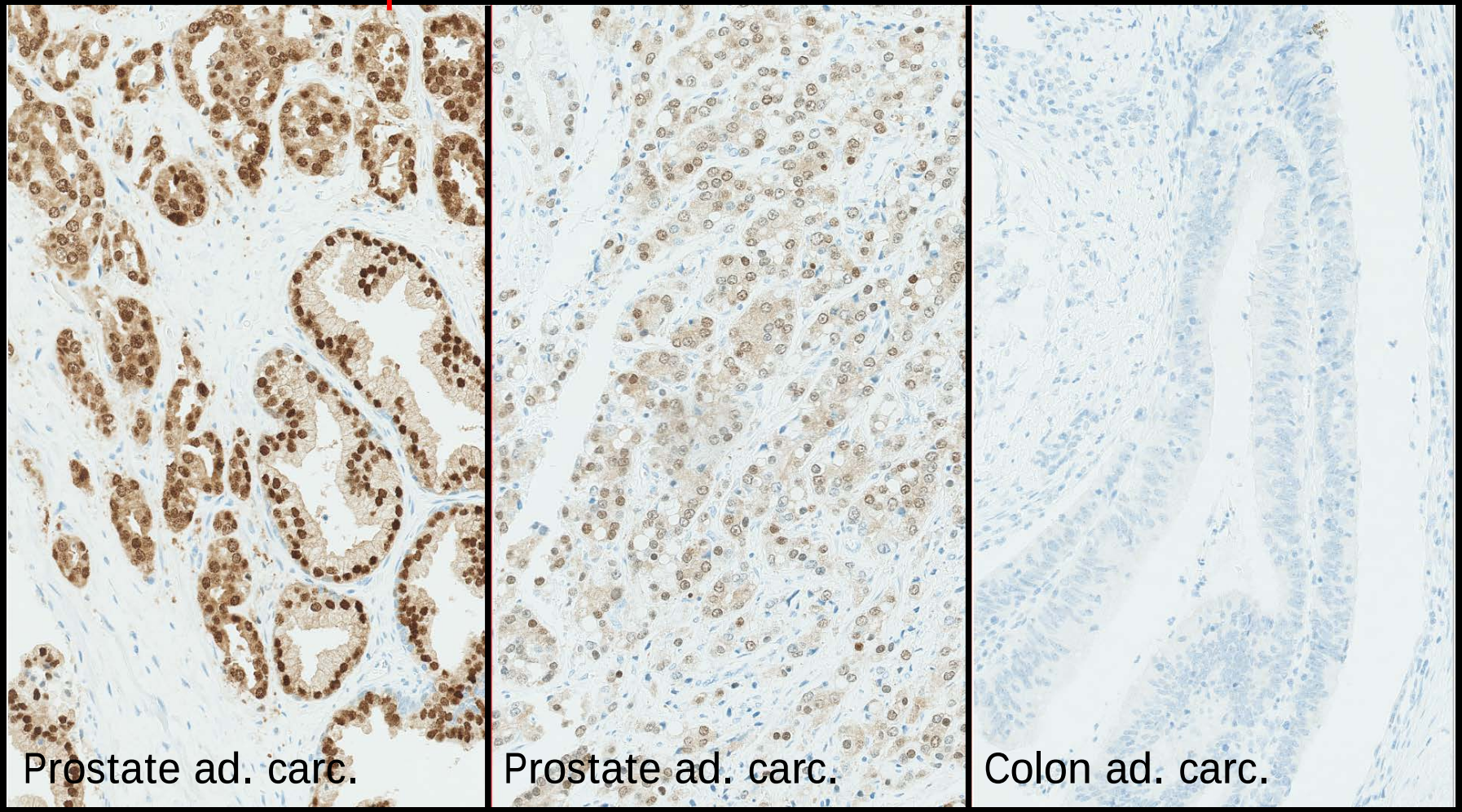


Appendix / Colon

No staining reaction.

IHC – Protocols and controls – End, Ren, Prost…

NKX3.1 reaction pattern



Internal studies:

+ 18 of 18 prostate adenocarcinomas
10% cut-off

- in 39 of 39 other neoplasias

IHC – Protocols and controls – End, Ren, Prost…

NKX3.1

	Retrieval	Titre	Detection	RTU	Detection
mAb UMAB196	HIER High	1:1000-5000	2- & 3-step	-	-
rmAb EP356	HIER High	1:50-100	3-step	Ventana	3-step
pAb CP422	HIER High	1:25-300	3-step	-	-

PSA

	Retrieval	Titre	Detection	RTU	Detection
mAb 35H9	HIER	1:100-800	2- & 3-step	Leica	3-step
mAb ER-PR8	HIER	1:10-200	2- & 3-step	-	-
rmAb EP109	HIER	1:25-100	-	-	-
pAb 0562	HIER	1:1000-10000	2- & 3-step	Dako VMS	2-step

IHC – Protocols and controls – End, Ren, Prost…

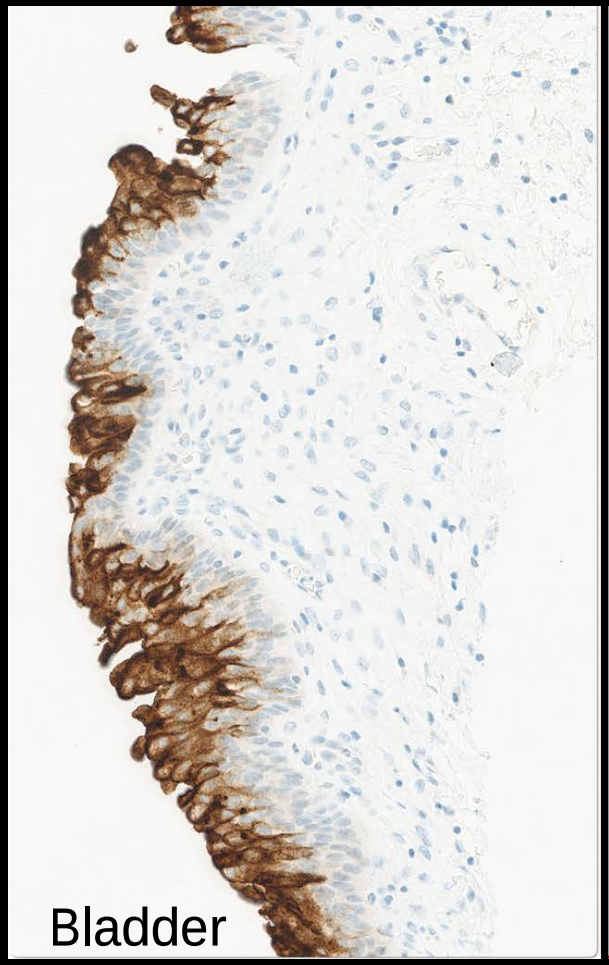
	Recommendable clones (conc.)	Less successful clones (conc.)	RTU ”plug and play” giving optimal result
SOX10	mAb BC34 mAb BS7 rmAb EP268 rmAb SP267	pAbs	
UP II	mAb BC21	mAb AU1 (UP III)	

IHC – Protocols and controls – End, Ren, Prost…

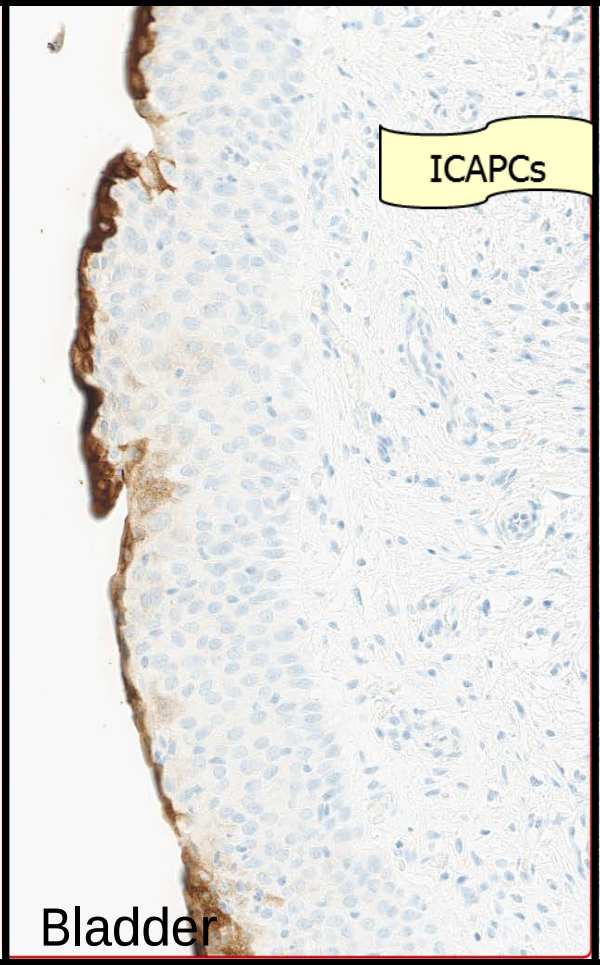
	Positive tissue control HE	Positive tissue control LE	Negative tissue control NE
SOX10	Skin: Melanocytes Appendix: Schwann cells	*Skin: Myoepithelial cells* *Appendix: Schwann cells*	Appendix: Epithelial cells
UP II	Bladder: Umbrella cells	*Bladder: Umbrella cells*	Appendix: Epithelial cells

IHC – Protocols and controls – End, Ren, Prost…

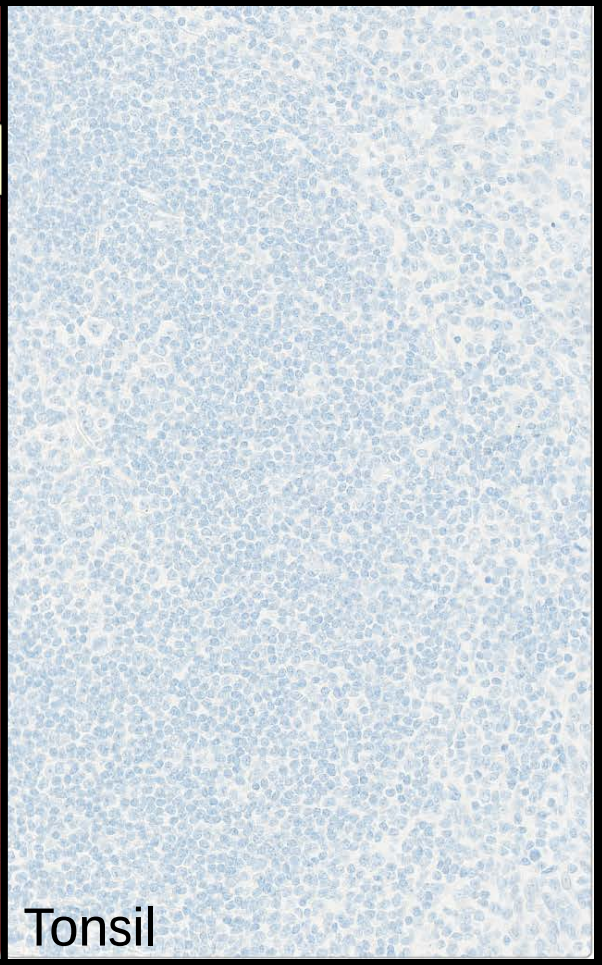
Uroplakin II reaction pattern



A moderate to strong predominantly cytoplasmic staining reaction of the vast majority of “umbrella cells”.

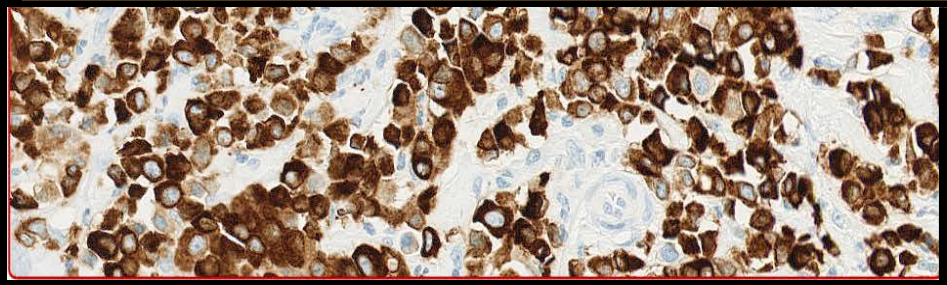
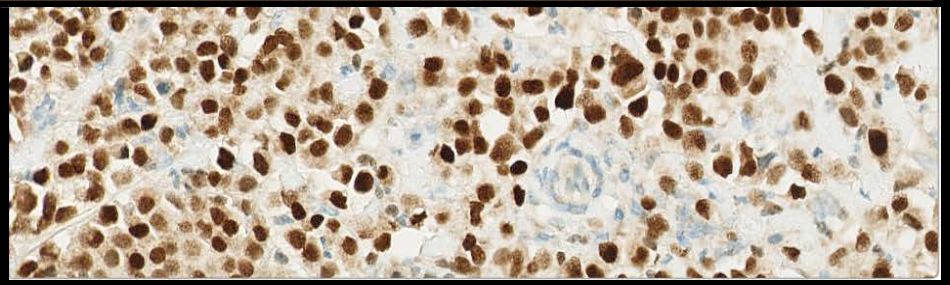
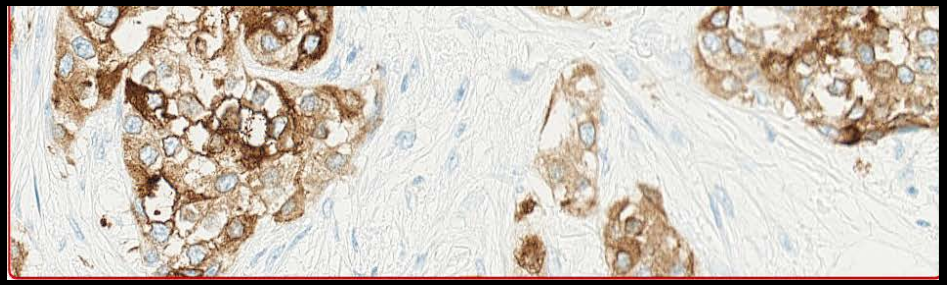
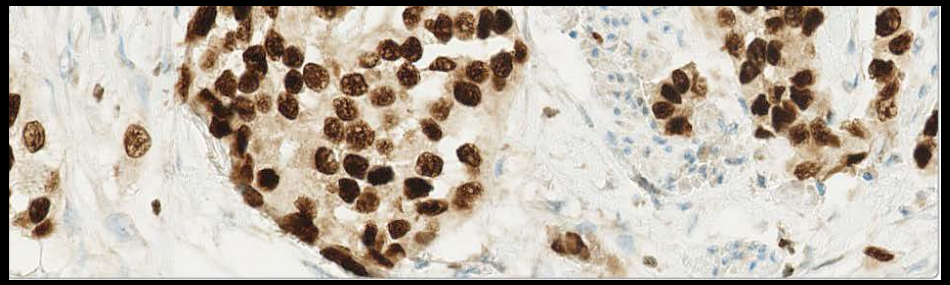
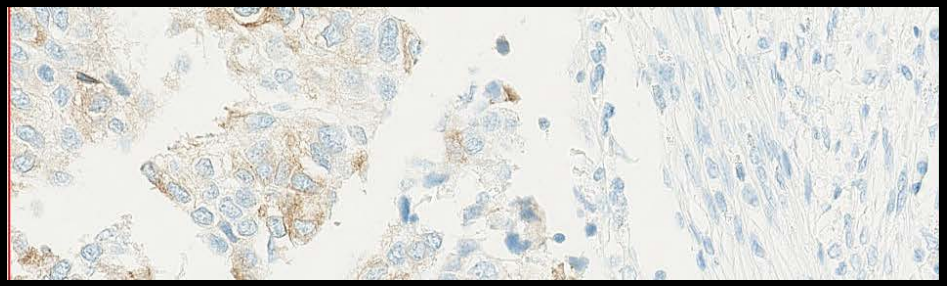
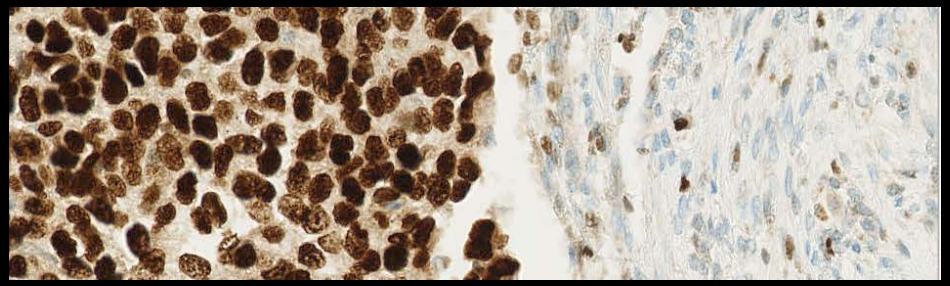
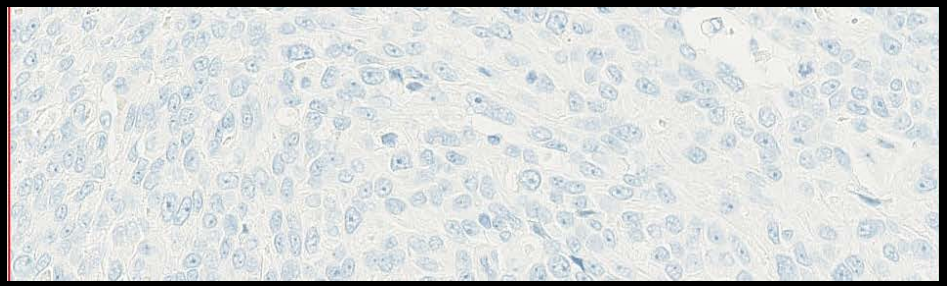
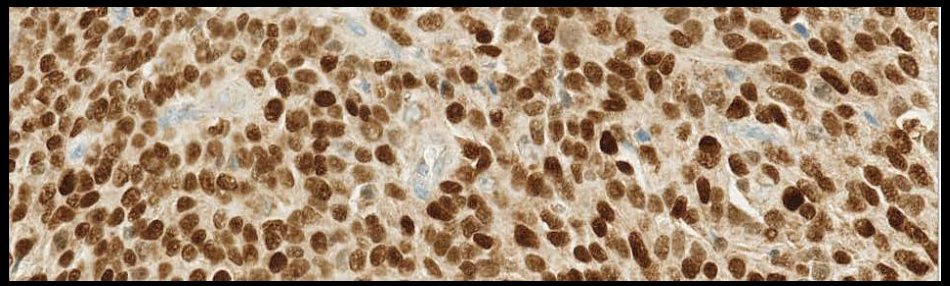


A moderate to strong predominantly cytoplasmic staining reaction of the vast majority of “umbrella cells”.



No staining reaction.

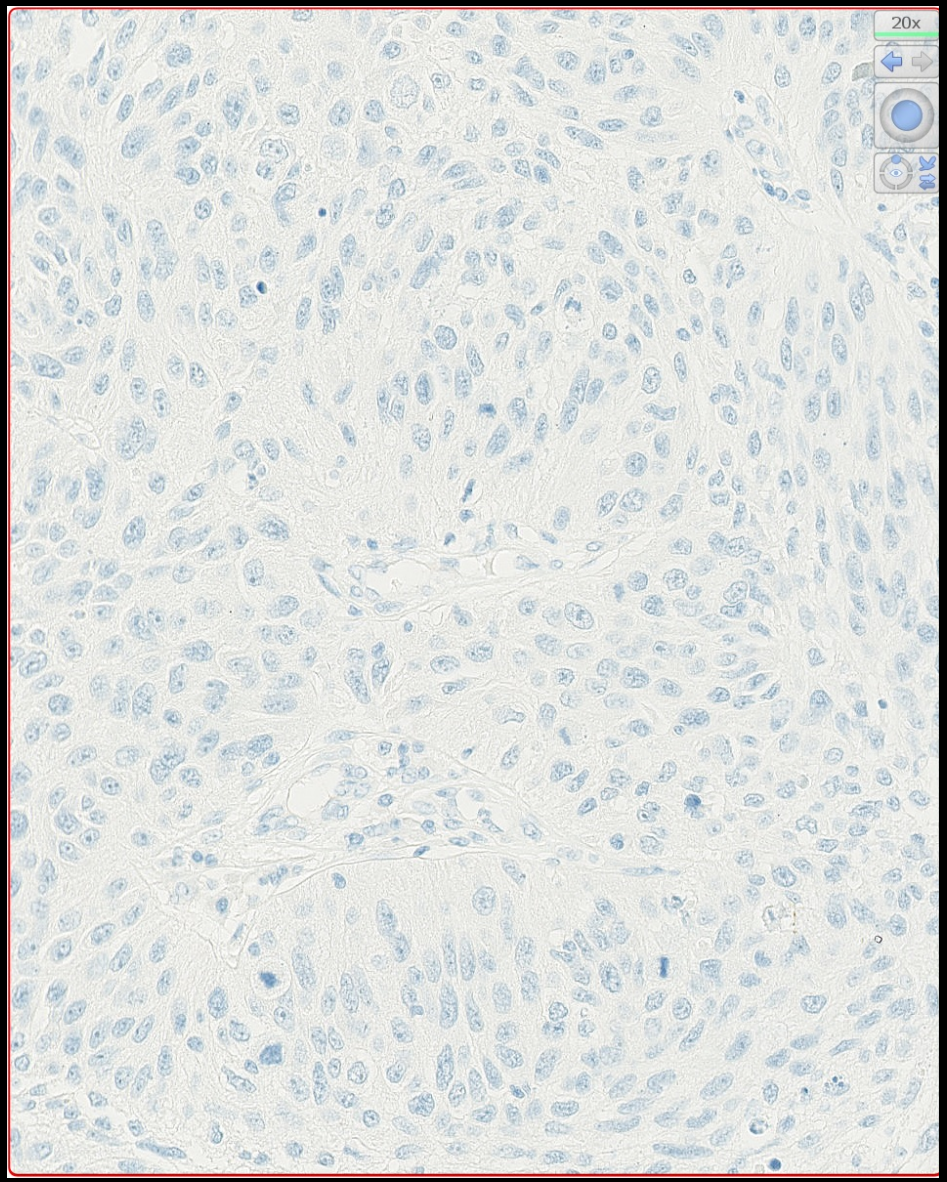
IHC – Protocols and controls – End, Ren, Prost…

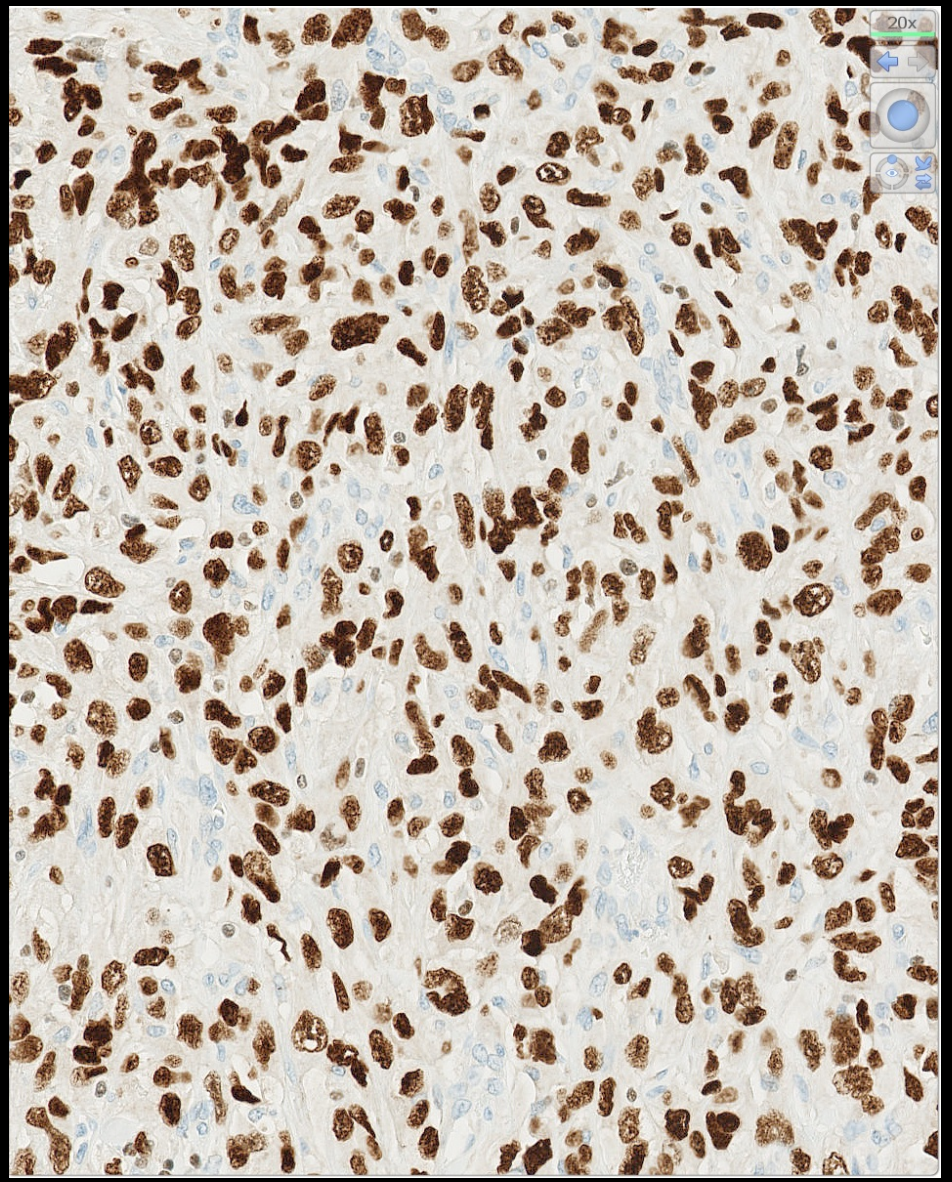
Uroth. carc. x 200 – Uroplakin II

Uroth. carc. x 200 – GATA3

IHC – Protocols and controls – End, Ren, Prost…



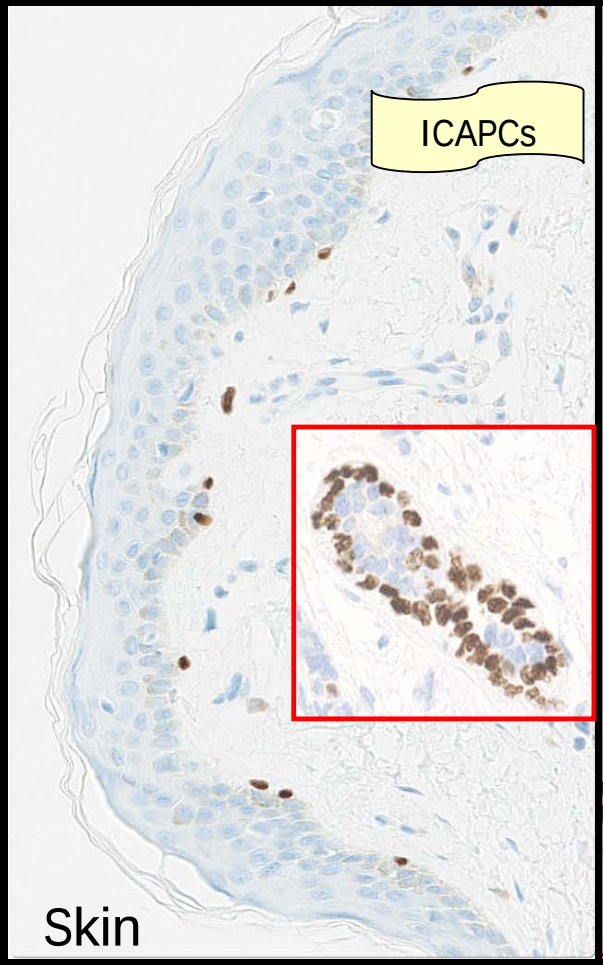
Lung. sq. carc. x 200 – Uroplakin II



Lung. sq. carc. x 200 – GATA3

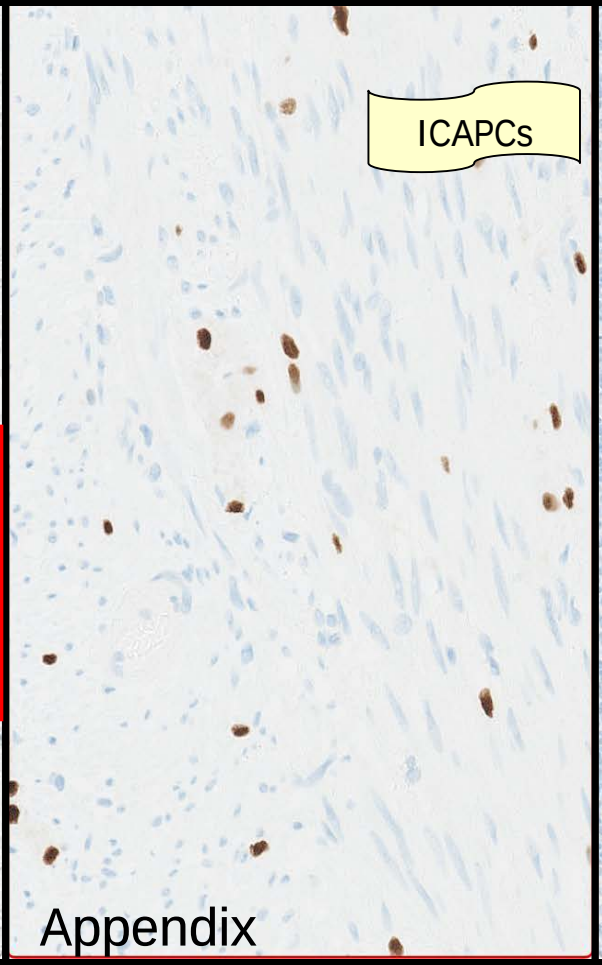
IHC – Protocols and controls – End, Ren, Prost…

SOX10 reaction pattern



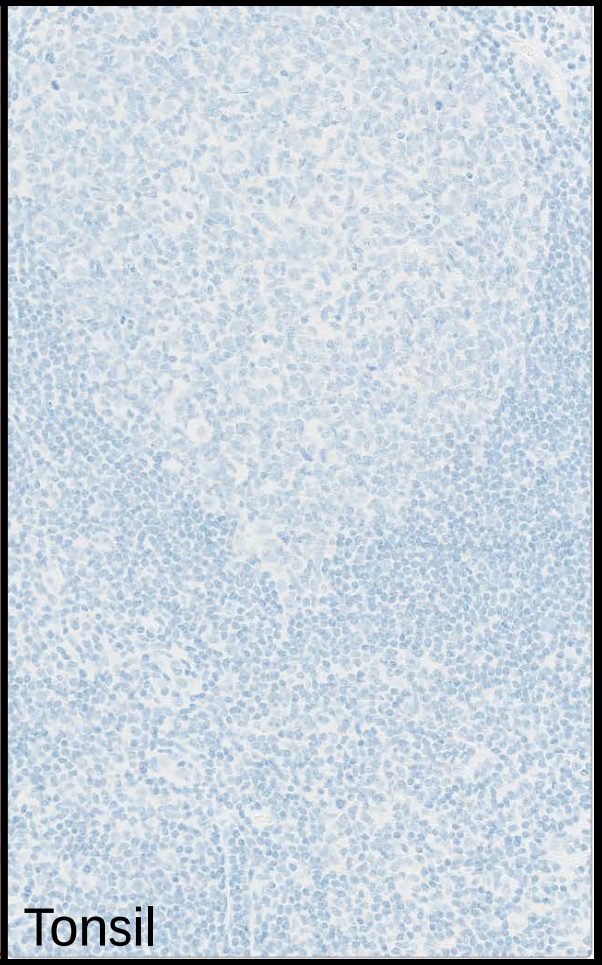
Skin

A moderate to strong nuclear staining reaction of virtually all melanocytes (and myoepithelial cells of sweat glands)



Appendix

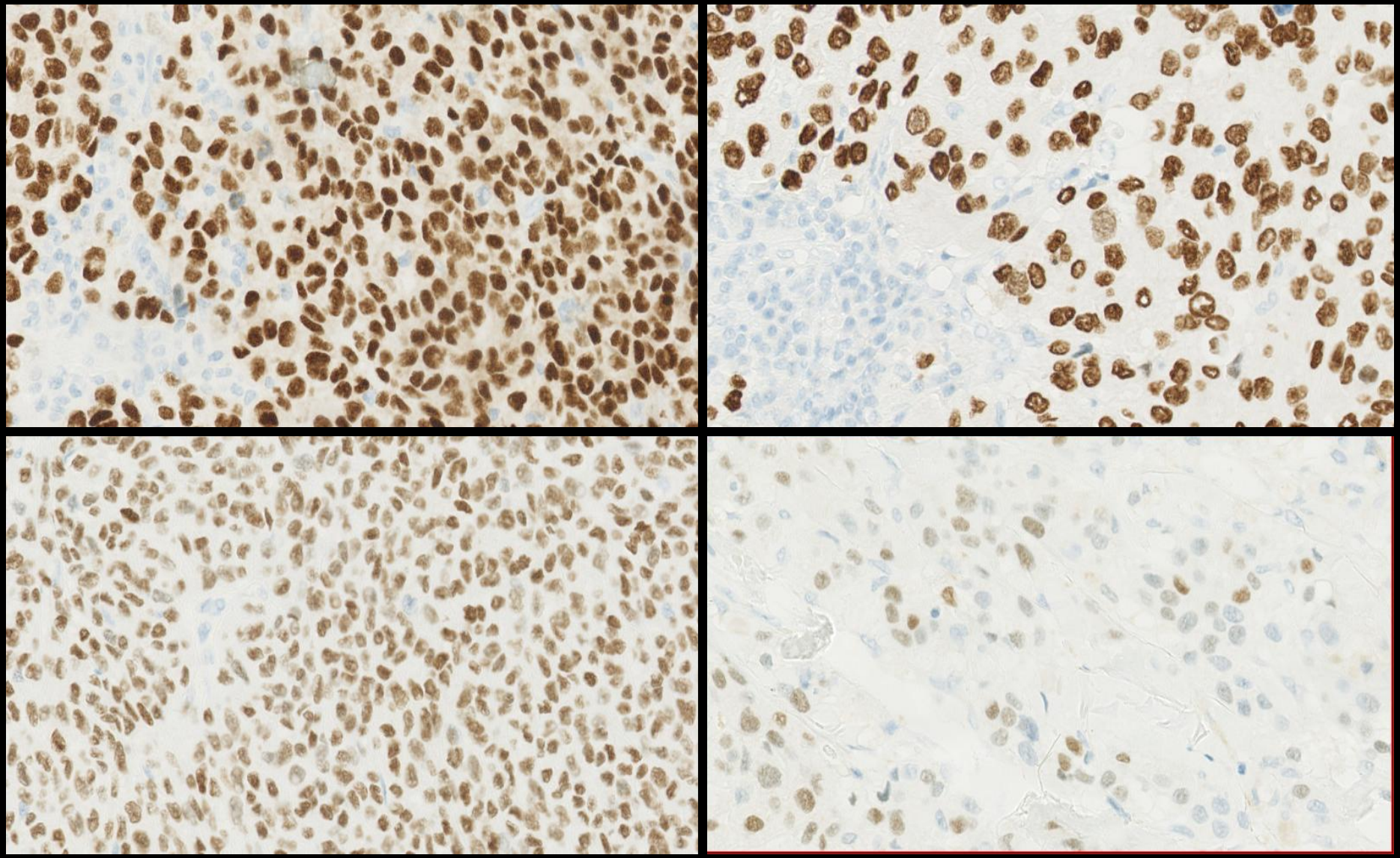
A moderate to strong nuclear staining of Schwann cells.



Tonsil

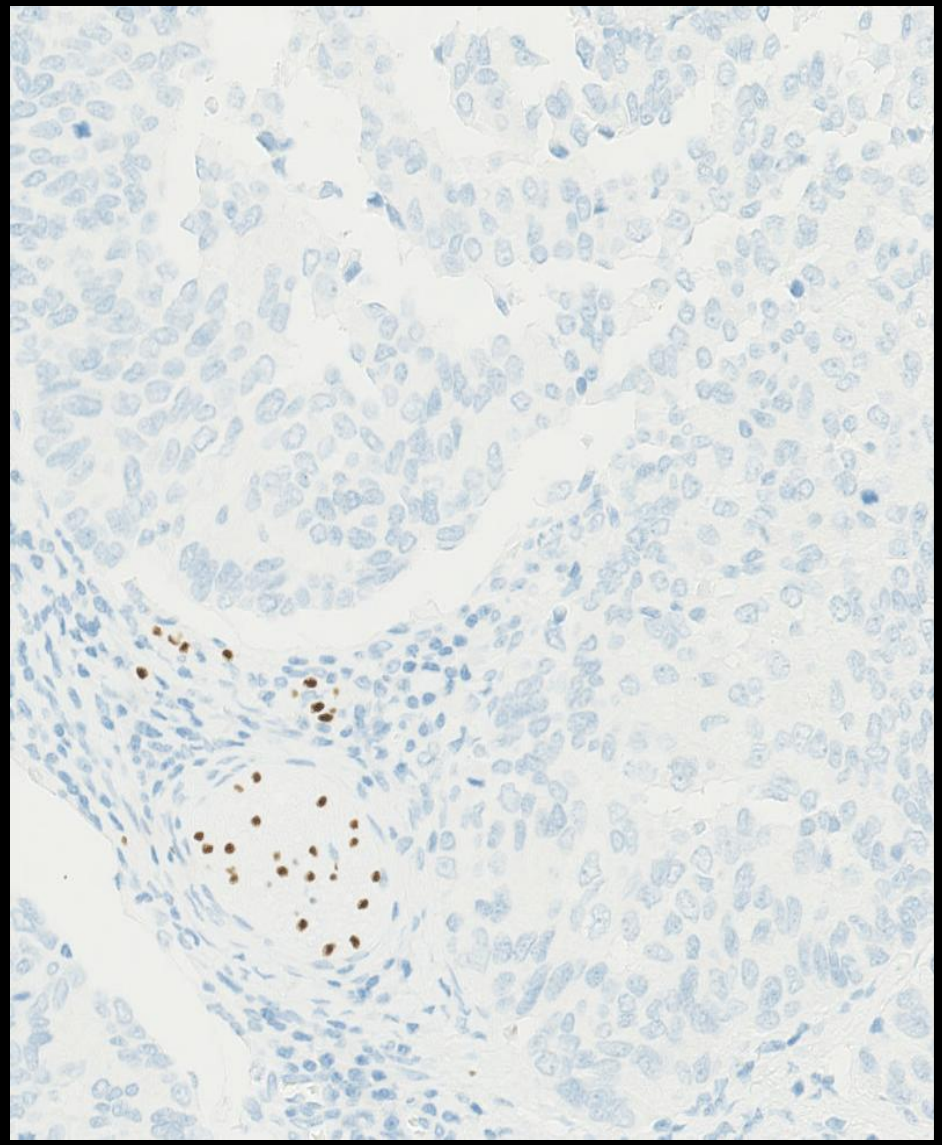
No staining reaction (apart from Schwann cells).

IHC – Protocols and controls – End, Ren, Prost…

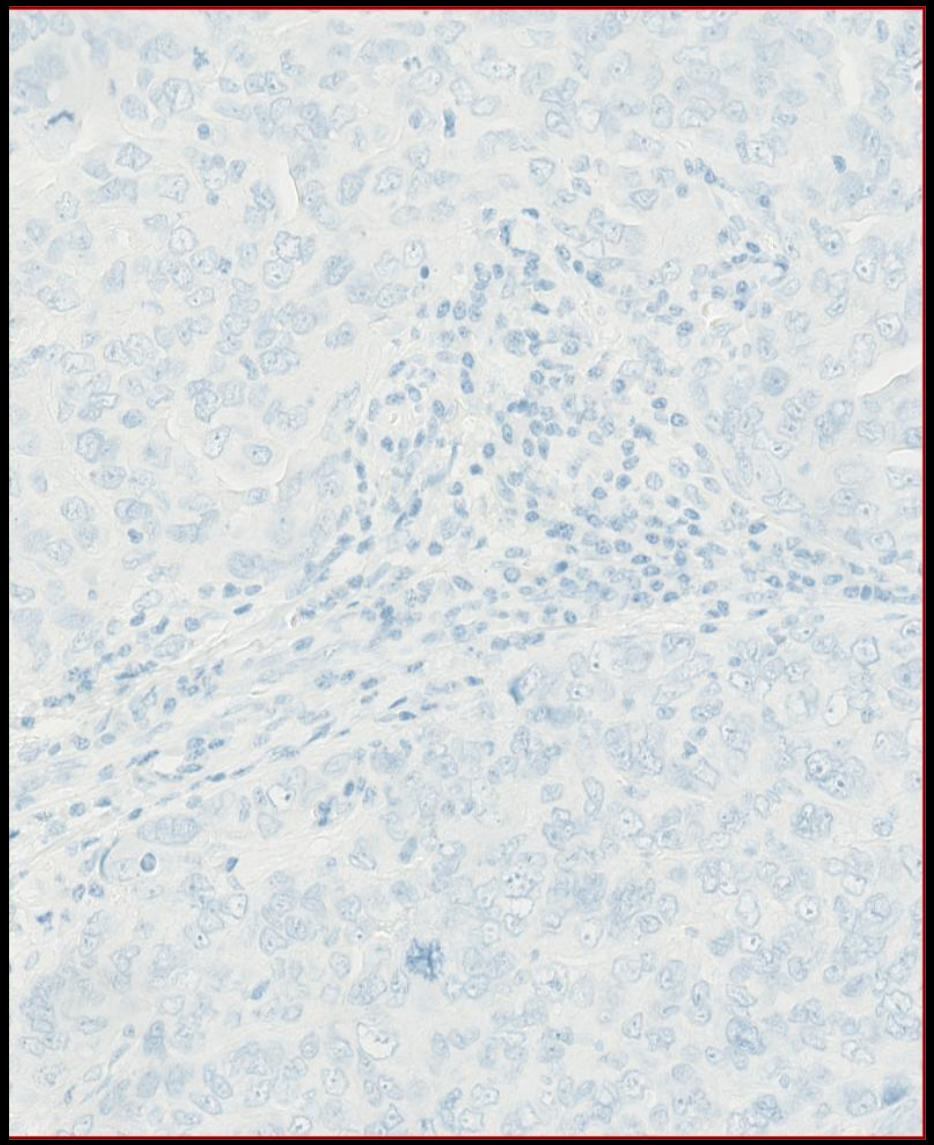


Melanomas x 200 – SOX 10

IHC – Protocols and controls – End, Ren, Prost…



Cerv. uteri, ad. carc. x 200 – SOX 10



Ovarian ser. carc. x 200 – SOX 10

IHC – Protocols and controls – End, Ren, Prost…

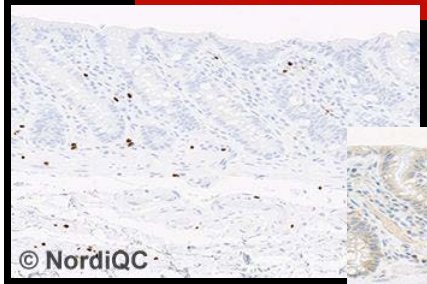
Table 1. **Antibodies and assessment marks for SOX10, run 48**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BC34**	36 1 1	Biocare Medical Abcam/Epitomics Klinipath	22	5	7	4	71%	77%
mAb clone **BS7**	5	Nordic Biosite	5	0	0	0	100%	100%
mAb clone **SOX10/1074**	5	Immunologic	0	2	1	2	-	-
mAb clone **DPM15.10**	1	Diagnostic Biosystem	0	1	0	0	-	-
mAb clone **ZM10**	1	Zeta Corporation	0	1	0	0	-	-
rmAb clone **EP268**	23 1 1 1	Cell Marque Epitomics BioSB Diagnostic Biosystems	13	6	3	4	73%	78%
rmAb clone **SP267**	2	Spring Bioscience	0	2	0	0	-	-
pAb **383A-76**	5	Cell Marque	0	0	5	0	0%	-
pAb **ILP3833-C1**	1	Immunologic	0	0	1	0	-	-
pAb **44-387**	1	Menarini	0	0	1	0	-	-
pAb **ab108408**	1	Abcam	0	0	0	1	-	-
pAb **RBK057-05**	1	ZytoMed	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **BC34** **API 3099 AA or H**	2	Biocare Medical	1	0	1	0	-	-
mAb clone **BC34** **API 3099 AA or H**[3]	5	Biocare Medical	2	0	3	0	-	-
rmAb clone **EP268**[4] **383R-10, -17 or -18**	13	Cell Marque	10	1	2	0	85%	91%
rmAb clone **EP268** **MAD-000656QD**	2	Master Diagnostica	0	1	1	0	-	-
rmAb clone **EP268** **RMA-0726**	1	Maixin	1	0	0	0	-	-
rmAb clone **EP268** **PR135**	1	PathSitu/Unknown	0	1	0	0	-	-
rmAb clone **SP267** **760-4968**	5	Ventana/Roche	5	0	0	0	100%	100%
rmAb clone **SP267** **M5671**	2	Spring Bioscience	1	1	0	0	-	-
pAb **383A-78**	2	Cell Marque	0	0	1	1	-	-
Total	120		60	21	26	13	-	
Proportion			50%	18%	22%	10%	68%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only (see below).
3) RTU formats developed for Biocare`s IHC system (IntelliPATH) but used by laboratories off-label on the platforms Ventana Benchmark/Ultra or Leica BOND III.
4) RTU format not developed for a specific IHC system and used by laboratories on different platforms as Ventana Benchmark Ultra/XT, Leica BOND III or Dako Autostainer Link+.

Select the right Ab!!!!

No room for pAbs



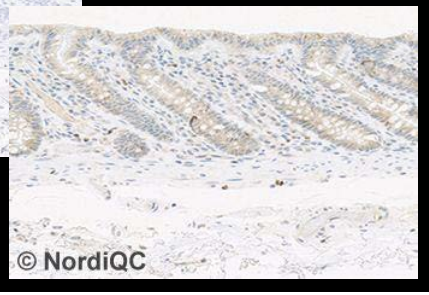


Table 2. **Proportion of sufficient results for SOX10 in the two NordiQC runs**

	Run 45 2015	Run 48 2016
Participants, n=	86	120
Sufficient results	45%	68%

IHC – Protocols and controls – End, Ren, Prost…

UPT II: SOX10

	Retrieval	Titre	Detection	RTU	Detection
mAb BC34	HIER High	1:25-200	3-step	-	-
mAb BS7	HIER High	1:100-300	3-step	-	-
rmAb EP268	HIER High	1:50-200	2- & 3-step	-	-
rmAb SP267	HIER High	1:30-50*	3-step	VMS SP267 – 3-step, CC1 mild.	-

UPT II: Uroplakin II*

	Retrieval	Titre	Detection	RTU	Detection
mAb BC21*	HIER High	1:25-50	3-step	-	-

**In-house data for best technical result*

IHC – Protocols and controls – End, Ren, Prost…

THANK YOU FOR

YOUR PATIENCE

AND ATTENTION