NordiQC

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

Optimization of antibodies, selection, protocols and controls

Unknown primary tumour - II

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IHC – Protocols and controls for UPT II

• TTF-1	Lung	• Arginase	Liver
• Napsin A		• Glypican 3	
• Ep-CAM	Mesothelioma	• ER	Breast
• Claudin 4		• GATA 3	
• Calretinin		• GCDFP15	
• Podoplanin		• Mammaglobin	
• CDX2	GI	• SYP	Neuroend.
• Cadherin 17		• CGA	
• CEA		• SOX10	Melanoma
• SMAD4		• Uroplakin	Bladder
• SATB2		• NKX3.1	Prostate
• PAX8	Fem. gen.		
• WT 1			

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- TTF-1
- Napsin A
- Ep-CAM
- Claudin 4
- Calretinin
- Podoplanin
- CDX2
- Cadherin 17
- CEA
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- PAX8
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Lung

- Arginase
- Glypican 3
- ER
- GATA 3
- GCDFP15
- Mammaglobin
- SYP
- CGA
- SOX10
- Uroplakin
- NKX3.1

Breast

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• Ep-CAM	Mesothelioma
• Claudin 4	
• CDX2	GI
• Cadherin 17	
• SATB2	
• SMAD4	
• PAX8	Fem. Gen.
• SOX10	Melanoma
• Uroplakin II	Bladder
• NKX3.1	Prostate

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	Recommendable clones (conc.)	Less successful clones (conc.)	RTU ”plug and play” giving optimal result
EPCAM	mAb Ber-EP4* mAb BS14 mAb MOC31 mAb SPM491	mAb C-10 rmAb E144 pAb 71916	Dako: mAb Ber-EP4 (GA637)
Claudin 4	mAb 3E2C1		

* Inferior performance on VMS/Leica stainer platforms (require HIER in TRS low pH)

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	Positive tissue control HE	Positive tissue control LE	Negative tissue control NE
EPCAM	Kidney: Epithelial cells of collecting ducts. Appendix: Epithelial cells.	Kidney: Epithelial cells lining Bowman capsule and in proximale tubules.	Kidney: Stroma Liver: Hepatocytes
Claudin 4	Appendix: Epithelial cells.	Placenta: Throphoblasts	Appendix: Smooth muscle cells Liver: Hepatocytes

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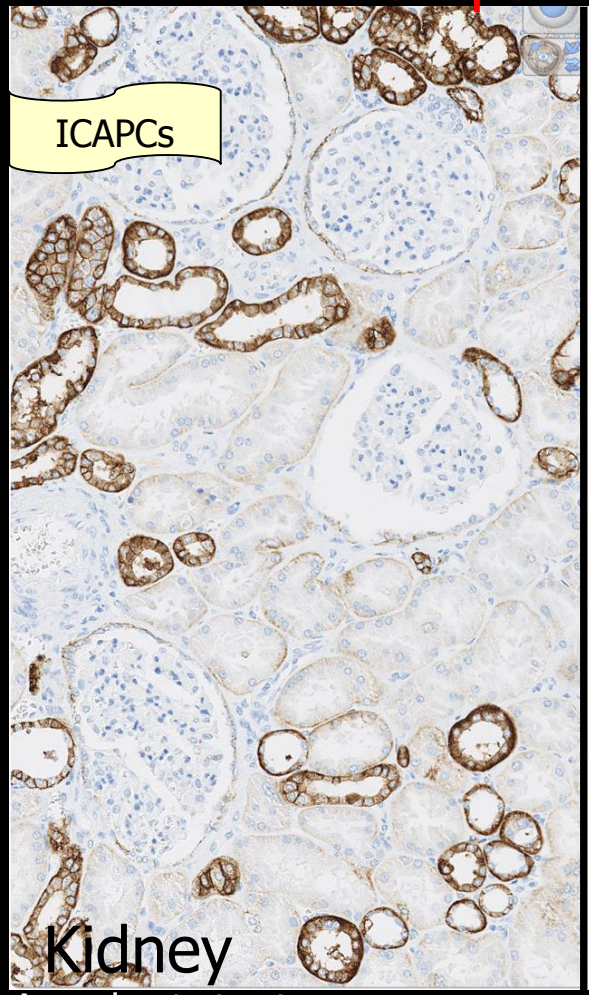
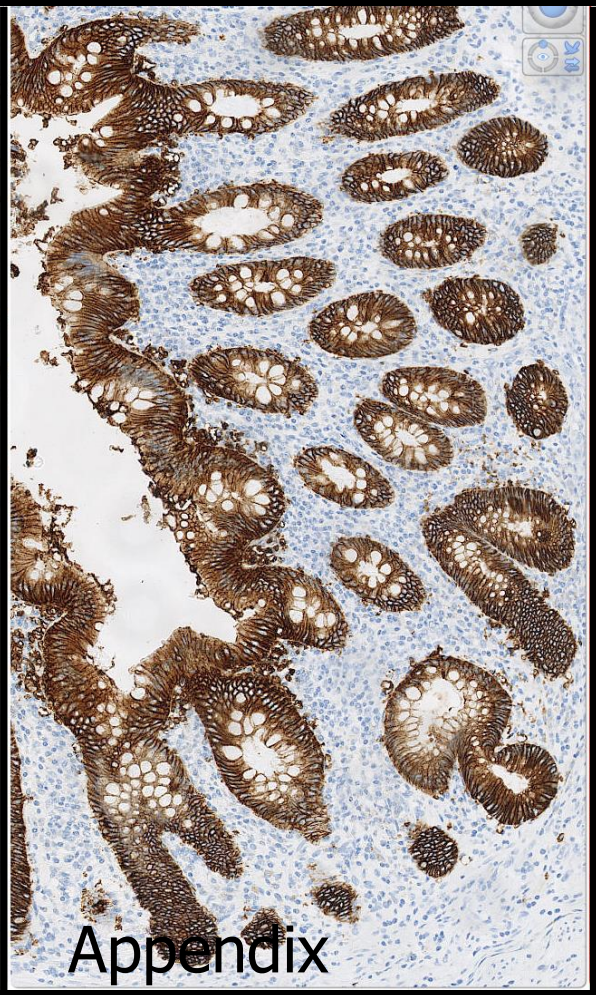
EP-CAM reaction pattern



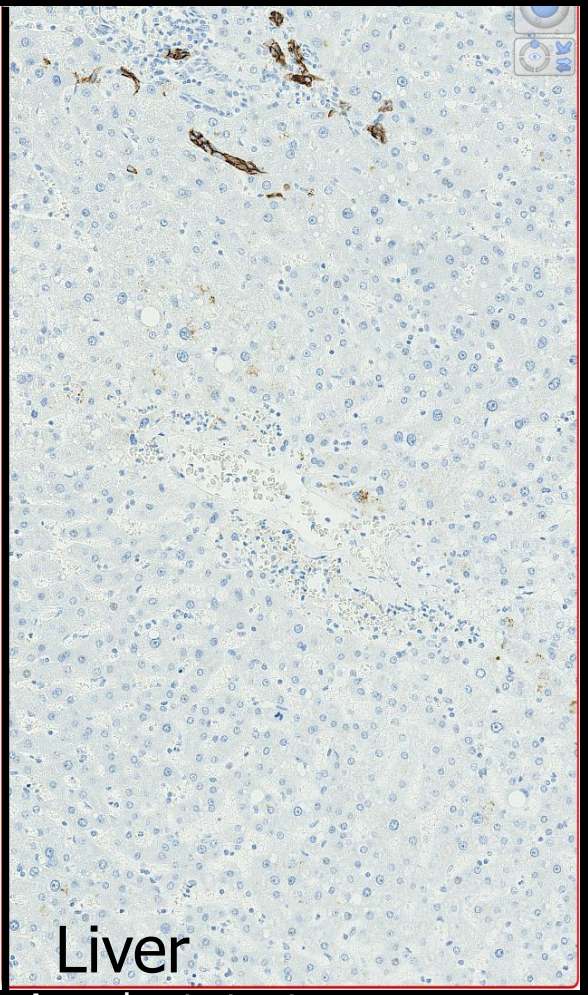
Kidney

A moderate to strong membranous staining of virtually all epithelial cells of collecting ducts. An at least weak staining of epithelial cells of proximal tubules and Bowman capsule.



Appendix

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



Liver

A moderate to strong membranous staining of virtually all epithelial cells of the bile ducts. No staining reaction in hepatocytes.

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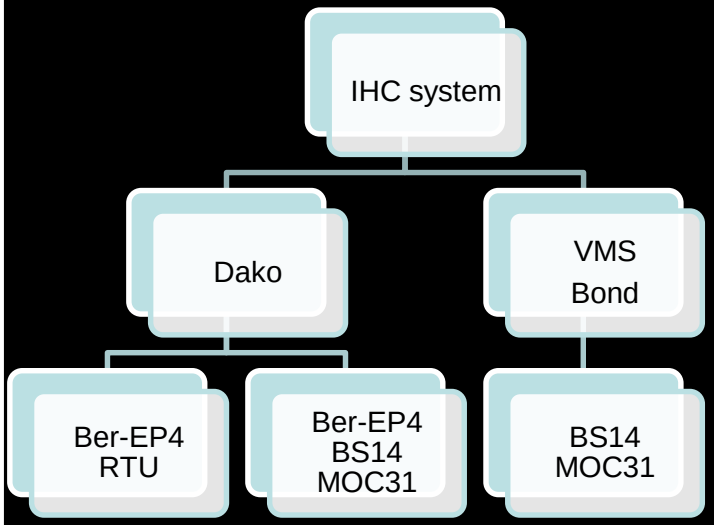
Table 1. **Antibodies and assessment marks for Ep-CAM, run 45**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **9C4**	1	BioLegend	0	0	0	1	-	-
mAb clone **BS14**	2	Nordic Biosite	2	0	0	0	-	-
mAb clone **C-10**	1	Santa Cruz Biotech	0	0	1	0	-	-
mAb clone **Ber-Ep4**	77 2 2	Dako Diagnostic BioSystems Thermo/NeoMarkers	9	16	38	18	31%	89%
mAb clone **MOC-31**	19 3 1 1	Dako Leica/Novocastra Cell Marque Monosan	9	6	6	3	63%	100%
mAb clone **VU-1D9**	3 3 1 1	Novocastra Thermo/LabVision Merck Millipore Thermo/Pierce	3	3	2	0	75%	75%
rmAb clone **E144**	1	Abcam	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **Ber-Ep4 760-4383**	36	Ventana/Cell Marque	0	6	21	9	17%	-
mAb clone **Ber-Ep4 IR/IS637**	19	Dako	4	12	1	2	84%	100%
mAb clone **Ber-Ep4 GA637**	9	Dako	7	1	1	0	89%	100%
mAb **Ber-Ep4 PM107**	1	Biocare	0	0	0	1	-	-
mAb **Ber-Ep4 MAD-001709QD**	1	Master Diagnostica	0	0	1	0	-	-
mAb clone **Ber-Ep4 MON-RTU1096**	1	Monosan	0	0	1	0	-	-
mAb clone **MOC-31 790-4561**	3	Ventana	0	1	2	0	-	-
mAb clone **MOC-31 248M-18**	1	Cell Marque	0	0	1	0	-	-
mAb clone **MOC-31 PA0797**	1	Leica/Novocastra	0	1	0	0		
mAb clone **MOC-31 MAB-0280**	1	Maixin	0	1	0	0	-	-
mAb clone **VU-1D9**	1	Unknown	0	0	1	0		
Total	192		34	47	76	35	-	
Proportion			18%	25%	39%	18%	43%	

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

Navigation path:



3-step systems seem to be superior

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Run 45	Proteolysis		HIER (all buffers)		HIER TRS 6.1	
	Suff.	Optimal	Suff.	Optimal	Suff.	Optimal
Ber-EP4	*9%*	*0*	*39%*	*16%*	*77%*	*46%*
LDT	*(2/22)*	*(0/22)*	*(22/56)*	*(9/56)*	*(10/13)*	*(6/13)*

In general 43% of submitted protocols were assessed as sufficient

The impact of selecting the right retrieval type and retrieval conditions……

But also the impact of the retrieval solution possible for the IHC platform…

24/28 RTU protocols from Dako sufficient (86%)

6/36 RTU protocols from Ventana sufficient (17%)

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ICAPCs:

epithelial cells of the tubules

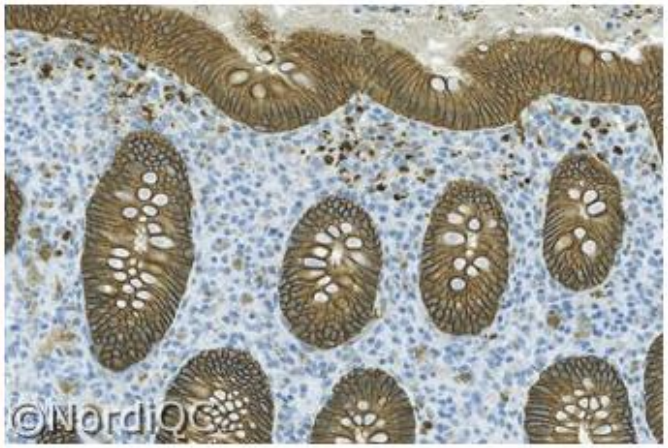

Fig. 1a. Optimal staining for Ep-CAM in the appendix using the mAb clone Ber-EP4 optimally calibrated and with HIER in DIVA epitope retrieval solution pH 6.2 (Biocare). The enterocytes show a strong distinct predominantly membranous staining. Macrophages having engulfed epithelial cells show an intracytoplasmic staining.

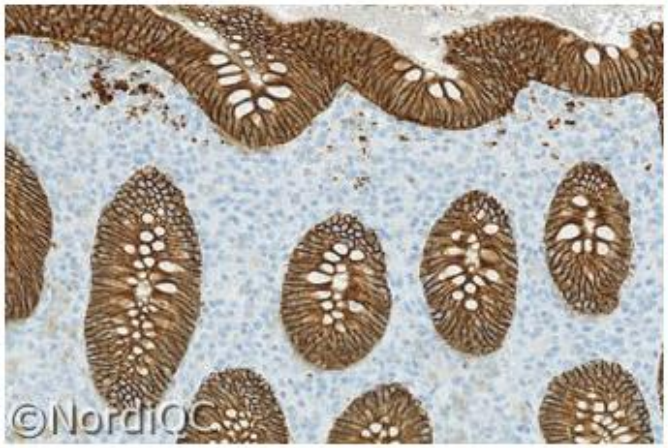

Fig. 1b. Staining for Ep-CAM in the appendix using an insufficient protocol based on the mAb clone Ber-ER4 with HIER in an alkaline buffer, CC1 pH 8.5 Ventana, same field as in Fig. 1a. The enterocytes show a strong distinct predominantly membranous staining. However also compare with Figs. 2b – 4b, same protocol. As an insufficient staining for Ep-CAM was seen in both renal cell carcinomas, appendix can not be recommended as a positive control due to a too high expression of Ep-CAM.

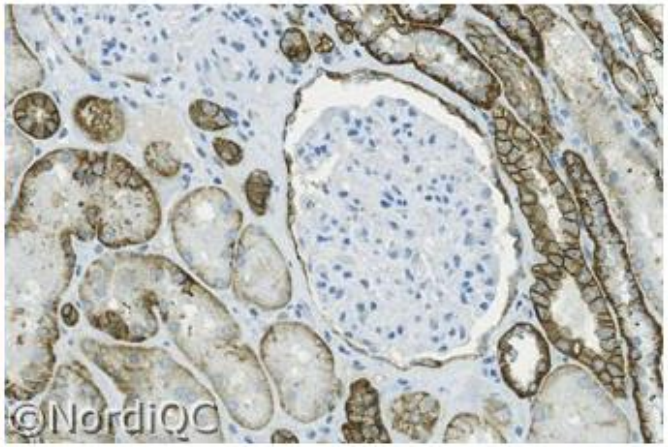

Fig. 2a. Optimal staining for Ep-CAM of the normal kidney using same protocol as in Fig. 1a.
The epithelial cells of the renal collecting tubules and the Bowman capsule show a moderate to strong membranous staining, while the epithelial cells of the proximale tubules only show a weak predominantly basolateral reaction.

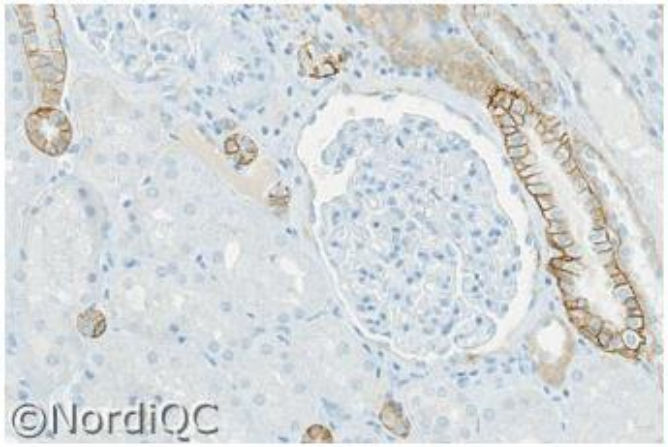

Fig. 2b. Insufficient staining for Ep-CAM of the normal kidney using same protocol as in Fig. 1b, same field as in Fig. 2a. Only the epithelial cells of the collecting tubules are demonstrated. Also compare with Figs. 3b and 4b.

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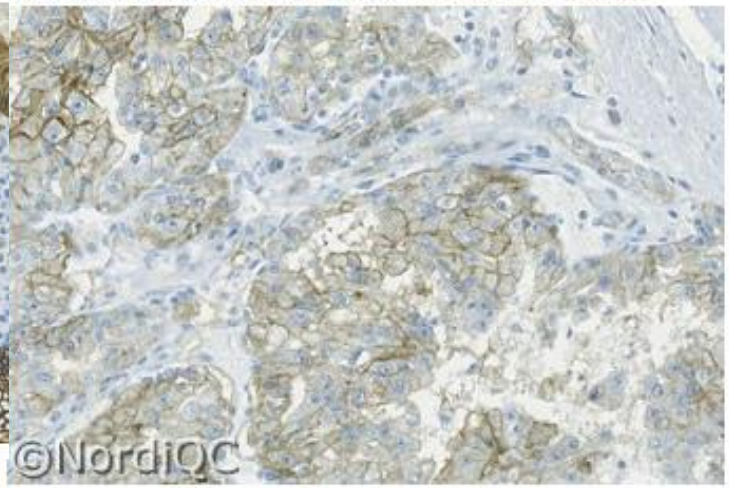


Fig. 3a. Optimal staining for Ep-CAM in the renal clear cell carcinoma no. 5 using same protocol as in Figs. 1a - 2a.
The majority of the neoplastic cells show a moderate and distinct membranous reaction.
No background reaction is seen.

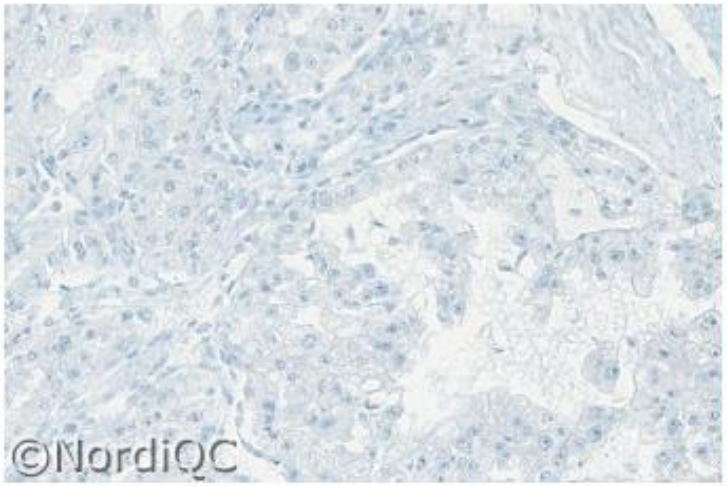
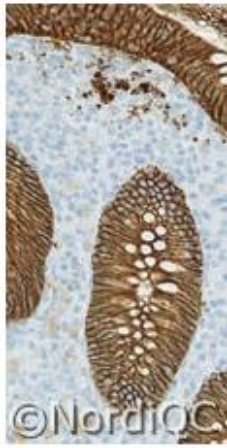


Fig. 3b. Insufficient staining staining for Ep-CAM in the renal clear cell carcinoma no. 5 using same protocol as in Figs. 1b – 2b, same field as in Fig. 3b.
The neoplastic cells are all false negative.



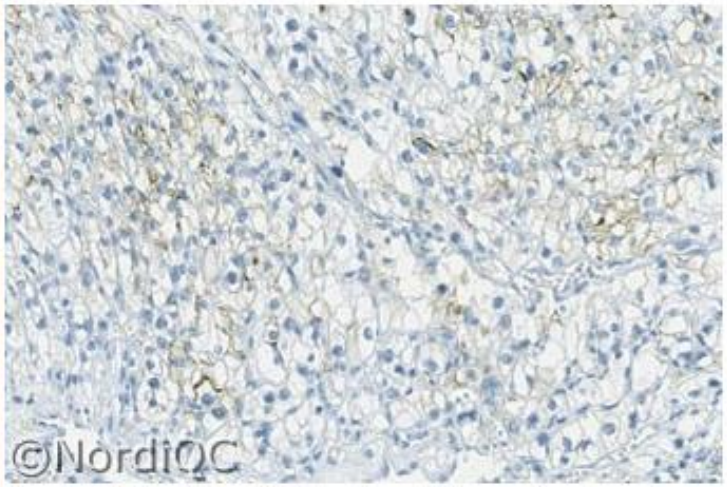


Fig. 4a. Optimal Ep-CAM staining of the renal cell carcinoma no. 6 using same protocol as in Figs. 1a – 3a. Scattered neoplastic cells show a weak to moderate distinct membranous reaction.

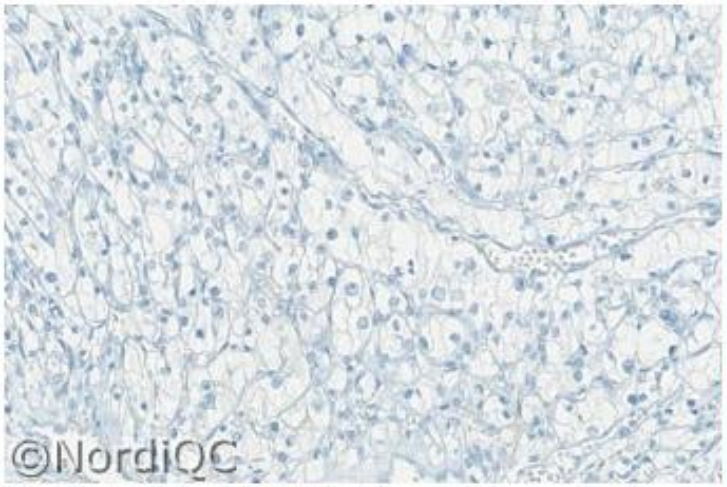


Fig. 4b. Insufficient Ep-CAM staining of the renal cell carcinoma no. 6 using same protocol as in Figs. 1b – 3b, same field as in Fig. 4a.
The neoplastic cells are all false negative.

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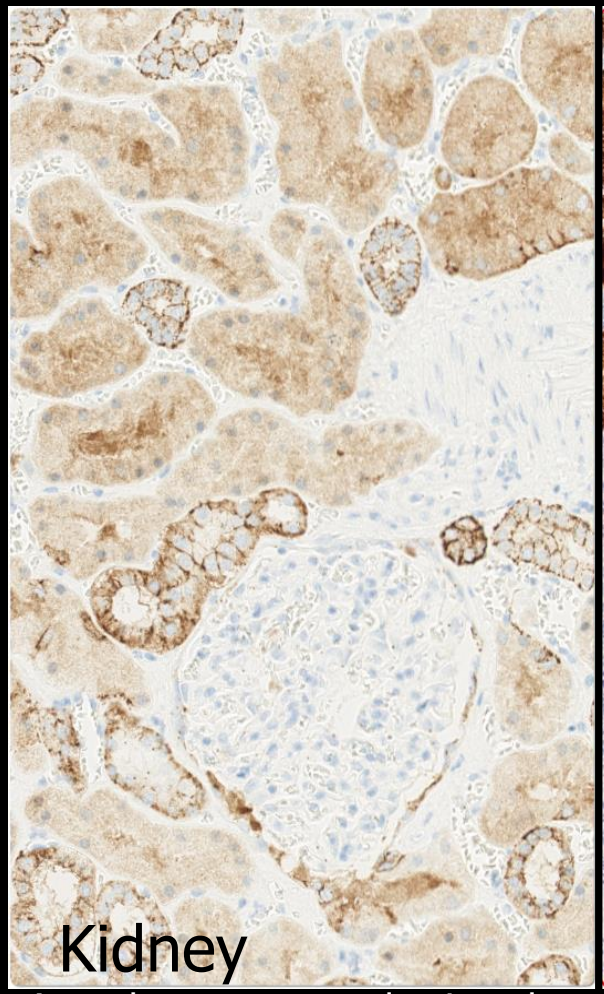
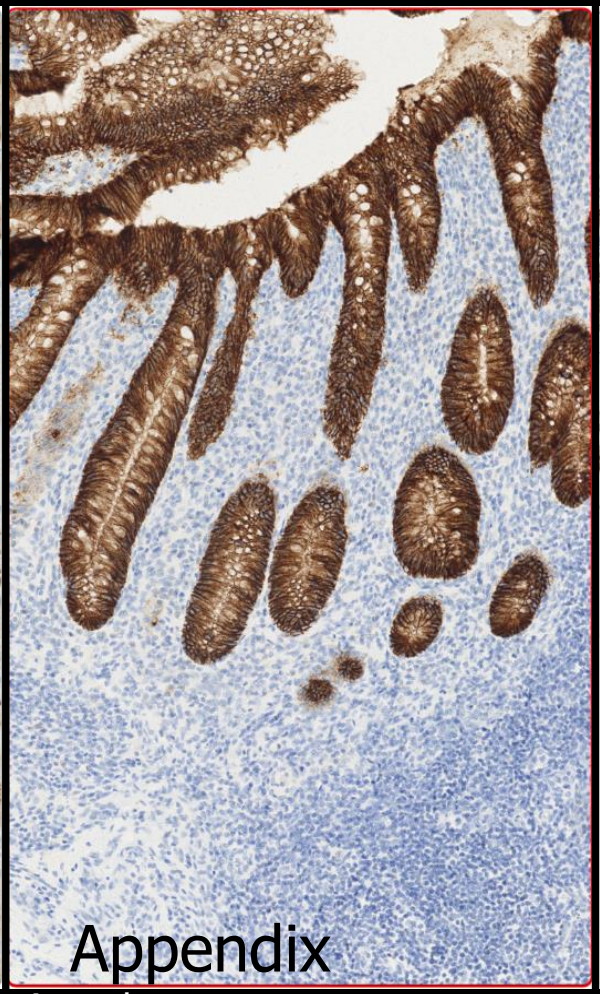
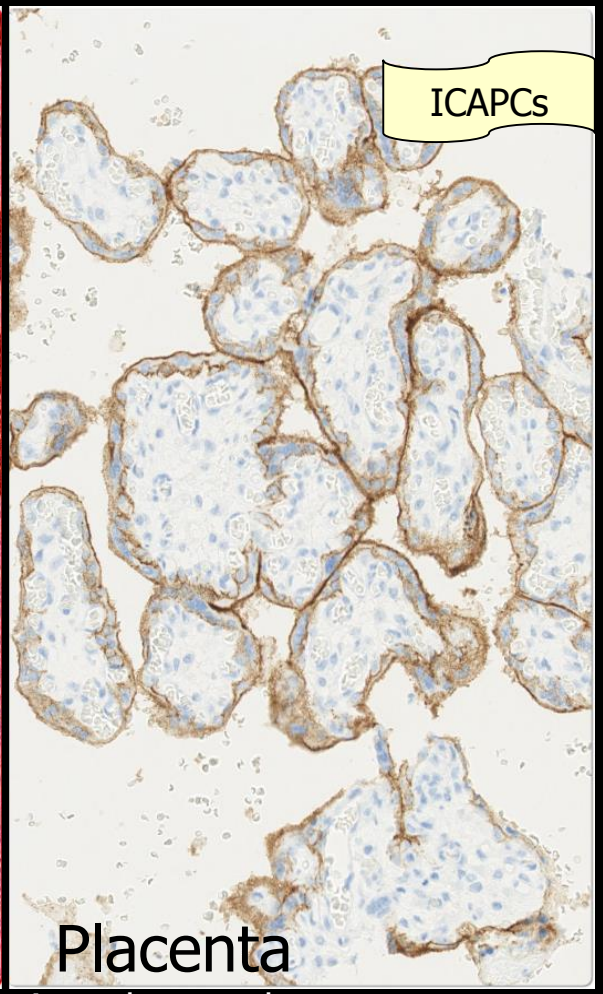
Claudin-4 reaction pattern



A weak to strong predominantly membranous but also cytoplasmic staining reaction of epithelial cells of collecting ducts and proximal tubules.



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



A weak to moderate predominantly membranous staining reaction of virtually all throphoblasts. No reaction in stromal cells.

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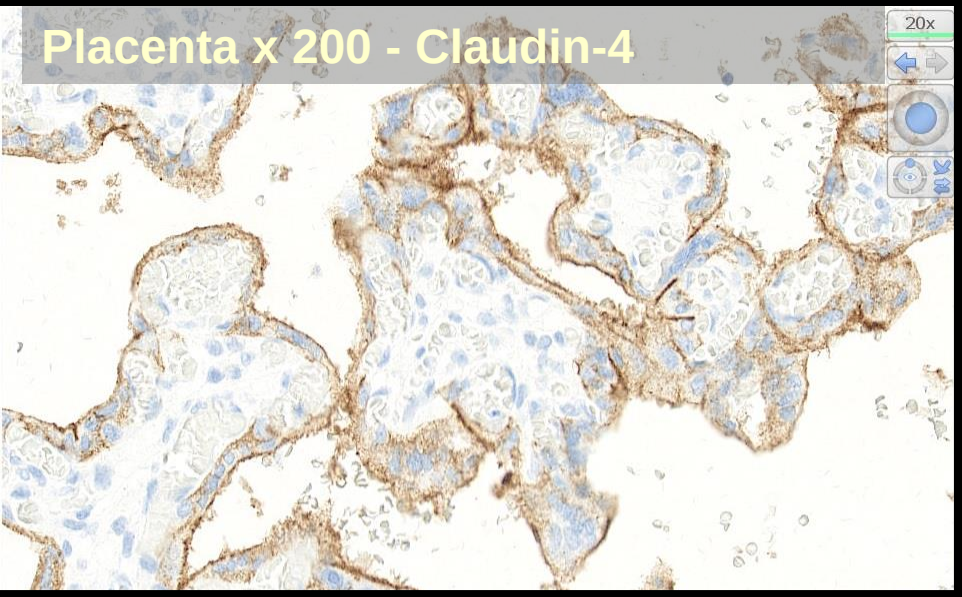

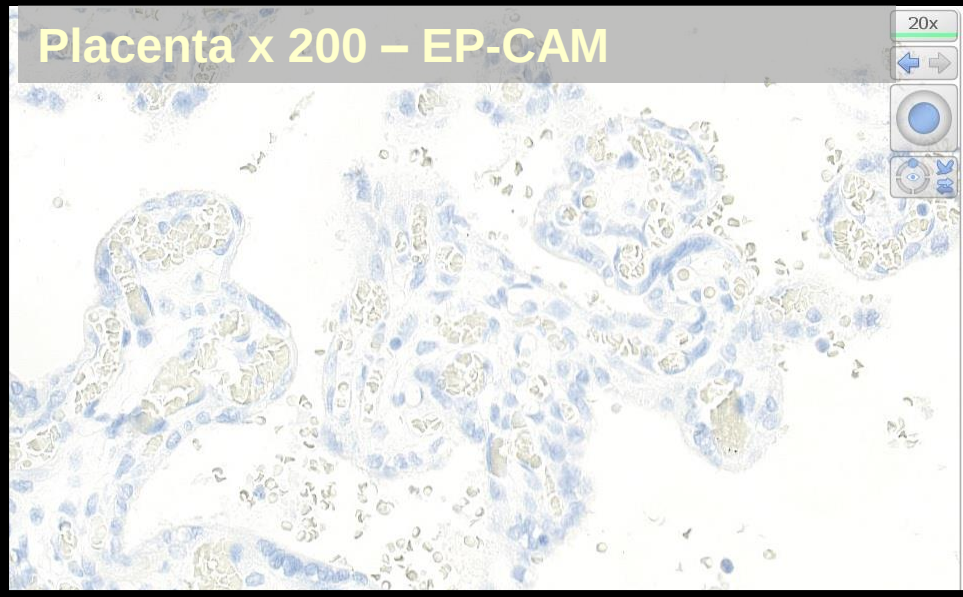

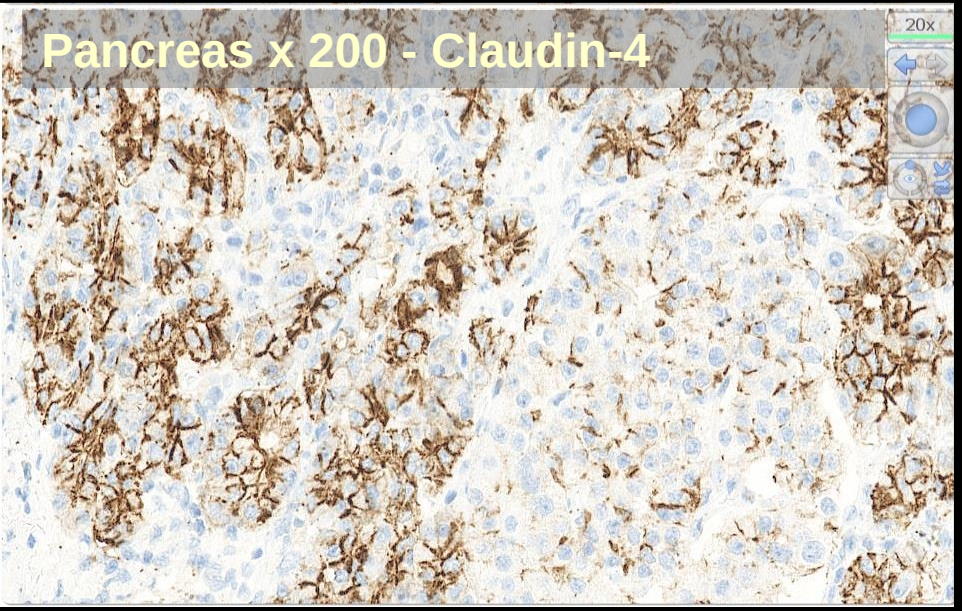

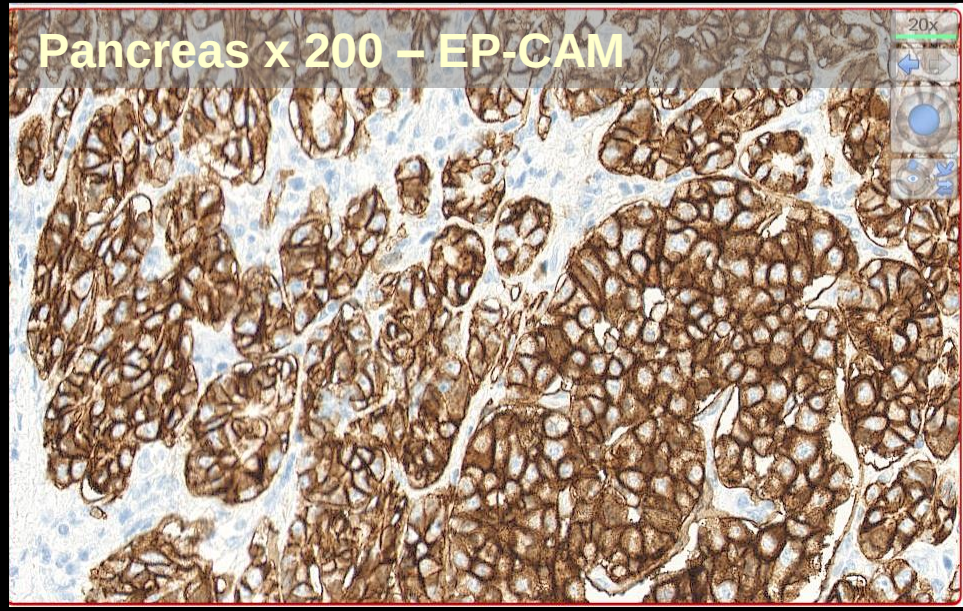

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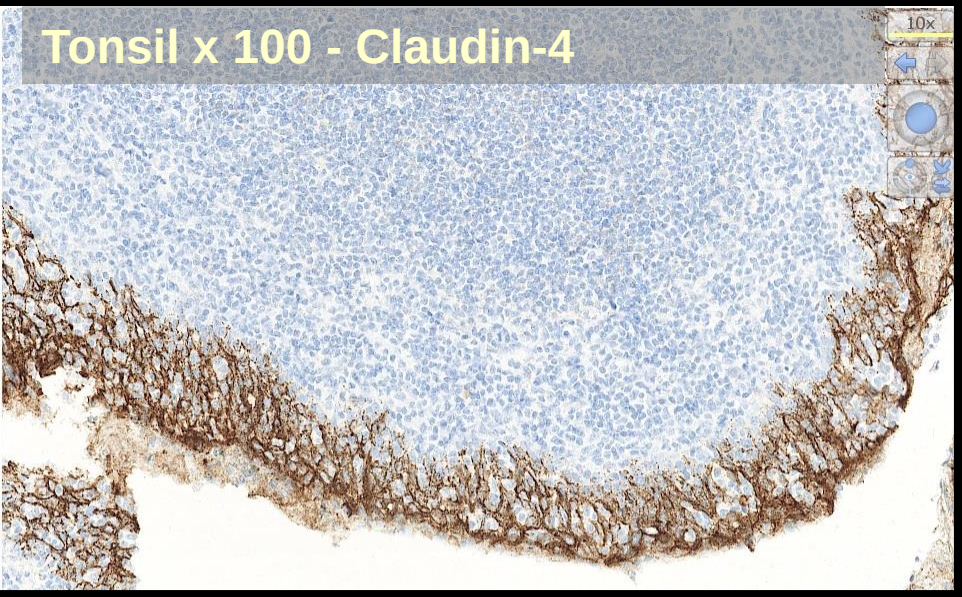

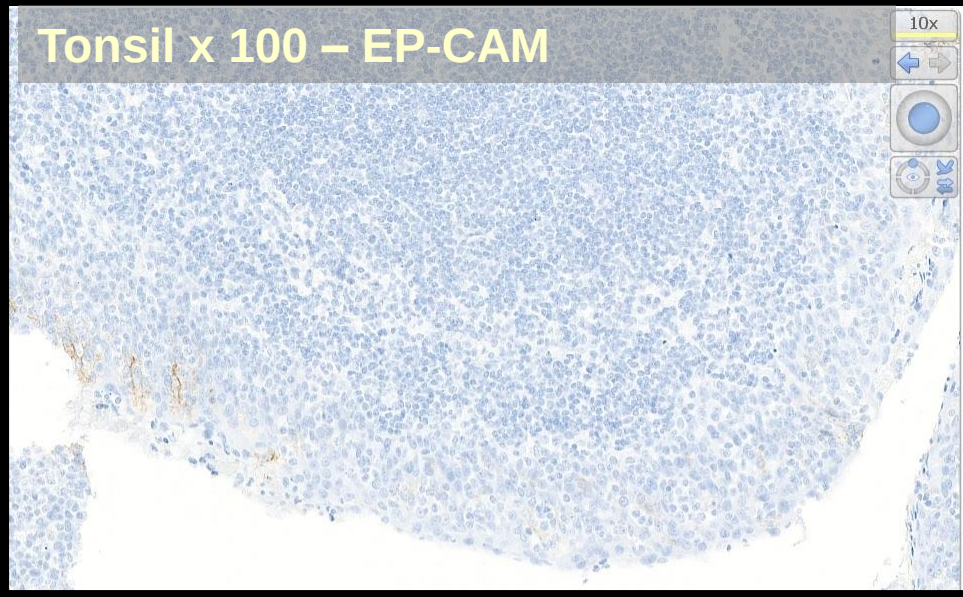

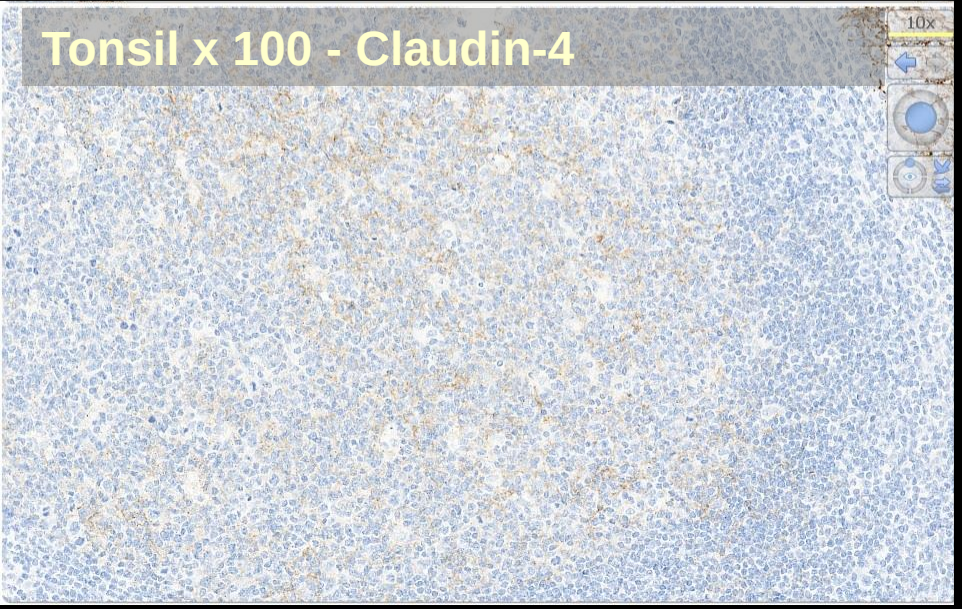

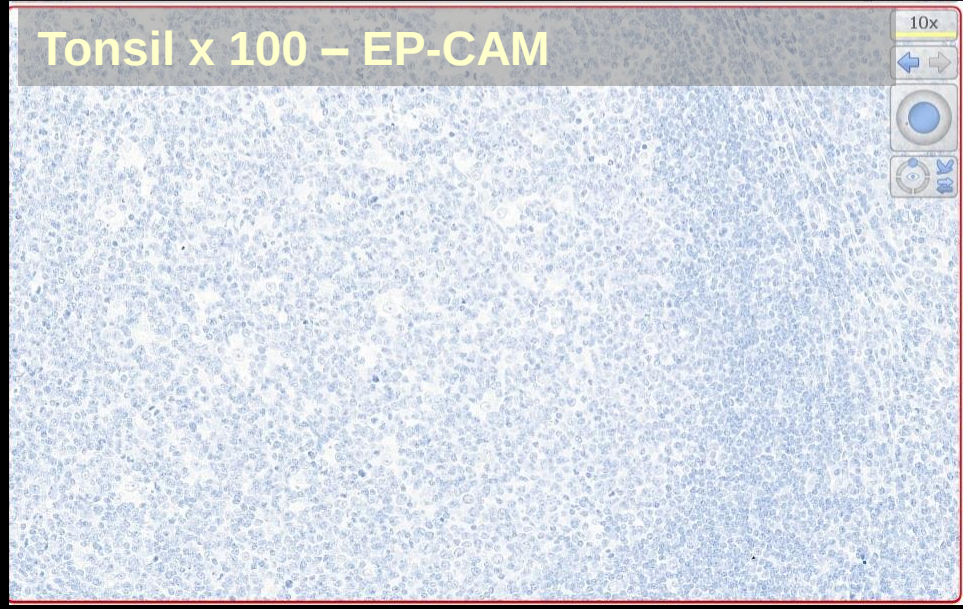

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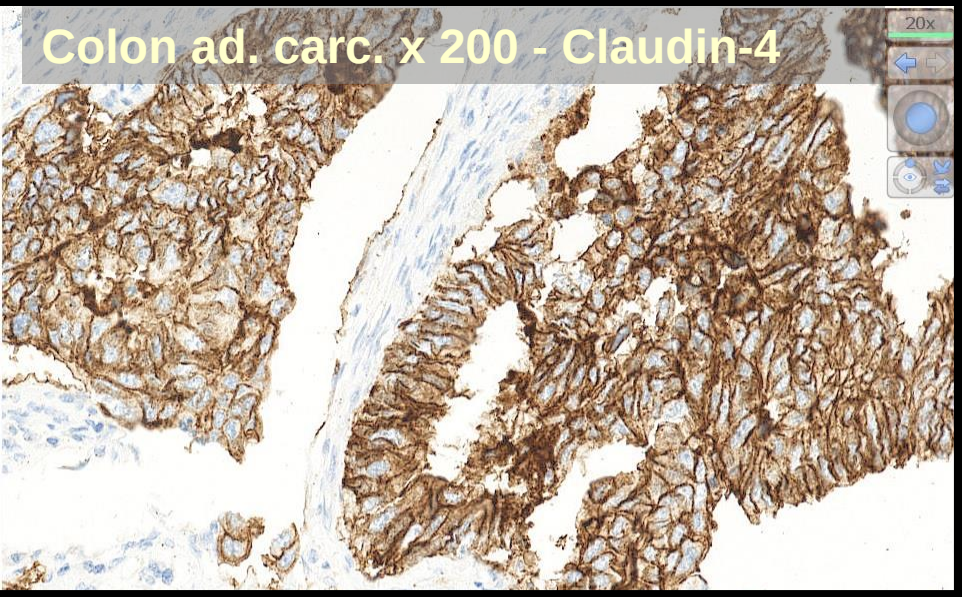

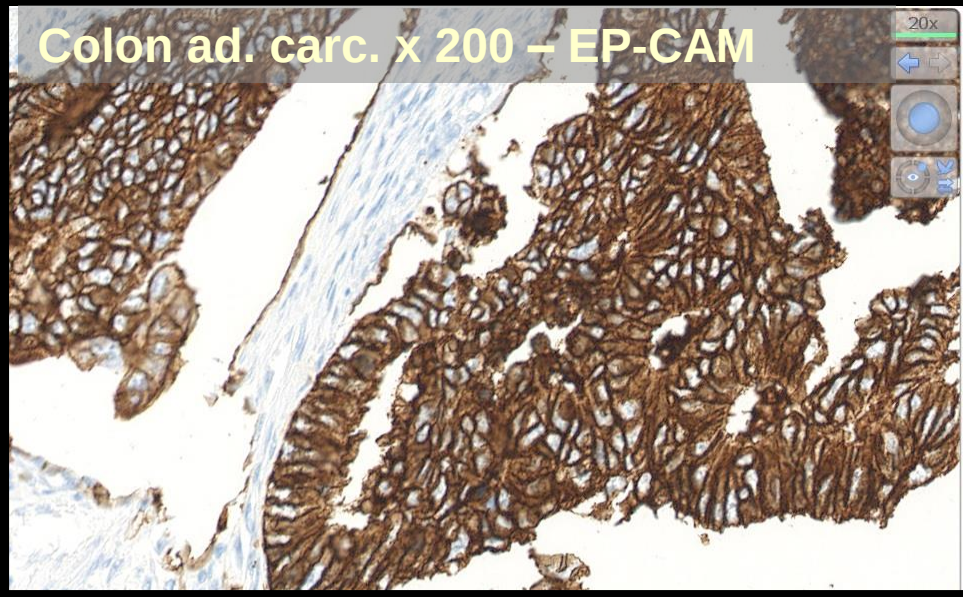

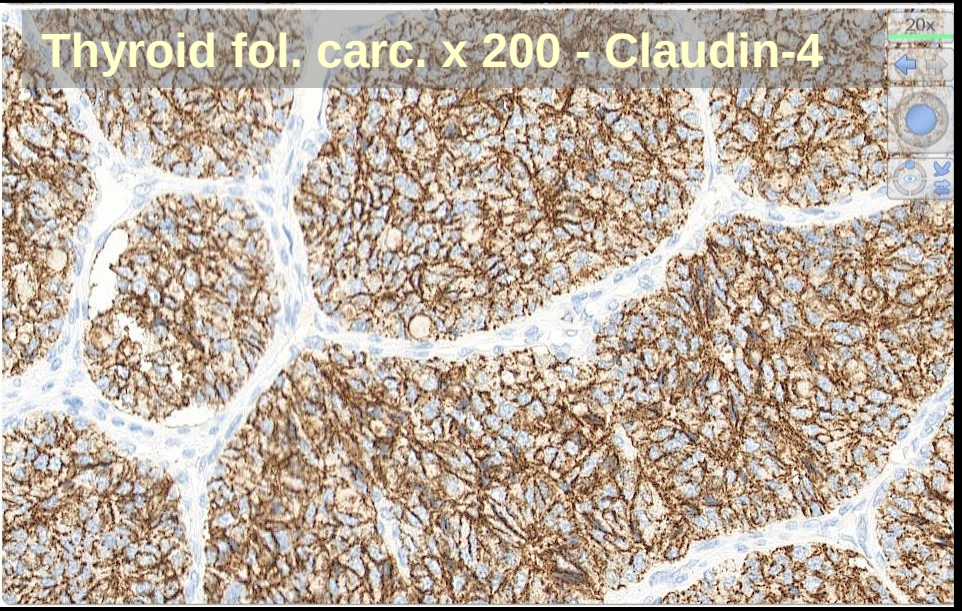

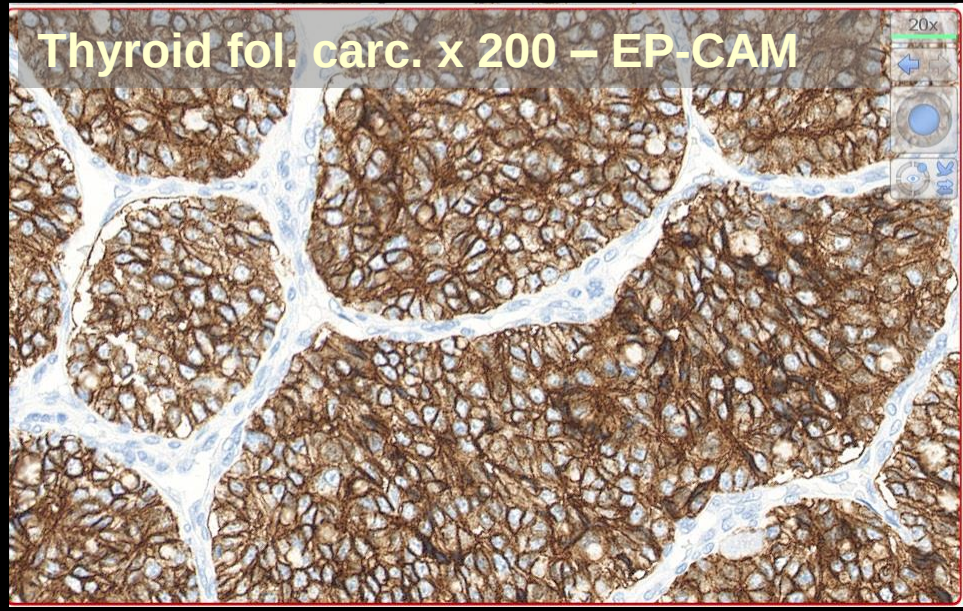

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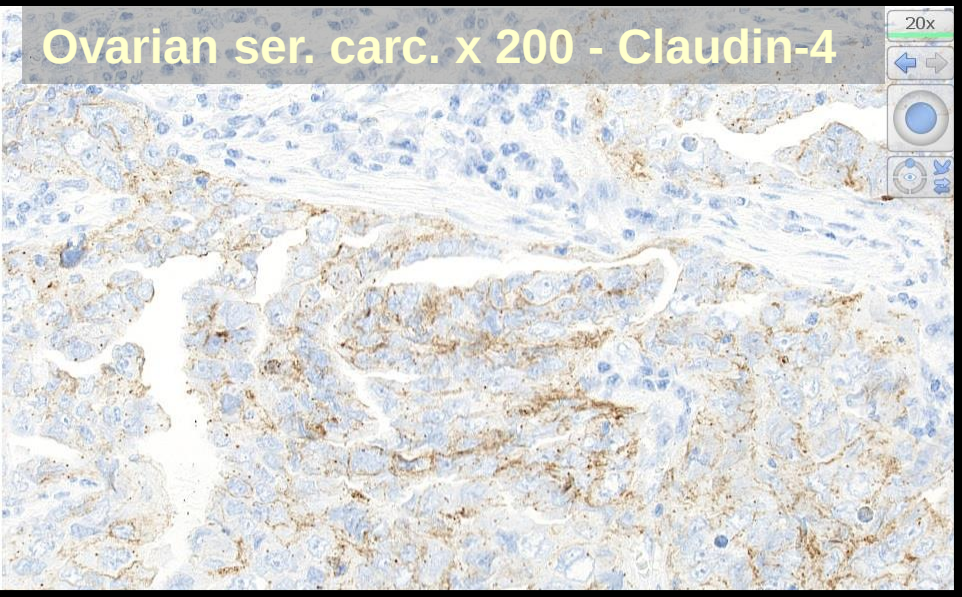

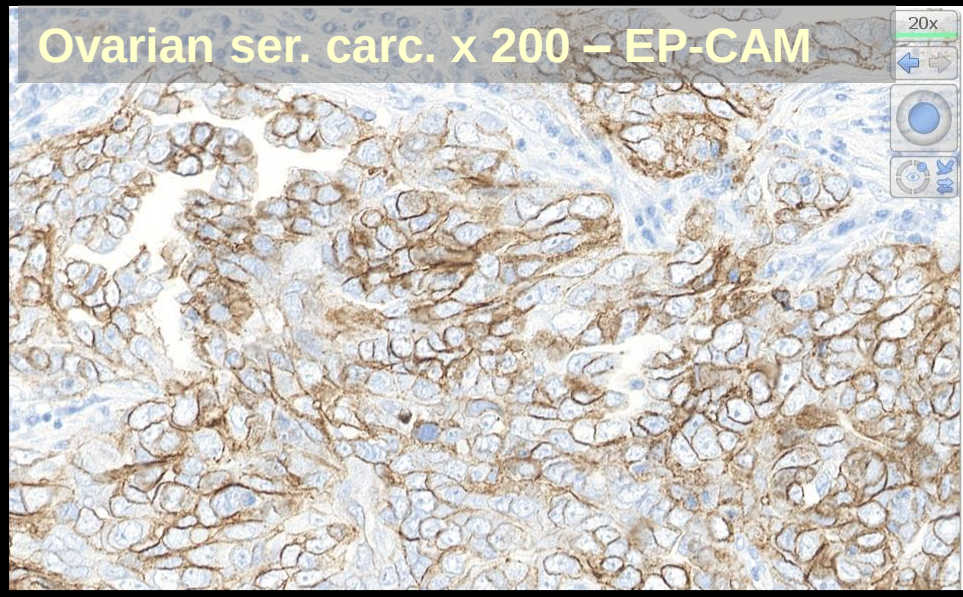

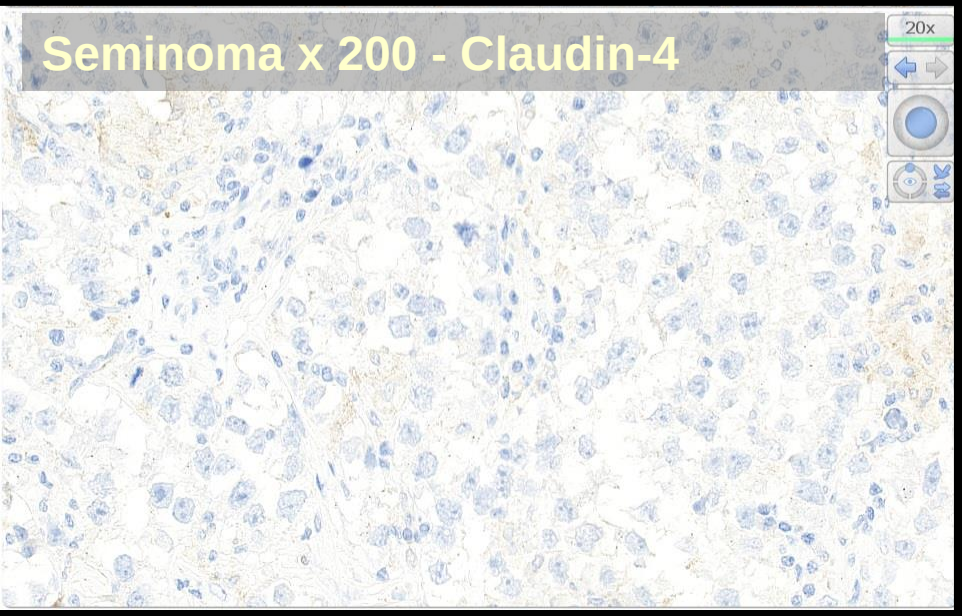

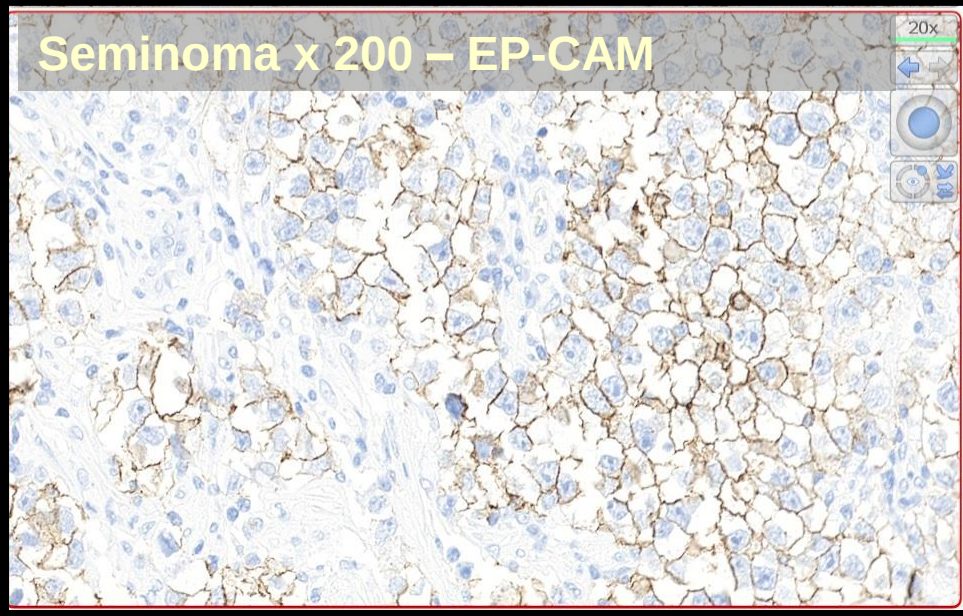

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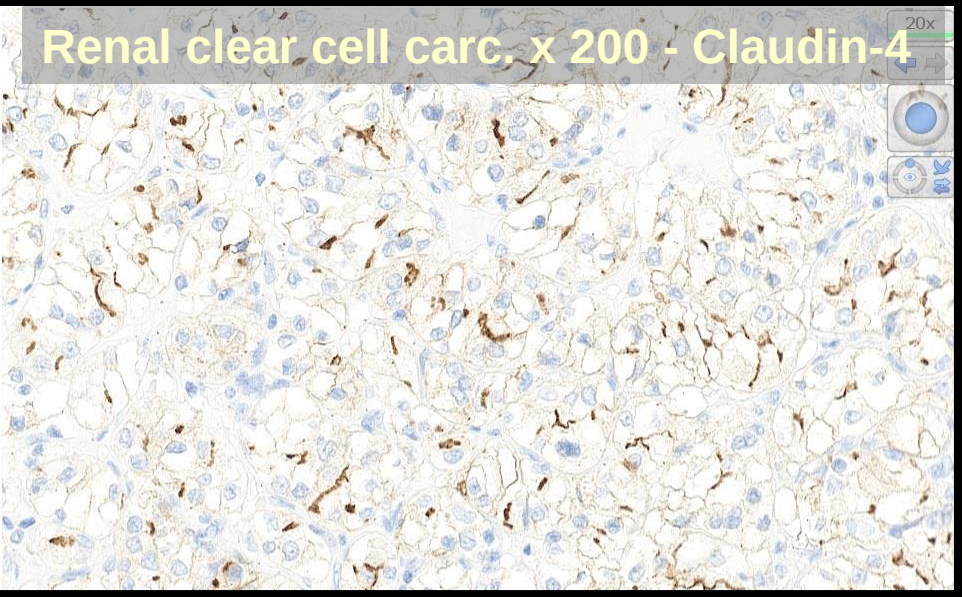

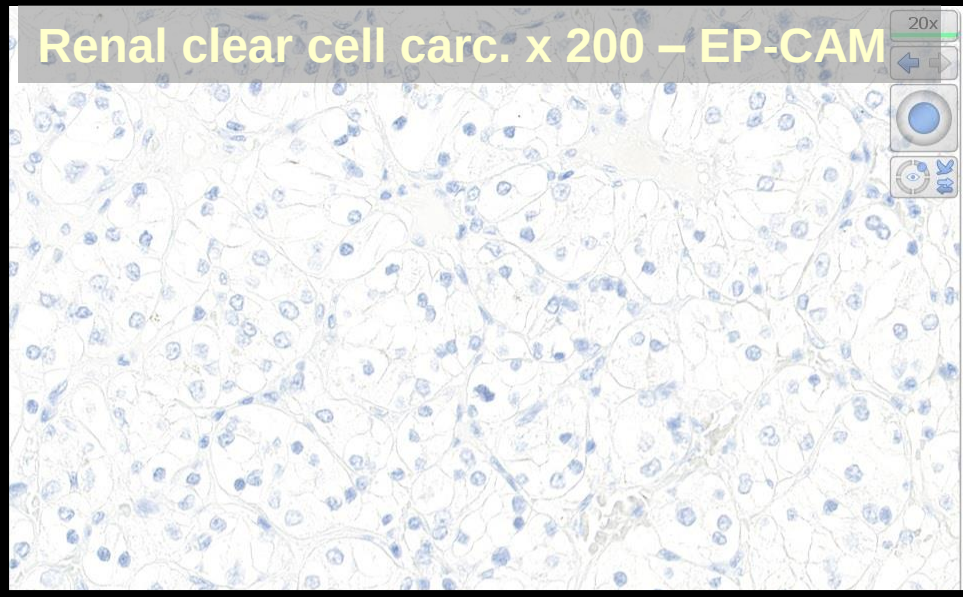

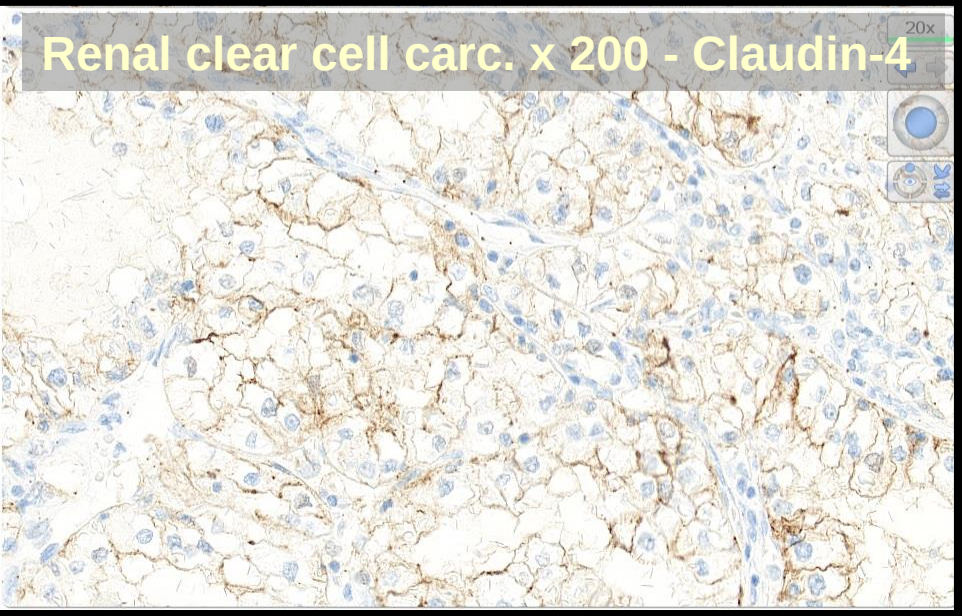

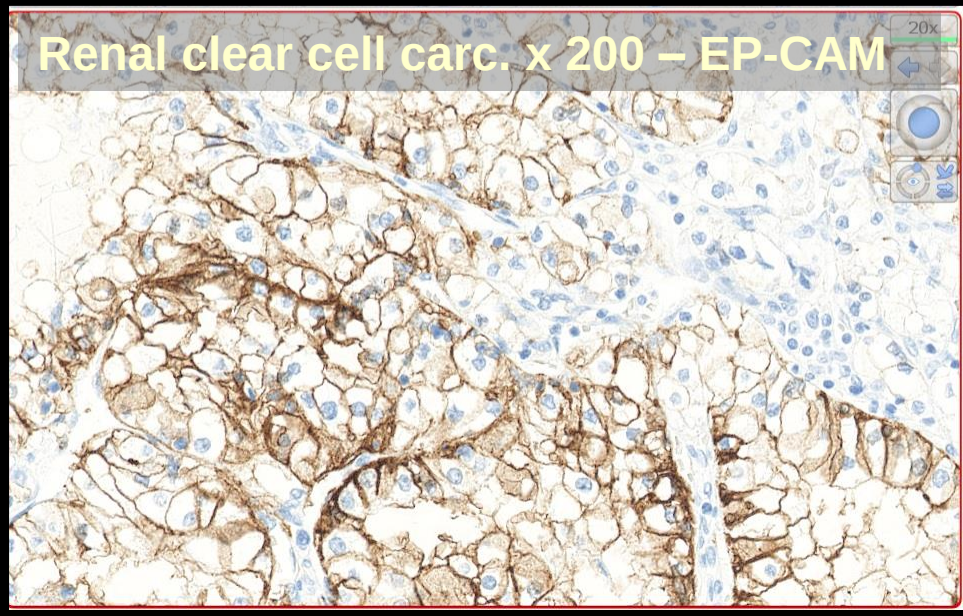

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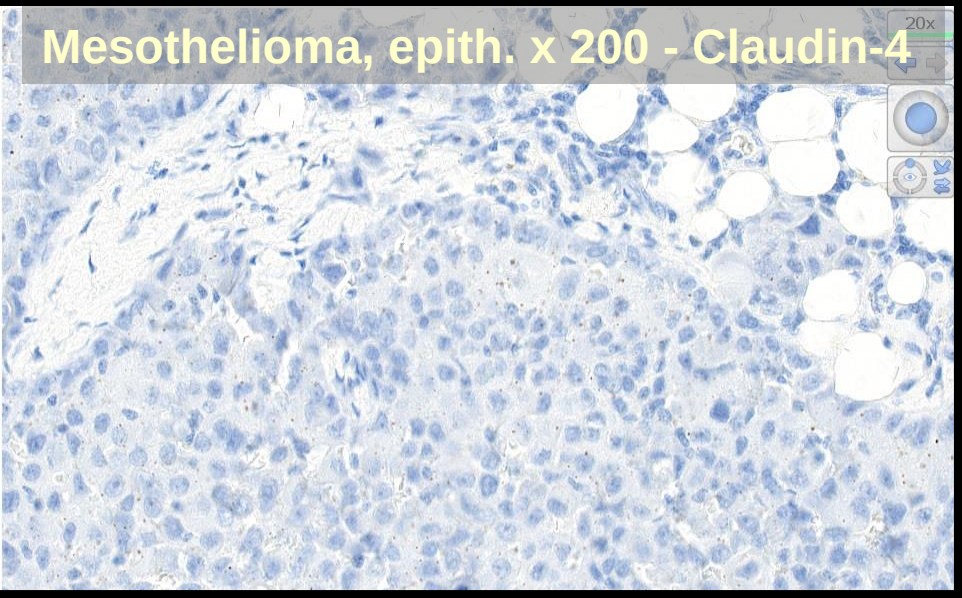
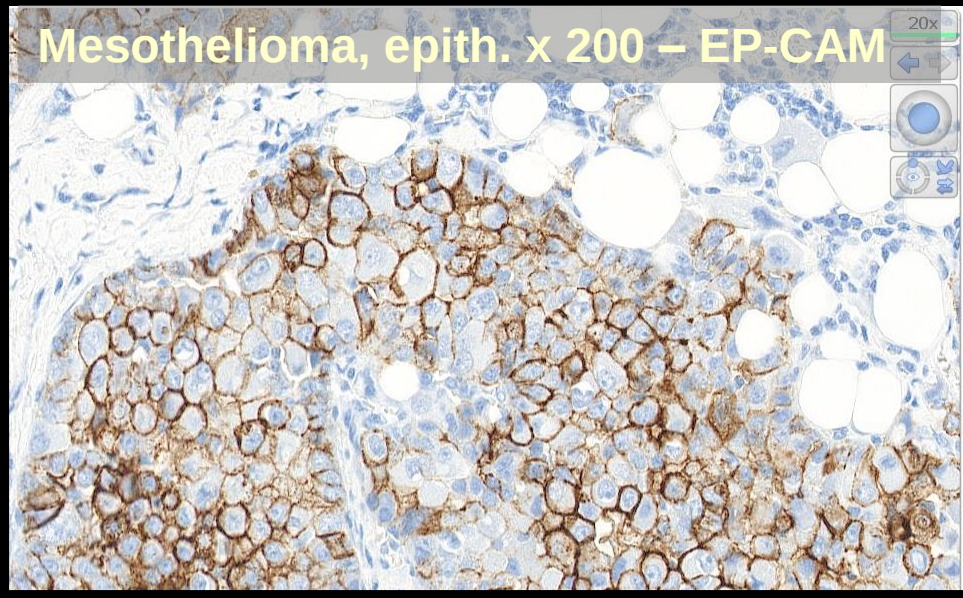
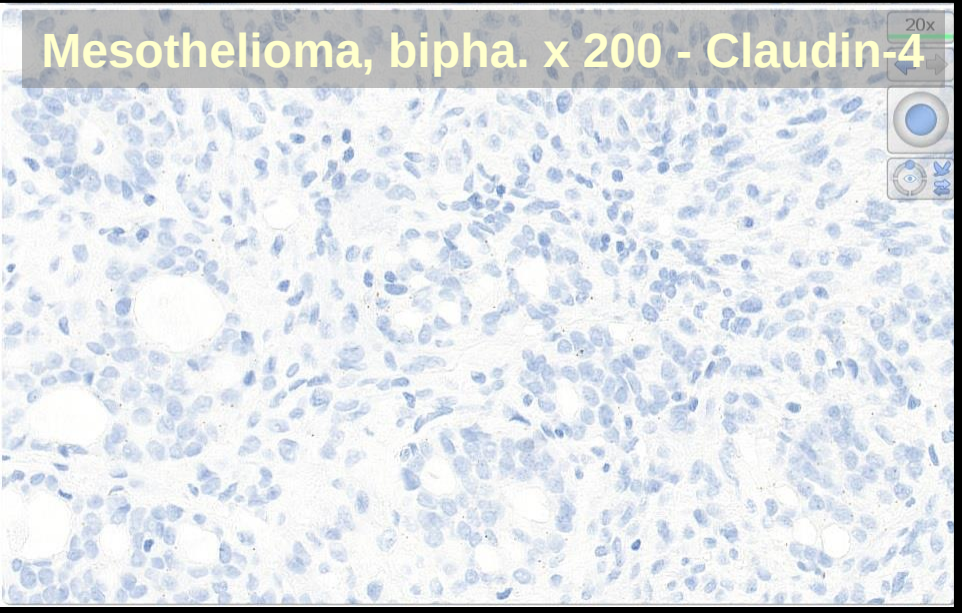
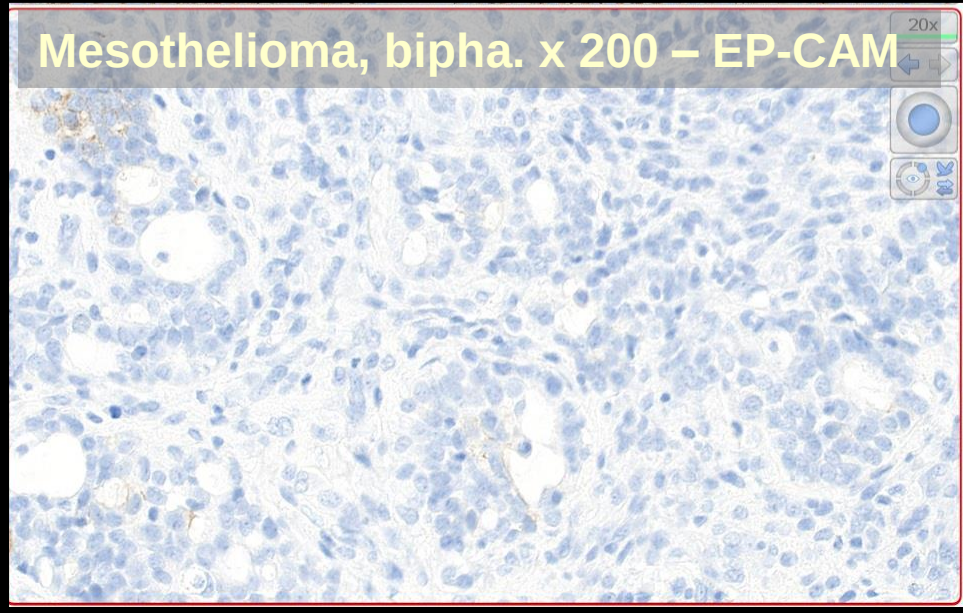








IHC – Protocols and controls for UPT II

UPT II: EPCAM

Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb Ber-EP4	HIER TRS 6.1 & Diva	1:50-200	3-step	Dako	3-step
mAb MOC31	HIER TRS 6.1, Diva & High	1:20-50	3-step	-	-
mAb BS14	HIER High / High + prot.	1:75-150	3-step	-	-

UPT II: Claudin-4

In-house pre-liminary data for best technical result

	Retrieval	Titre	Detection	RTU	Detection
mAb 3E2C1	HIER TRS 6.1	1:100	3-step	-	-

IHC – Protocols and controls for UPT II

GI	Recommendable clones (conc.)	Less successful clones (conc.)	RTU ”plug and play” giving optimal result
CDX2	mAb DAK-CDX2* rmAb EPR2764Y	mAb AMT28 rmAb CDX88	Dako: mAb DAK-CDX2 VMS: rmAb EPR2764Y
Cadh. 17	rmAb SP183		
SATB2	rmAb EP281 rmAb SP281		
SMAD4	mAb B8*,** mAb BCB8 rmAb EP618Y		

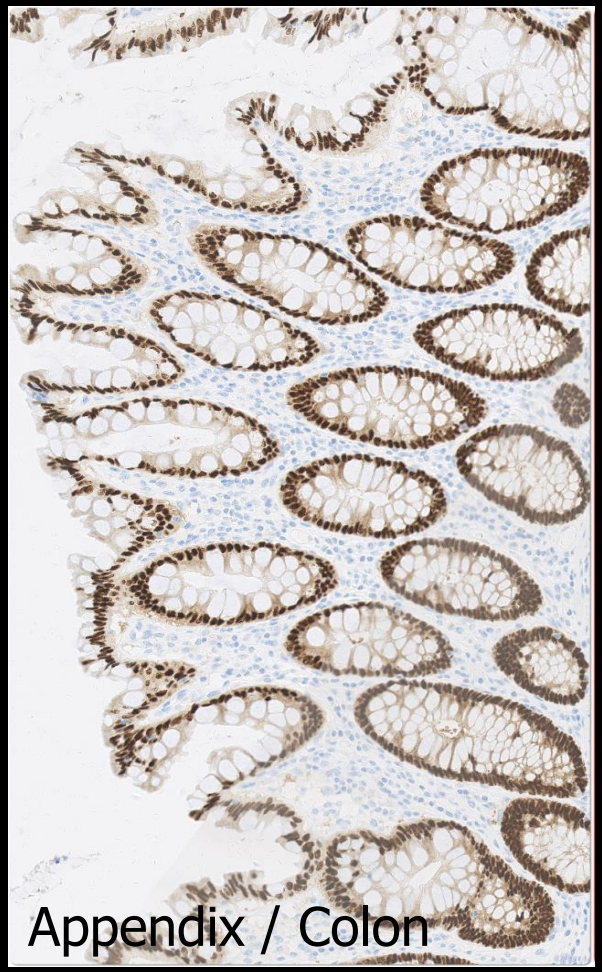
* Inferior performance on VMS/Leica stainer platforms
** Inferior performance on Dako Omnis stainer platform

IHC – Protocols and controls for UPT II

	Positive tissue control HE	Positive tissue control LE	Negative tissue control NE
CDX2	Appendix: Epithelial cells.	Pancreas: Epithelial cells of intercalating ducts	Liver: Hepatocytes
Cadh. 17	Appendix: Epithelial cells.	Pancreas: Epithelial (few) cells of large ducts	Liver: Hepatocytes
SATB2	Appendix: Epithelial cells.	Kidney: Epithelial cells of collecting ducts	Pancreas: Epithelial cells (all)
SMAD4	Tonsil: Scattered squamous epithelial cells Internal control	Tonsil: All T- and B-cells Internal control	Tumour with loss

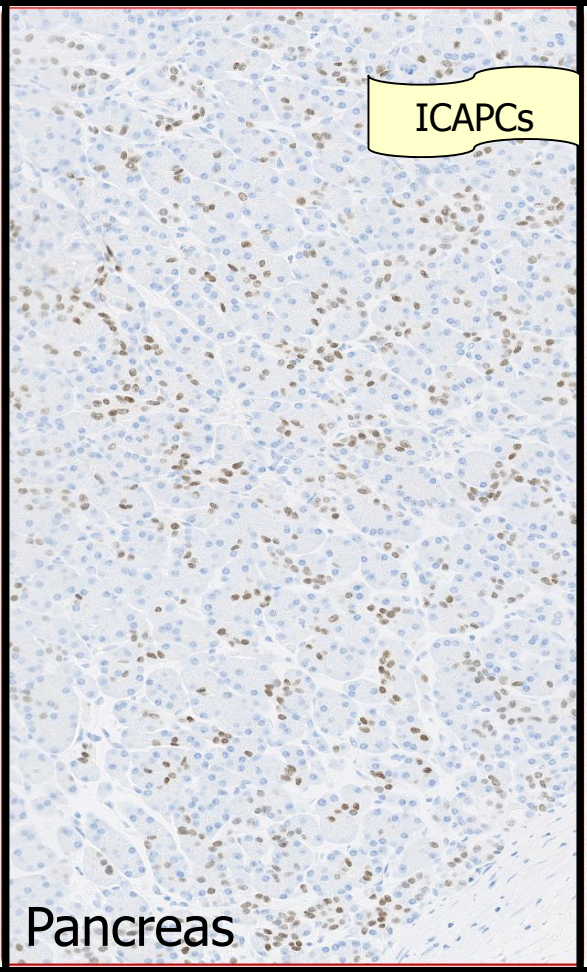
IHC – Protocols and controls for UPT II

CDX2 reaction pattern



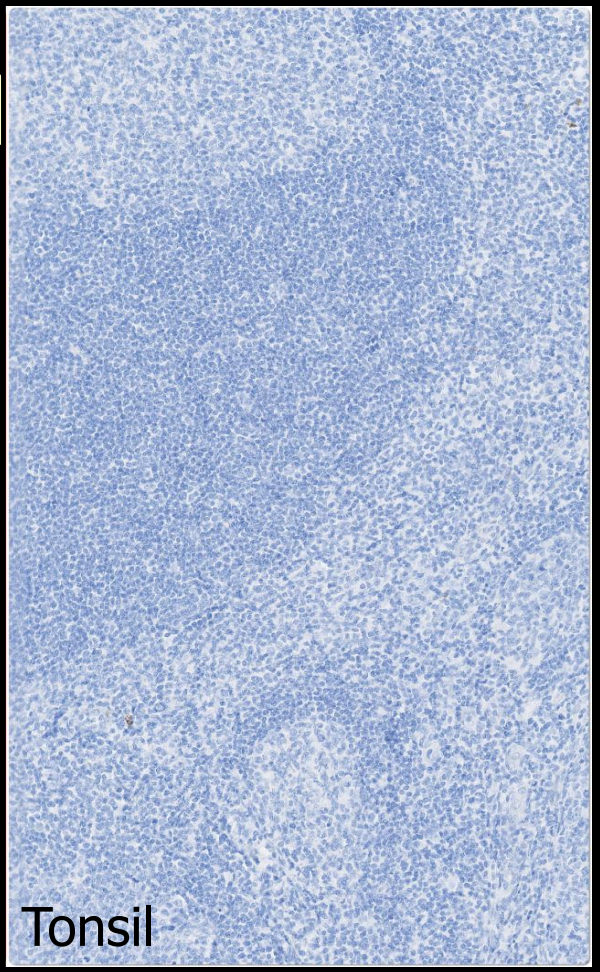
Appendix / Colon

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



Pancreas

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



Tonsil

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

IHC – Protocols and controls for UPT II

Table 1. Abs and assessment marks for CDX2, run 38

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **AMT28**	9	Leica/Novocastra	0	3	3	3	33 %	0 %
mAb clone **CDX2-88**	15 4	Biogenex Biocare	2	3	2	12	26 %	50 %
mAb clone **DAK-CDX2**	31	Dako	6	9	9	7	48 %	80 %
rmAb clone **EP25**	2 1	Epitomics Zhongshan	0	2	0	1	-	-
rmAb clone **EPR2764Y**	23 3 3 1 1 1 1	Cell Marque Thermo/Neomarkers Zytomed Epitomics Gene Tech Immunologic Nordic Biosite	20	11	2	0	94 %	94 %
Ready-To-Use Abs								
mAb clone **AMT28 PA0535**	3	Leica	0	1	2	0	-	-
mAb clone **AMT28 NCL-CDX2**	1	Novocastra	0	0	1	0	-	-
mAb clone **CDX2-88 PM226**	2	Biocare	1	0	1	0	-	-
mAb clone **CDX2-88 PH22 6AA**	1	Menarini	0	1	0	0	-	-
mAb clones **CDX2-88 ZA-0520**	1	Zhongshan	0	0	1	0	-	-
mAb clone **DAK-CDX2 IS/IR080**	44	Dako	11	27	6	0	86 %	95 %
rmAb clone **EPR2764Y 760-4380**	48	Ventana/Cell Marque	35	10	2	1	94 %	95 %
rmAb clone **EPR2764Y 235R-18**	3	Cell Marque	2	1	0	0	-	-
rmAb clone **EPR2764Y GT201902**	1	Gene Tech	0	0	0	1	-	-
rmAb clone **EPR2764Y MAD-000343QD**	1	Master Diagnostica	1	0	0	0	-	-
Total	200		78	68	29	25		
Proportion			39 %	34 %	15 %	12 %	73 %	

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

AMT28 and CDX2-88 lower pass rate – used by a significantly lower proportion – 18% in this run vs 70% in run 27 2009.

DAK-CDX2 & EPR2764Y

Conc & RTU

Control tissue !

IHC – Protocols and controls for UPT II

Table 2: **Optimal results for CDX2 using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **CDX2-88**	50 % 1/2**	-	0 % 0/8	-	50 % 1/2	-
mAb clone **DAK-CDX2**	67 % 6/9	-	0 % 0/7	-	-	-
rmAb clone **EPR2764Y**	60 % 3/5	100 % 1/1	63 % 10/16	-	80 % 4/5	0 % 0/2

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

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

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

Table 3: Proportion of sufficient results for CDX2 in the four NordiQC runs performed

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

< use of mAb clones CDX2-88 and AMT28

IHC – Protocols and controls for UPT II

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

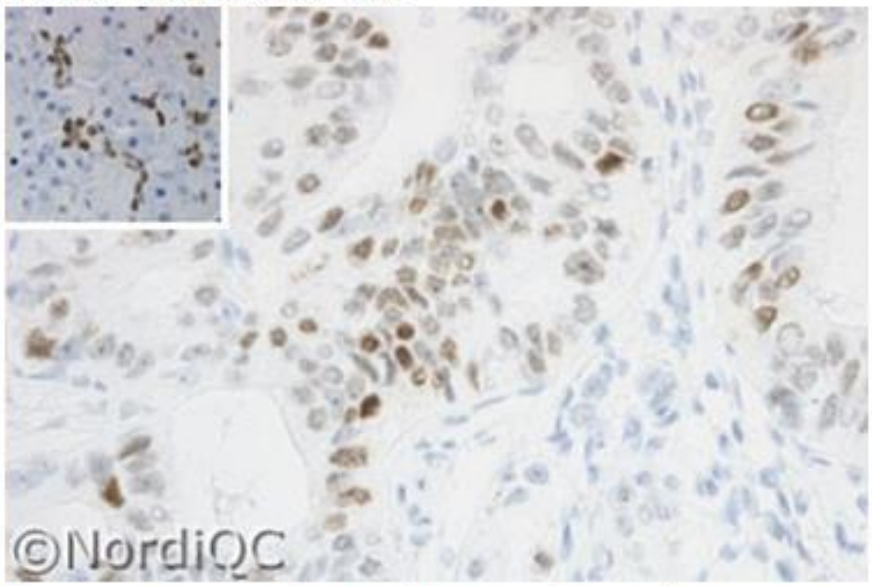


Fig. 4a. Pancreas adenocarcinoma and normal pancreas (insert) showing optimal staining for CDX2 with clone the mAb clone DAK-CDX2 in a Ready-To-Use format and performed at the Autostainer platform. A weak to moderate staining is seen in the majority of the ductal epithelial cells of the pancreas and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

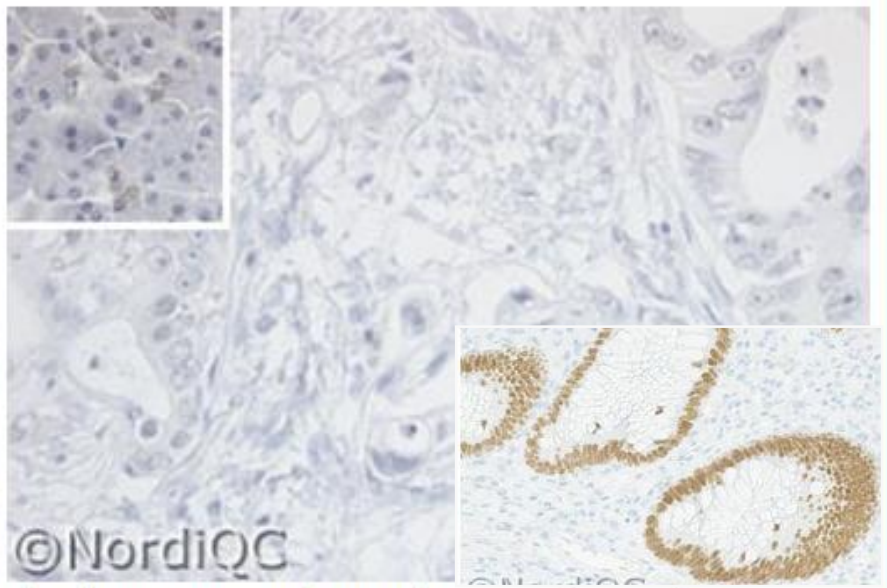
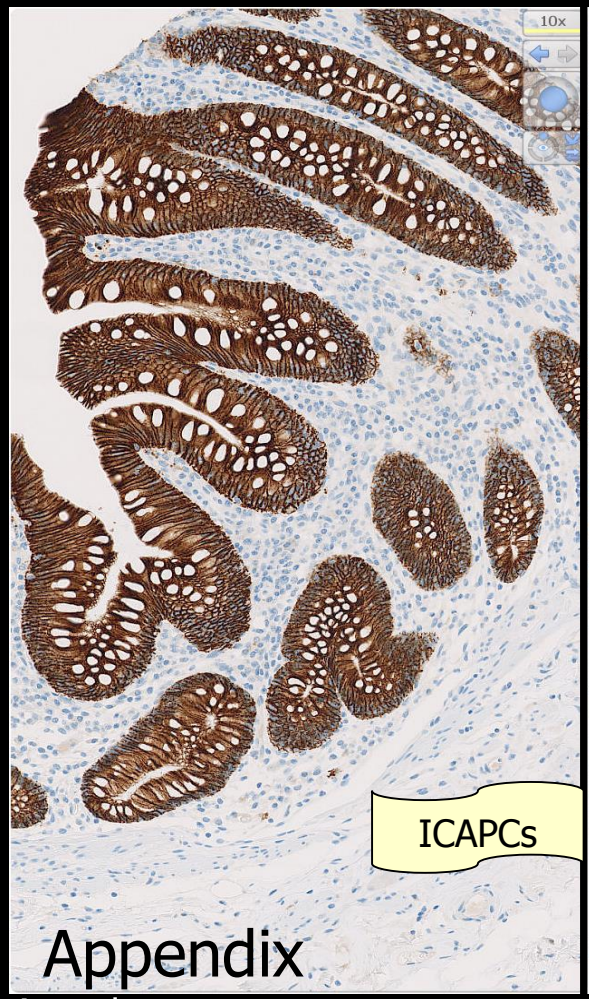


Fig. 4b. Pancreas adenocarcinoma and normal pancreas (insert) showing an insufficient staining for CDX2. Same Ready-To-Use product of the mAb clone DAK-CDX2 as in Fig. 4a was used, but performed at the Ventana Benchmark platform. Only a faint staining in very few ductal epithelial cells is seen and the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

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

IHC – Protocols and controls for UPT II

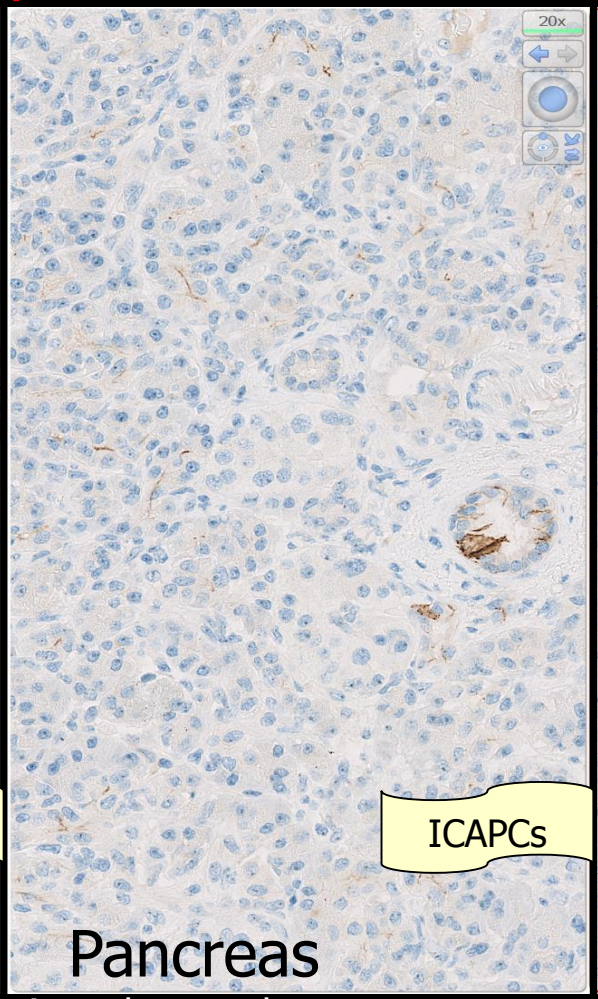
Cadherin 17 reaction pattern



Appendix

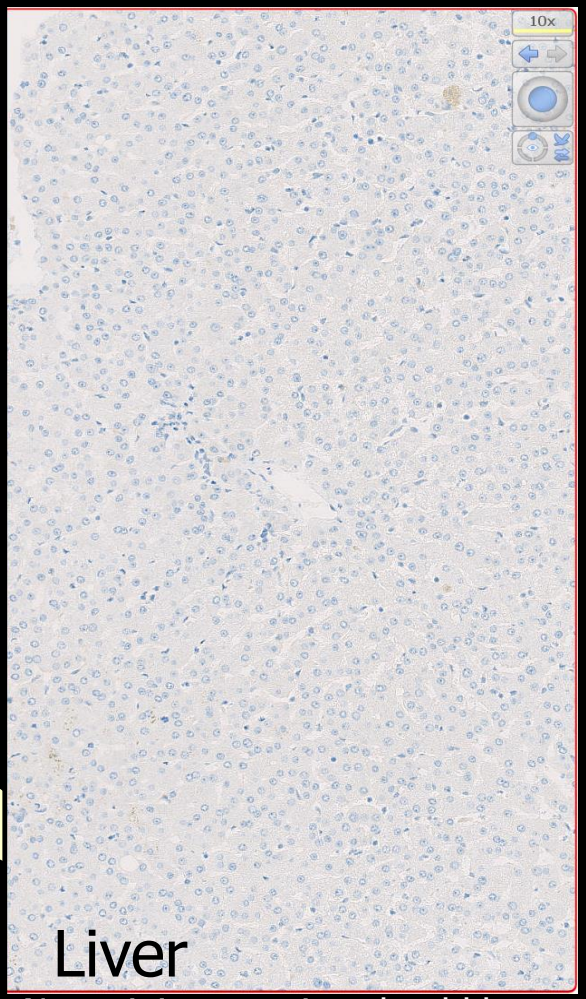
A moderate to strong membranous staining of virtually all columnar epithelial cells – both luminal and crypt base.

.



Pancreas

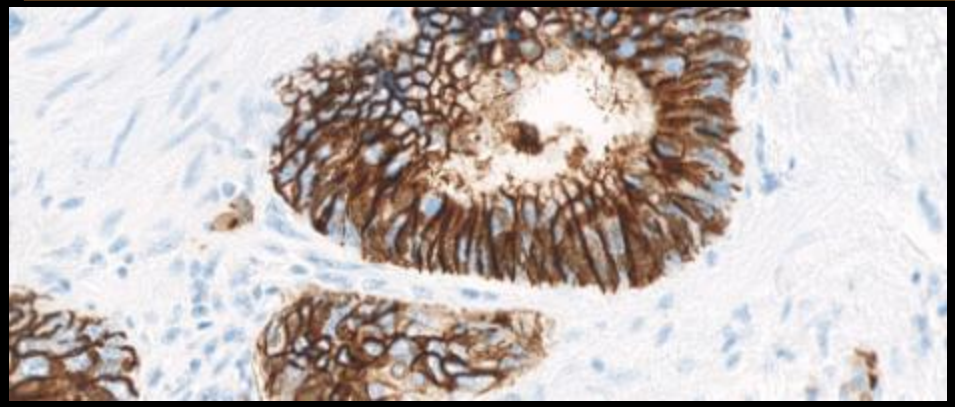
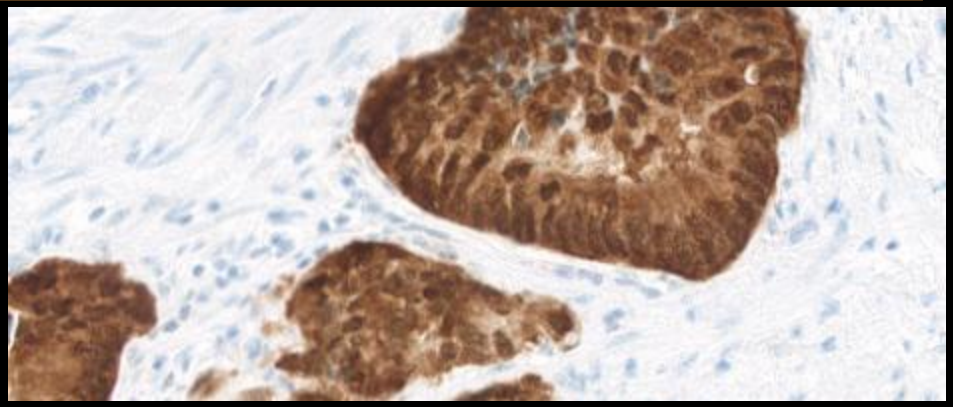
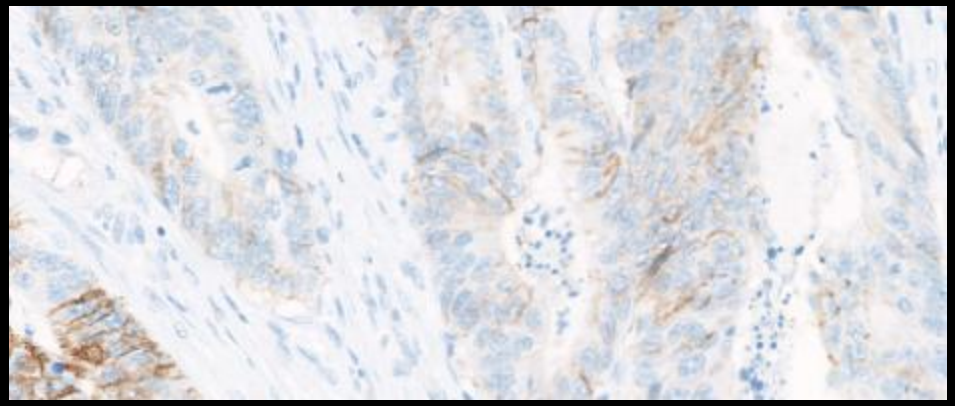
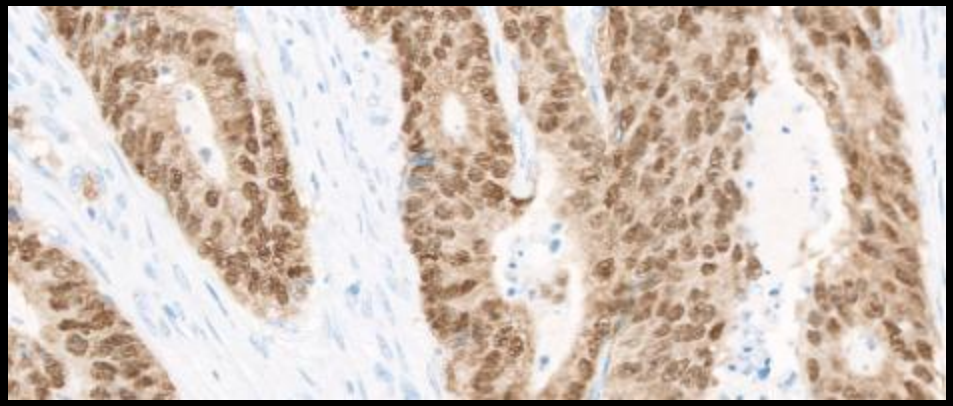
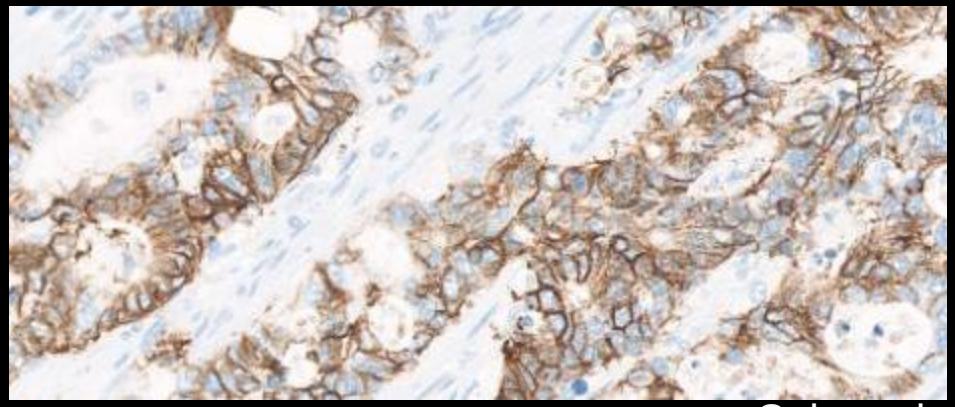
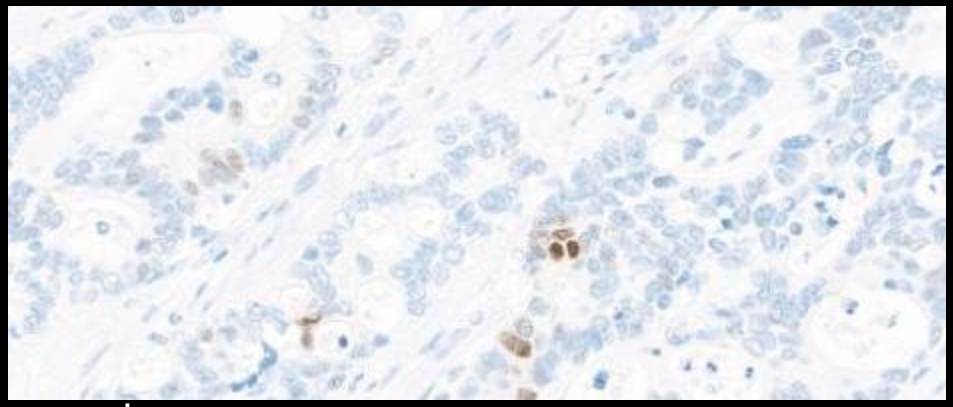
A weak to moderate membranous staining of dispersed columnar epithelial cells of large ducts and exocrine acini.



Liver

No staining reaction should be seen.

IHC – Protocols and controls for UPT II

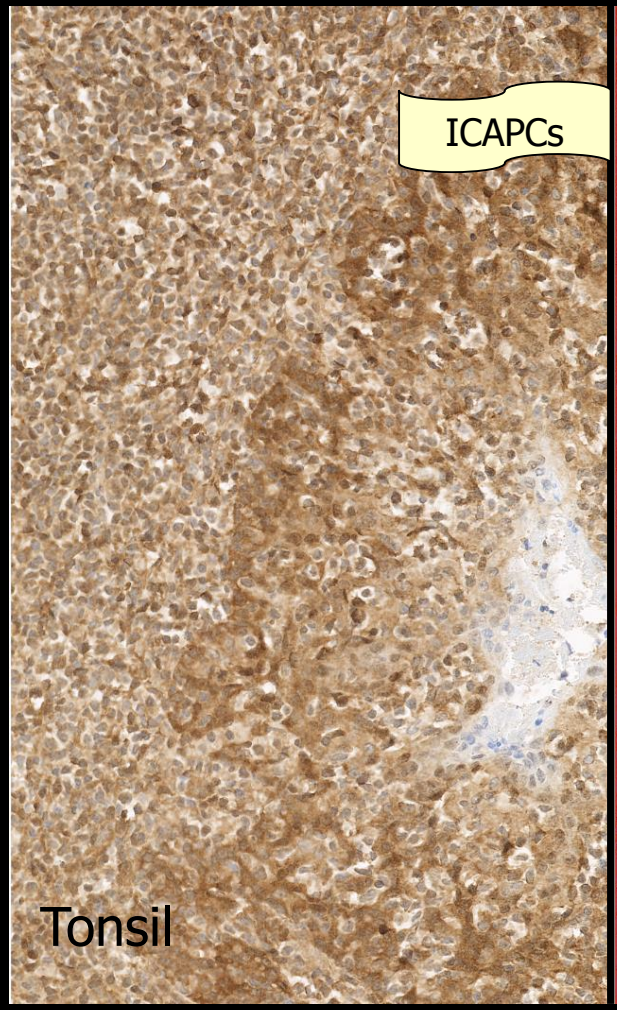
Colon adenocarcinomas

Cadherin 17, rmAb SP183

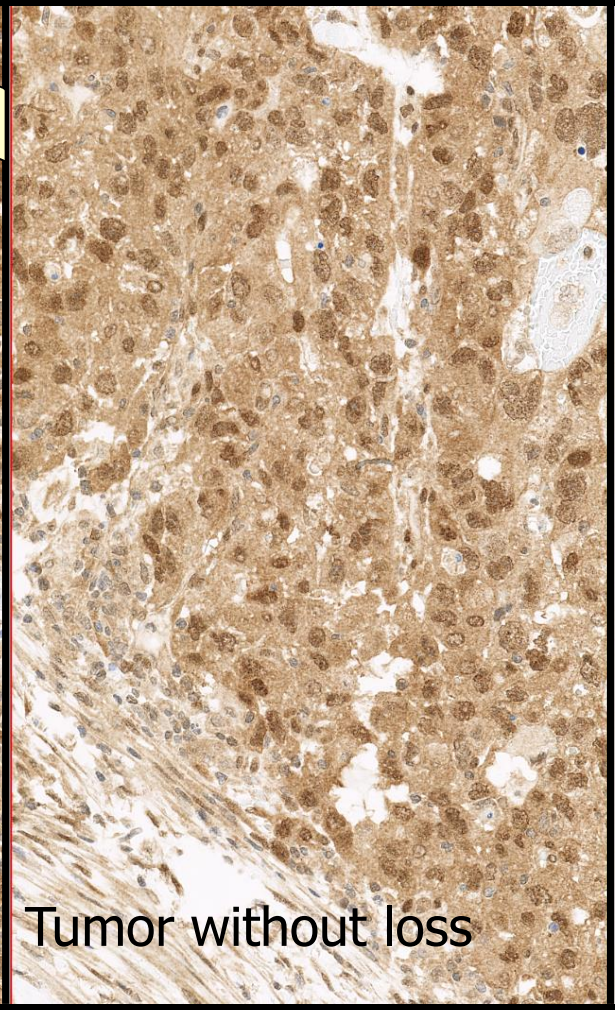
CDX2, rmAb EPR2764Y

IHC – Protocols and controls for UPT II

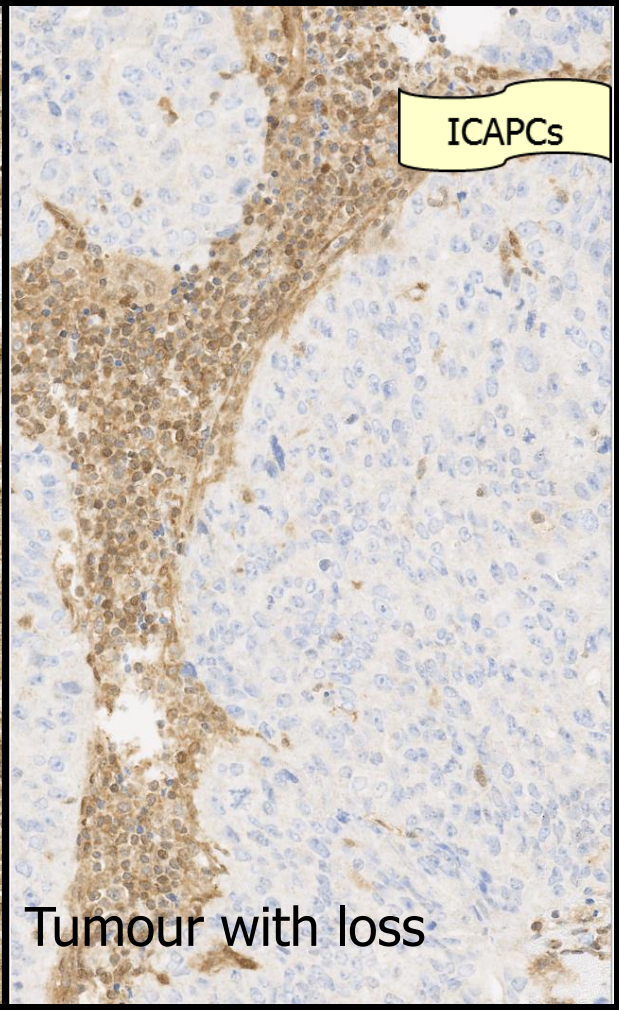
SMAD4 reaction pattern in external tissue controls



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



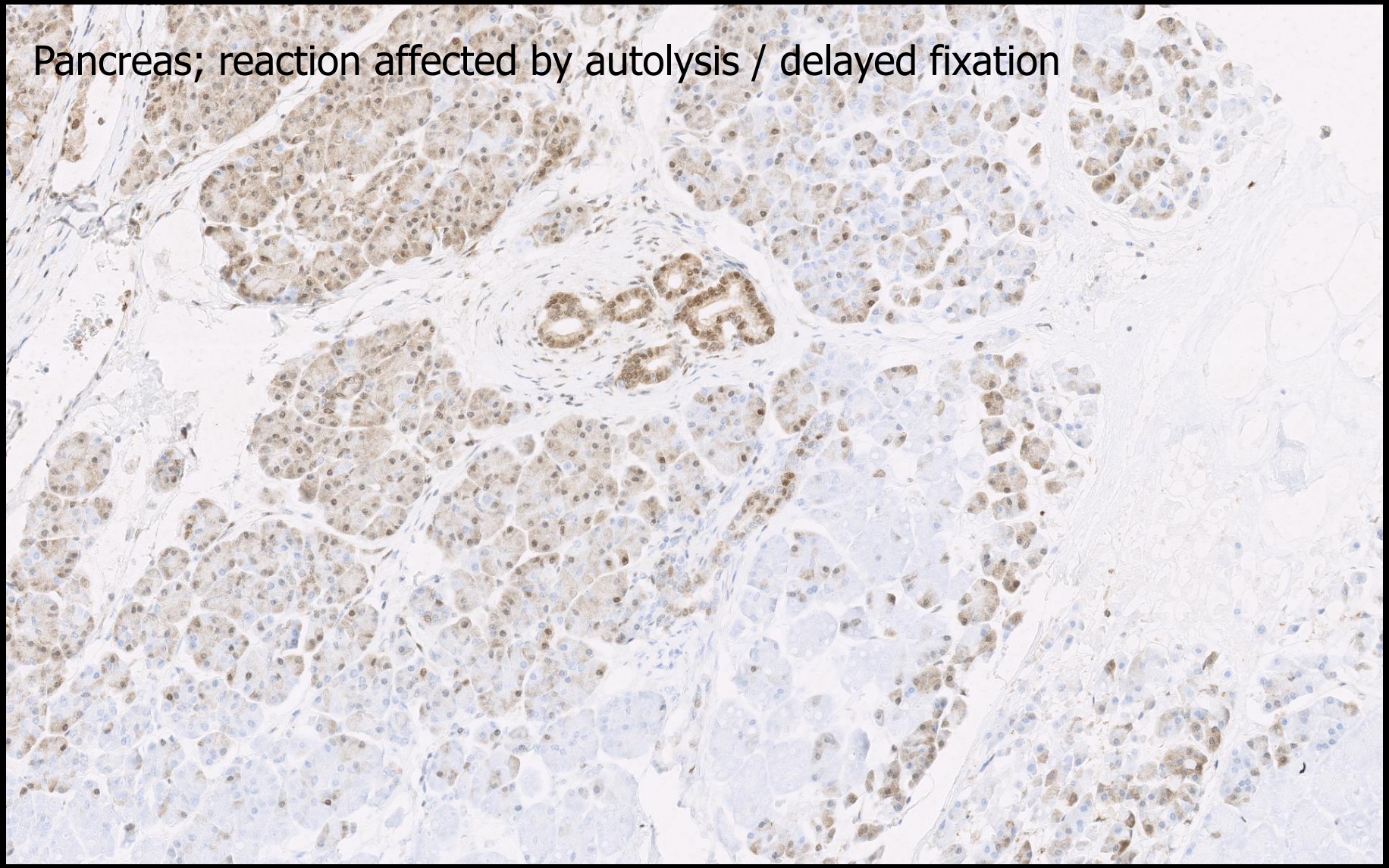
A moderate to strong nuclear staining reaction of the majority of all cells. A weak to moderate cytoplasmic staining reaction can be expected.



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

IHC – Protocols and controls for UPT II

SMAD4 reaction pattern



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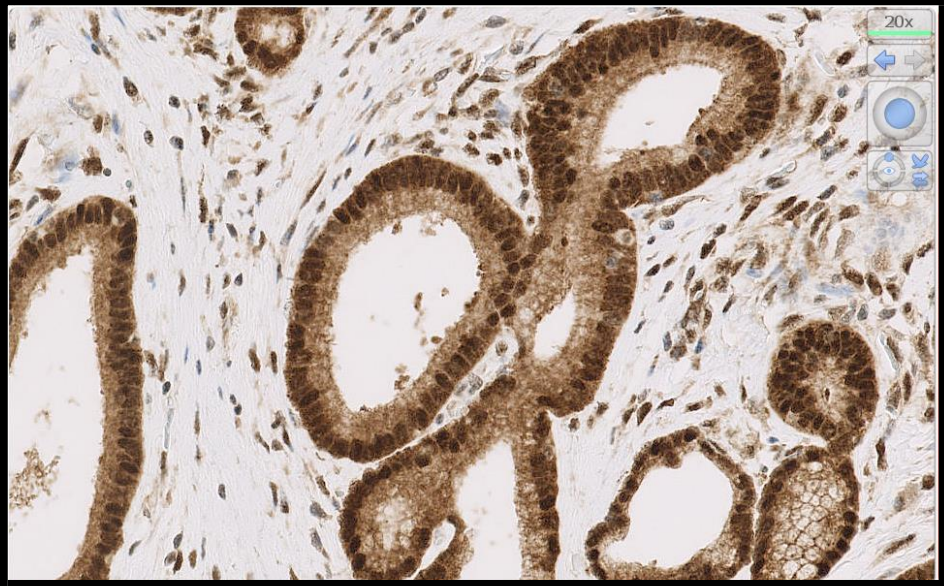
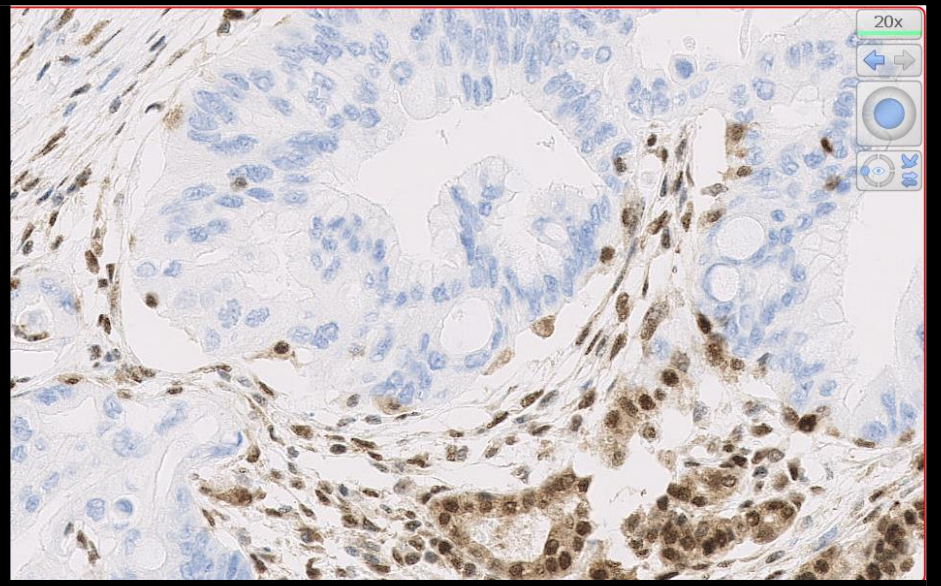
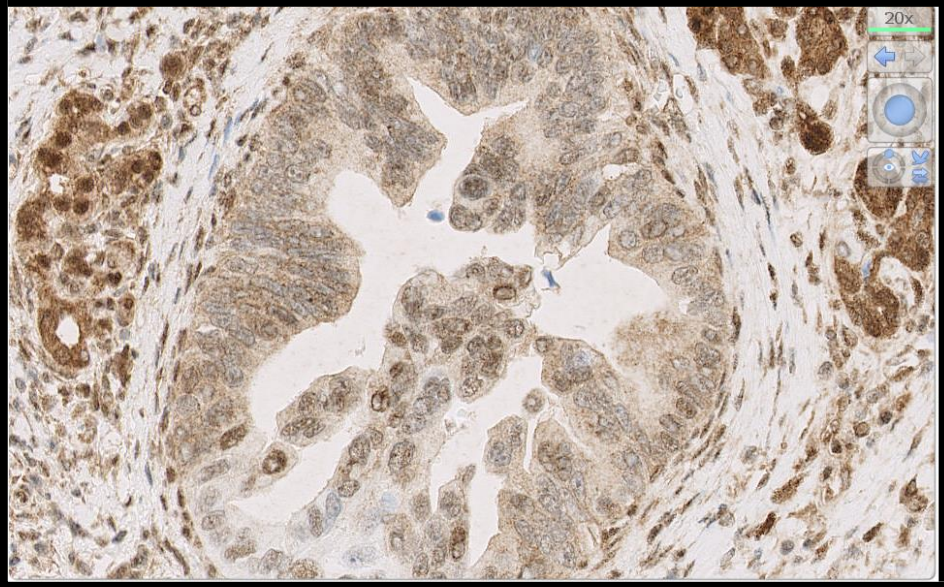
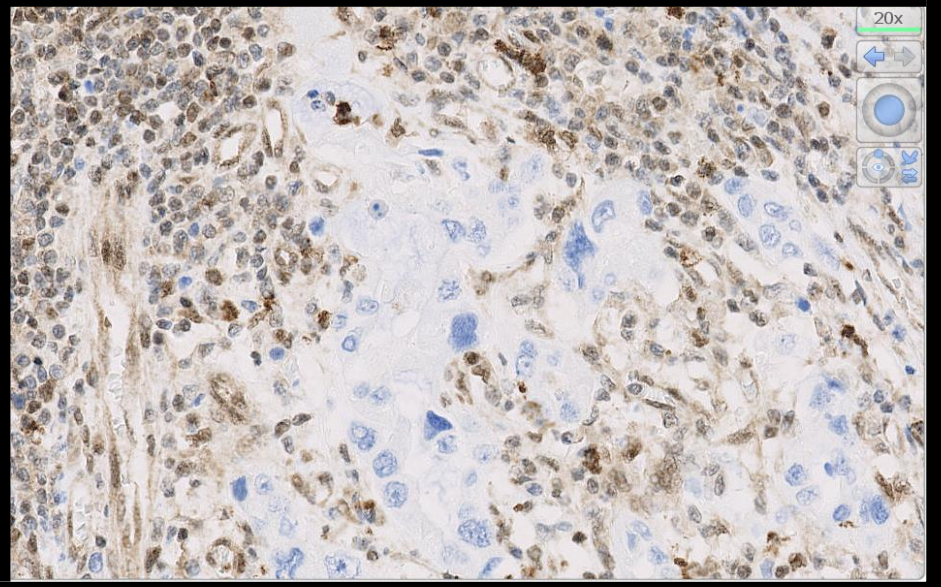








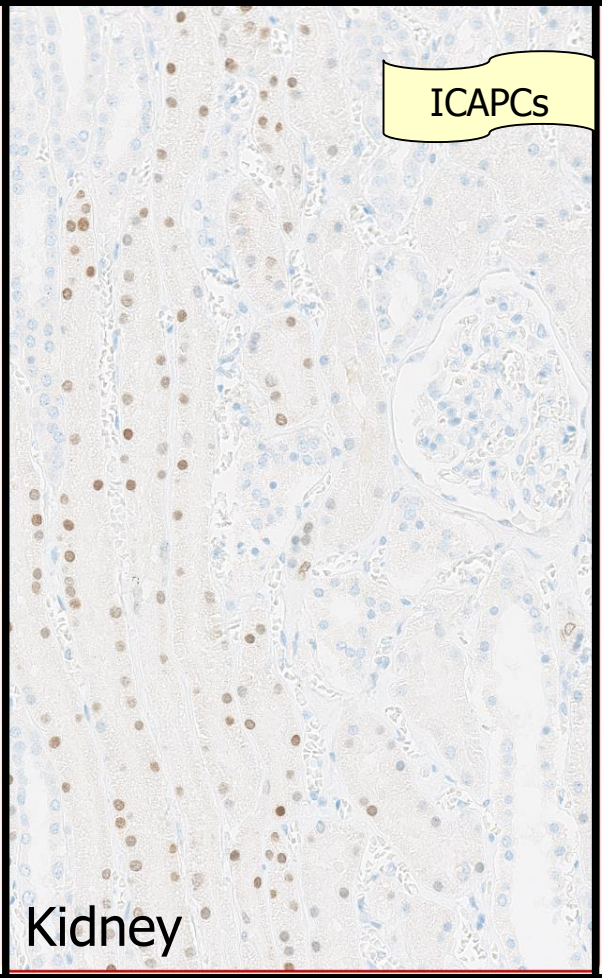
SMAD4 staining: mAb BC8, TE pH 9, FLEX+

IHC – Protocols and controls for UPT II

SATB2 reaction pattern

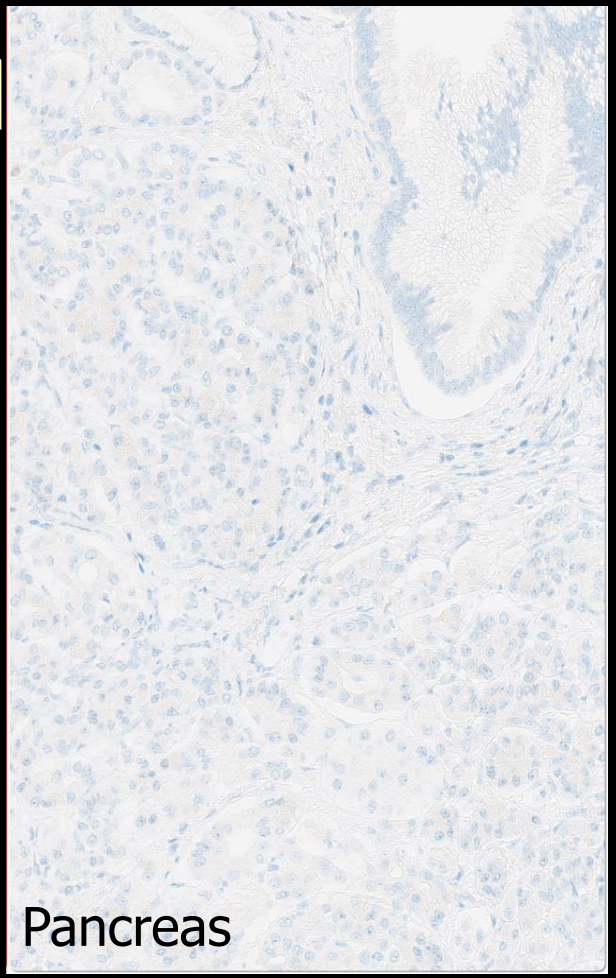
Appendix / Colon

A strong nuclear staining reaction of virtually all epithelial cells. A weak cytoplasmic staining reaction can be seen.



Kidney

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

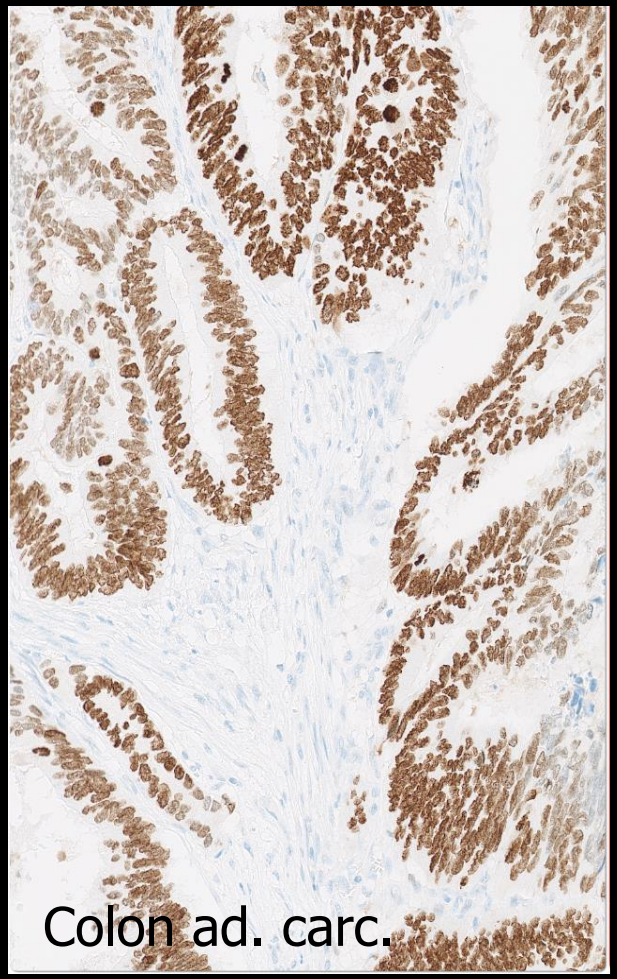


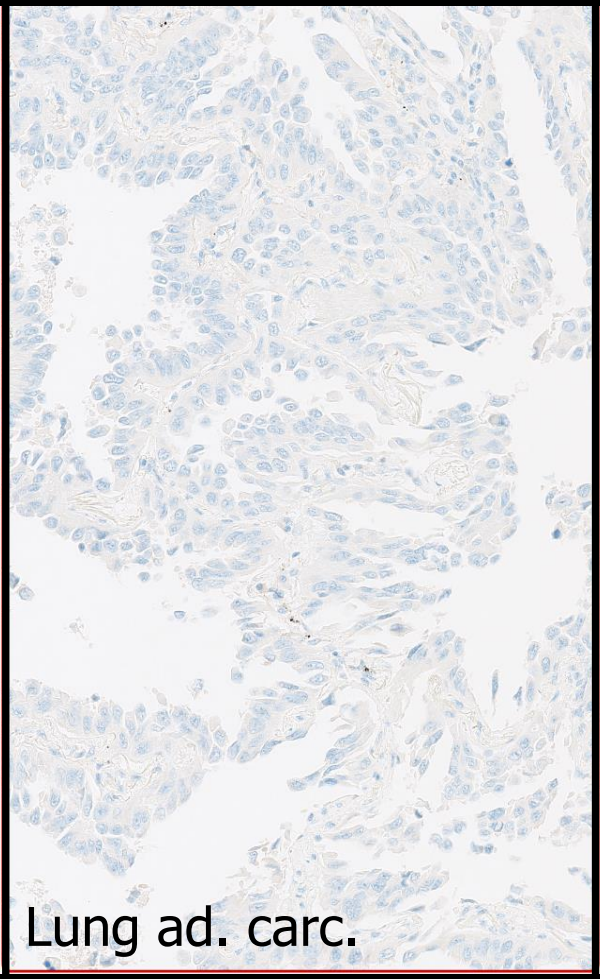
Pancreas

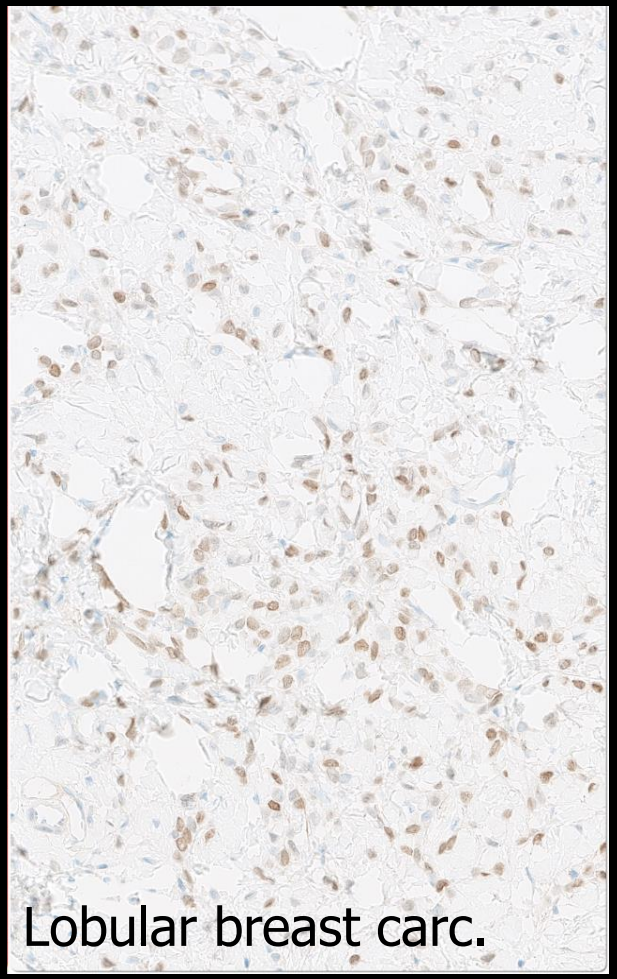
No staining reaction.

IHC – Protocols and controls for UPT II

SATB2 reaction pattern







Internal studies:

+ 7 of 8 colon adenocarcinomas
10% cut-off

- in 50 of 52 other carcinomas

+ in 1 of 4 lobular breast carc.
+ in 1 of 4 RCC

IHC – Protocols and controls for UPT II

UPT II: CDX2
Basic protocol settings for an optimal staining result (NQC)

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

* Inferior performance on VMS stainer platform

UPT II: Cadherin 17
Basic protocol settings for an optimal staining result (Internal data)

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

IHC – Protocols and controls for UPT II

UPT II: SMAD4

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

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

* Inferior peformance on VMS stainer platform and Dako Omnis.
** Benefits from Renoir Red diluent

UPT II: SATB2

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

	Retrieval	Titre	Detection	RTU	Detection
rmAb EP281, SP281	HIER TE	1:200-400	3-step	-	-

IHC – Protocols and controls for UPT II

	Recommendable clones (conc.)	Less successful clones (conc.)	RTU ”plug and play” giving optimal result
PAX8	mAb BC12*, ** mAb ILQ-50 mAb MRQ-50*,** rmAb ZR-1 pAb 10336-1-AP	pAb 363	

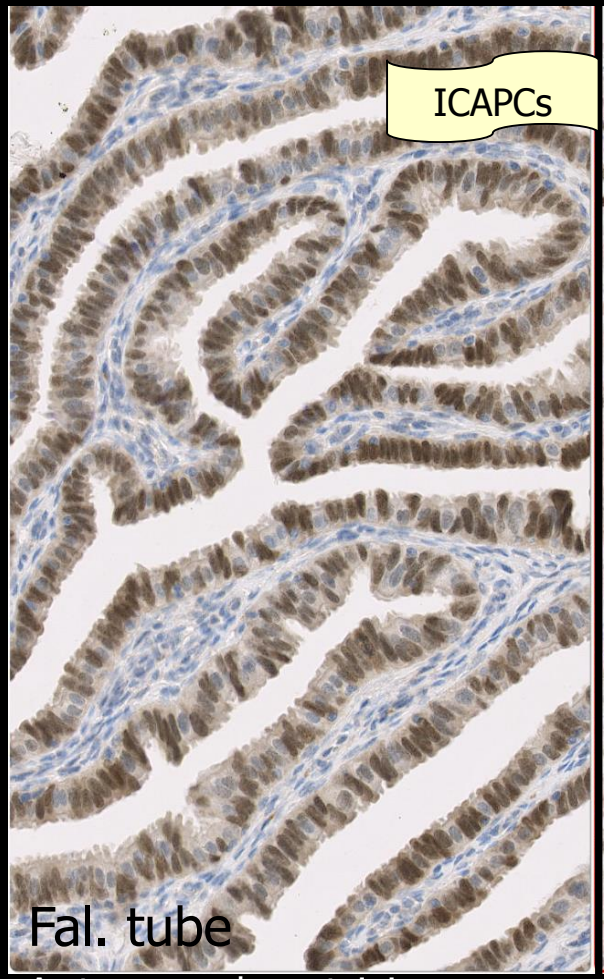
* Inferior performance on VMS/Leica stainer platforms
** Inferior performance on Dako Omnis stainer platform

IHC – Protocols and controls for UPT II

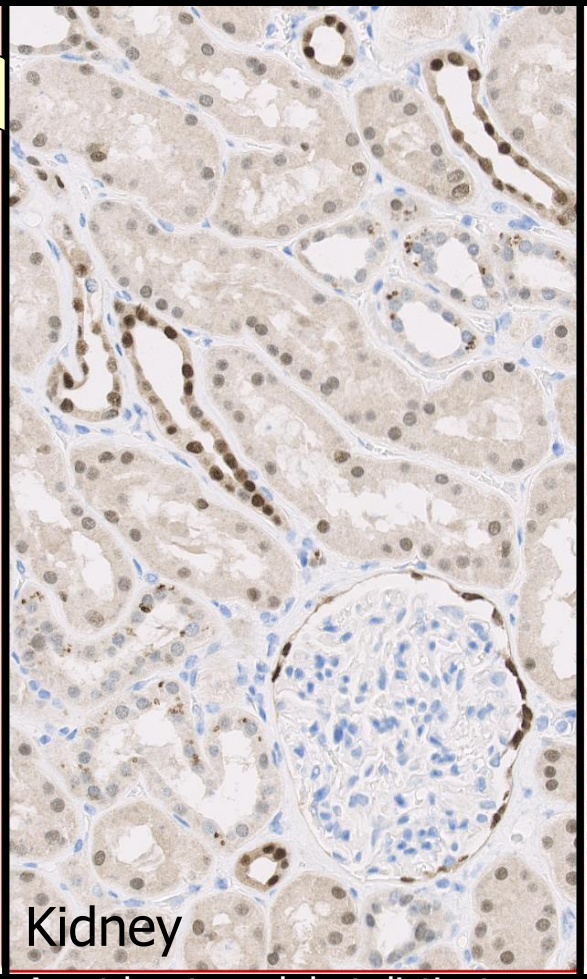
	Positive tissue control HE	Positive tissue control LE	Negative tissue control NE
PAX8	Fallopian tube: Secretory epithelial cells. Kidney: Epithelial cells of collecting ducts and lining Bowman capsules.	Fallopian tube: Ciliated epithelial cells. Kidney: Epithelial cells of proximale tubules.	Appendix: Epithelial cells

IHC – Protocols and controls for UPT II

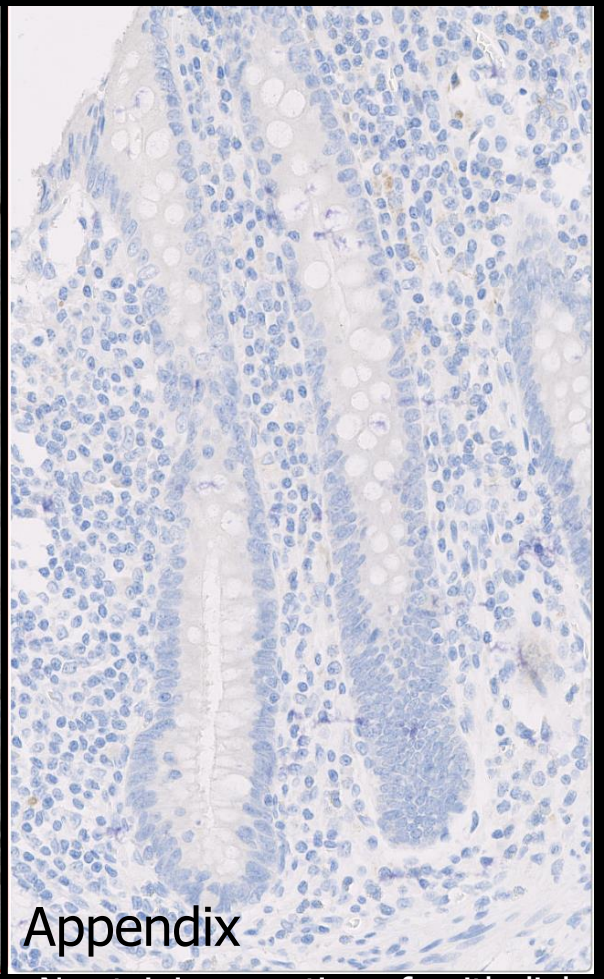
PAX8 reaction pattern



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



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



No staining reaction of epithelial cells.

IHC – Protocols and controls for UPT II

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

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

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

Choose clone depending on platform...

IHC – Protocols and controls for UPT II

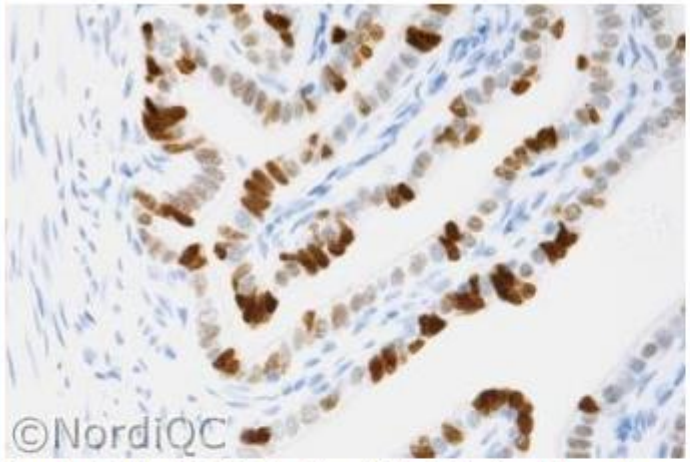


Fig. 1a. Optimal PAX8 staining of the Fallopian tube using the mAb clone MRQ-50 as a concentrate, HIER in TRS pH 6.1 and a 3-step polymer based detection system. Virtually all the ciliated epithelial cells show a distinct, weak to moderate nuclear staining reaction, while the secretory epithelial cells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

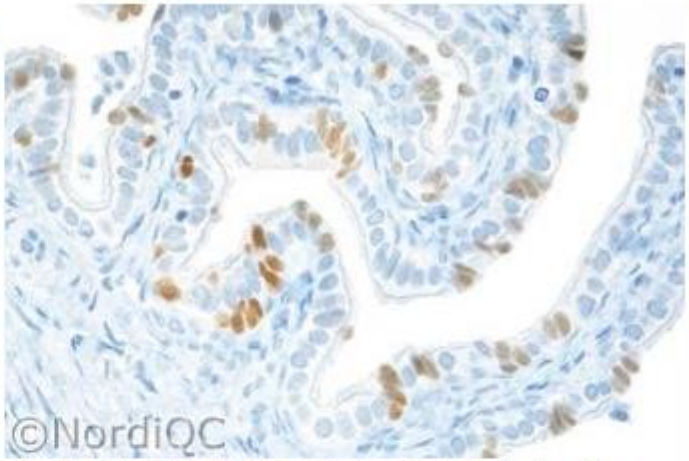


Fig. 1b. Insufficient PAX8 staining of the Fallopian tube using the mAb clone MRQ-50 as a concentrate with a protocol giving a too low sensitivity (a too low concentration of the primary Ab and a 2-step multimer based detection system) - same field as in Fig. 1a. The proportion of positive cells and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

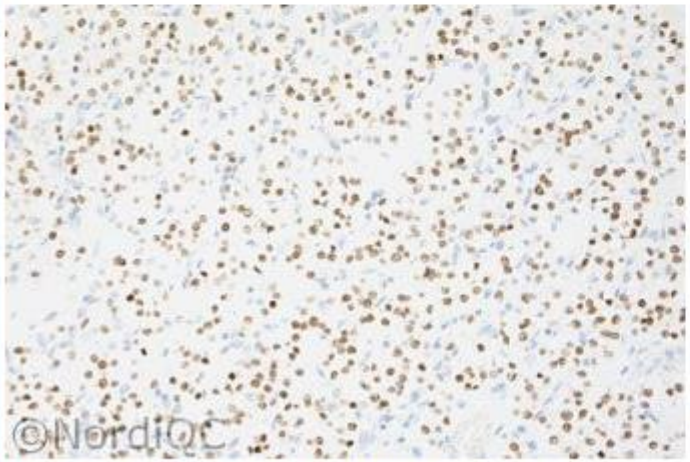


Fig. 2a. Optimal PAX8 staining of the renal clear cell carcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a moderate to strong nuclear staining reaction.

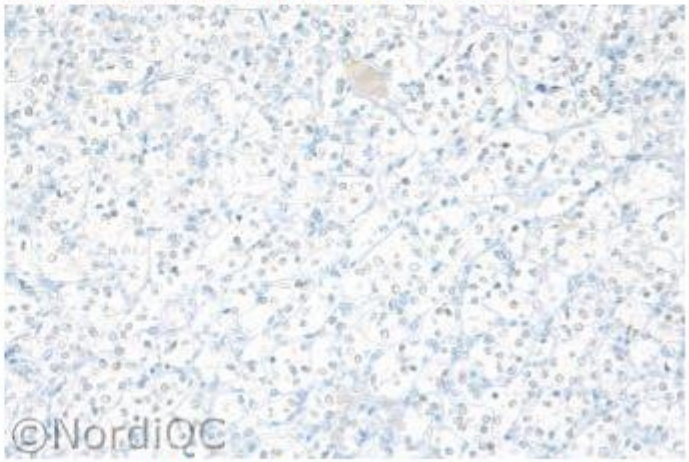


Fig. 2b. Insufficient PAX8 staining of the renal clear cell carcinoma using same protocol as in Fig. 1b - same field as in Fig. 2a. Only scattered neoplastic cells show an equivocal staining reaction.

Dako AS48:

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

VMS, Dako OMNIS

ZR1 or Prot. Tech

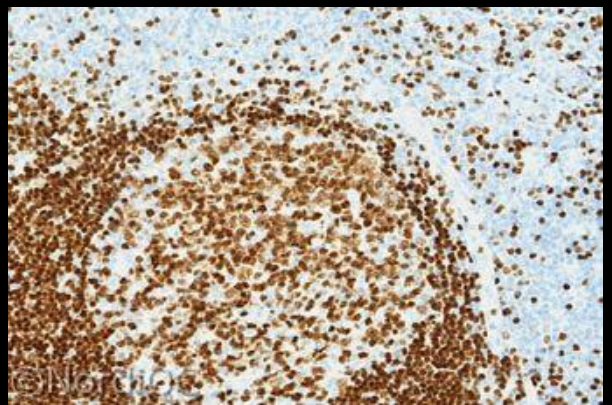
IHC – Protocols and controls for UPT II

MODERN PATHOLOGY (2011), 1–6

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

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

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



Tonsil stained for PAX8 =

Same pattern as for PAX5

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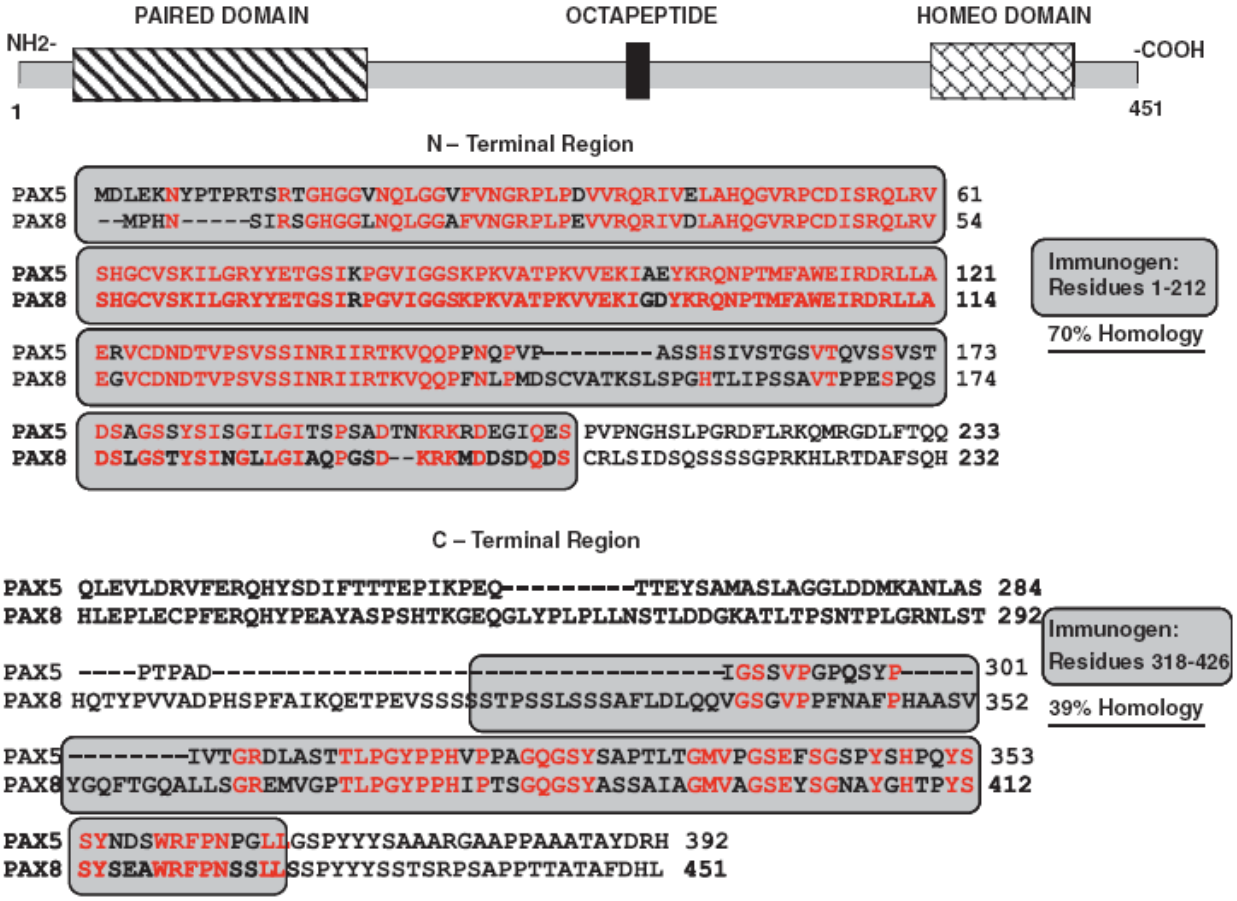

Figure 1 Schematic representation of PAX8 protein, and human PAX5 and PAX8 protein sequence comparison. The region in gray cover the sequence of the PAX8 antibodies (against N-terminal region top and C-terminal region bottom) and their homology with the sequences of PAX-5, N-terminal region (top) and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

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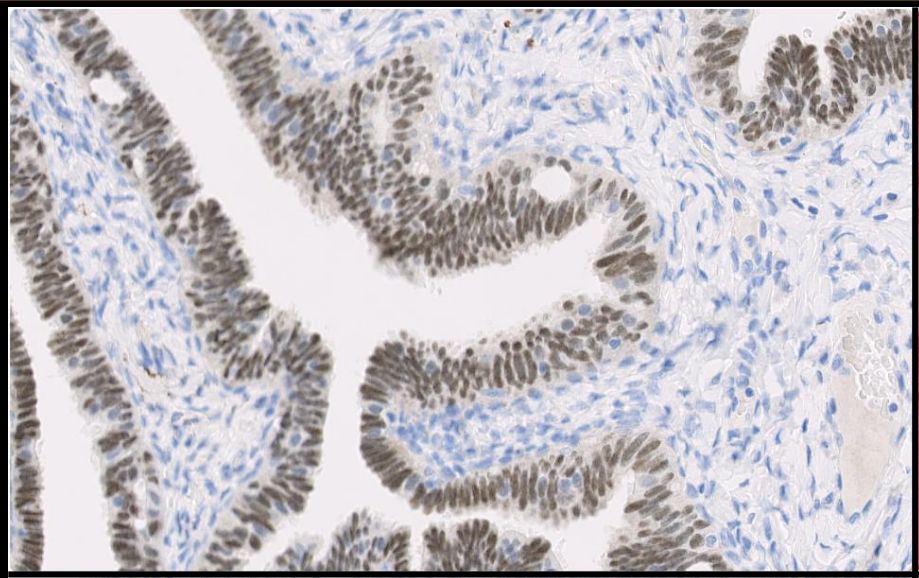

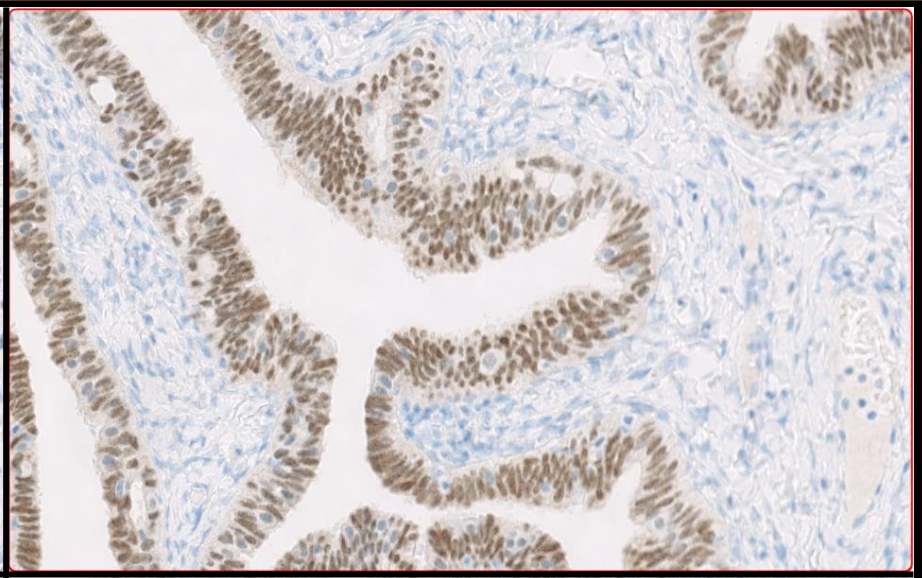

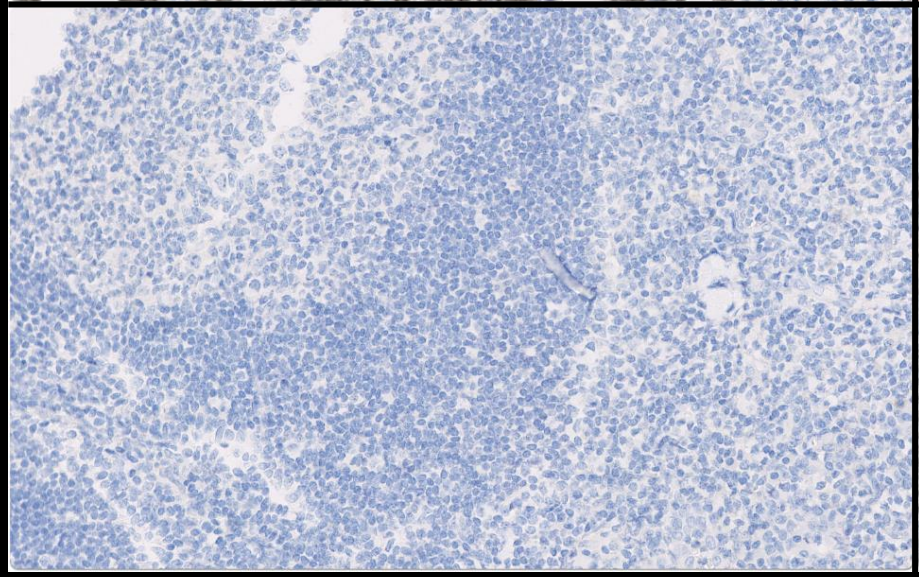

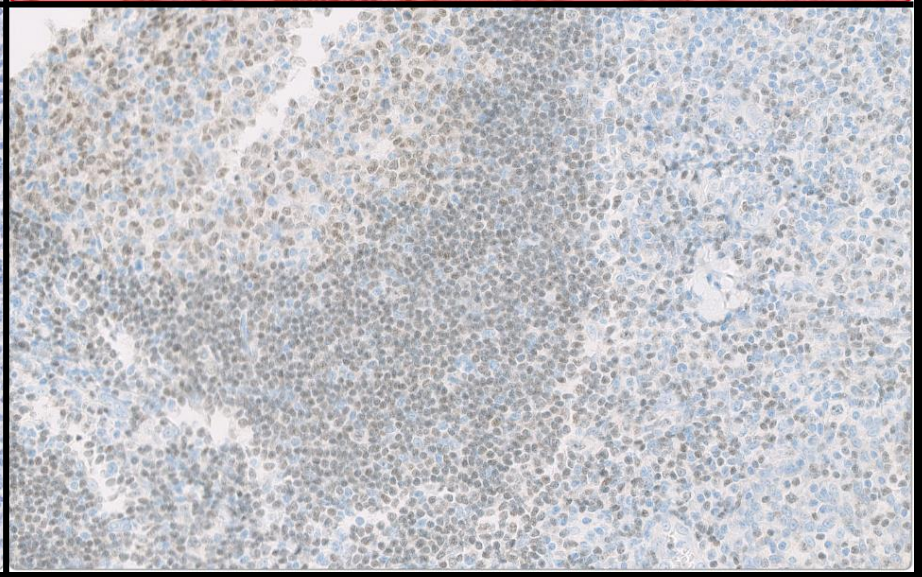

mAb clone BC12 (C-term.)

mAb clone MRQ-50 (N-term.)

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

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

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

- mAb clone BC12 (Biocare)
- mAb clone PAX8R1 (Abcam)

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

Fallopian tube – secretory & ciliated cells

Kidney – epithelial cells lining the collecting tubules

Thyroid – epithelial cells lining the follicles

Tonsil – B-lymphocytes

Pancreas – neuroendocrine cells

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

Fallopian tube – secretory & ciliated cells

Kidney – epithelial cells lining the collecting tubules

Thyroid – epithelial cells lining the follicles

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UPT II: PAX8

Basic protocol settings for an optimal staining result (NQC)

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

* Inferior performance on VMS & Dako Omnis stainer platform

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

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

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

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

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

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	Recommendable clones (conc.)	Less successful clones (conc.)	RTU ”plug and play” giving optimal result
SOX10	mAb BC34 rmAb EP268 rmAb SP267	pAbs	
UP II	mAb BC21	mAb AU1 (UP III)	
NKX3.1	rmAb EP356 pAb CP422		

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	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
NKX3.1	Prostate: Luminal epithelial cells.	*Prostate: Basal cells* Testis: Germ cells	Appendix: Epithelial cells

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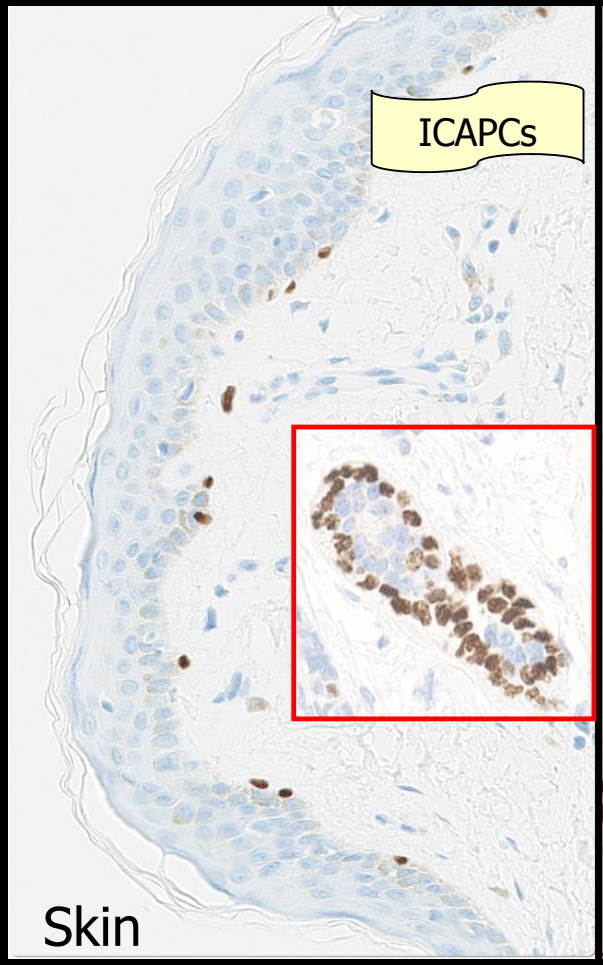
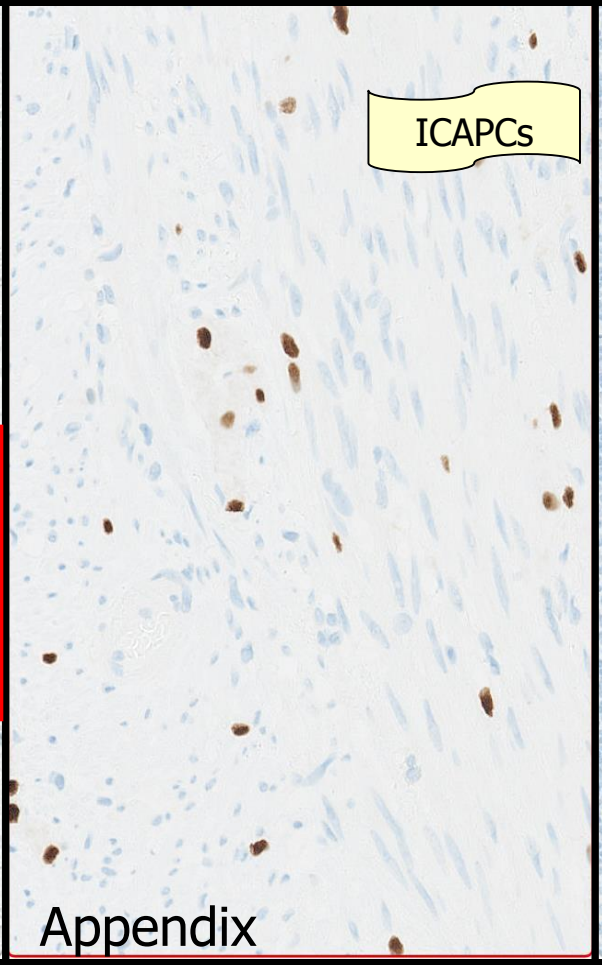
SOX10 reaction pattern



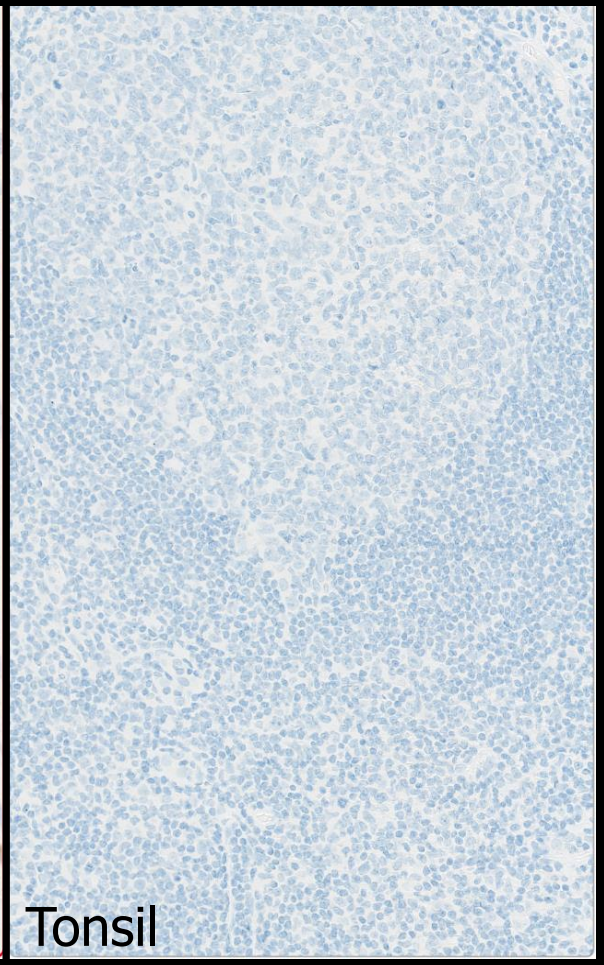
Skin

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).

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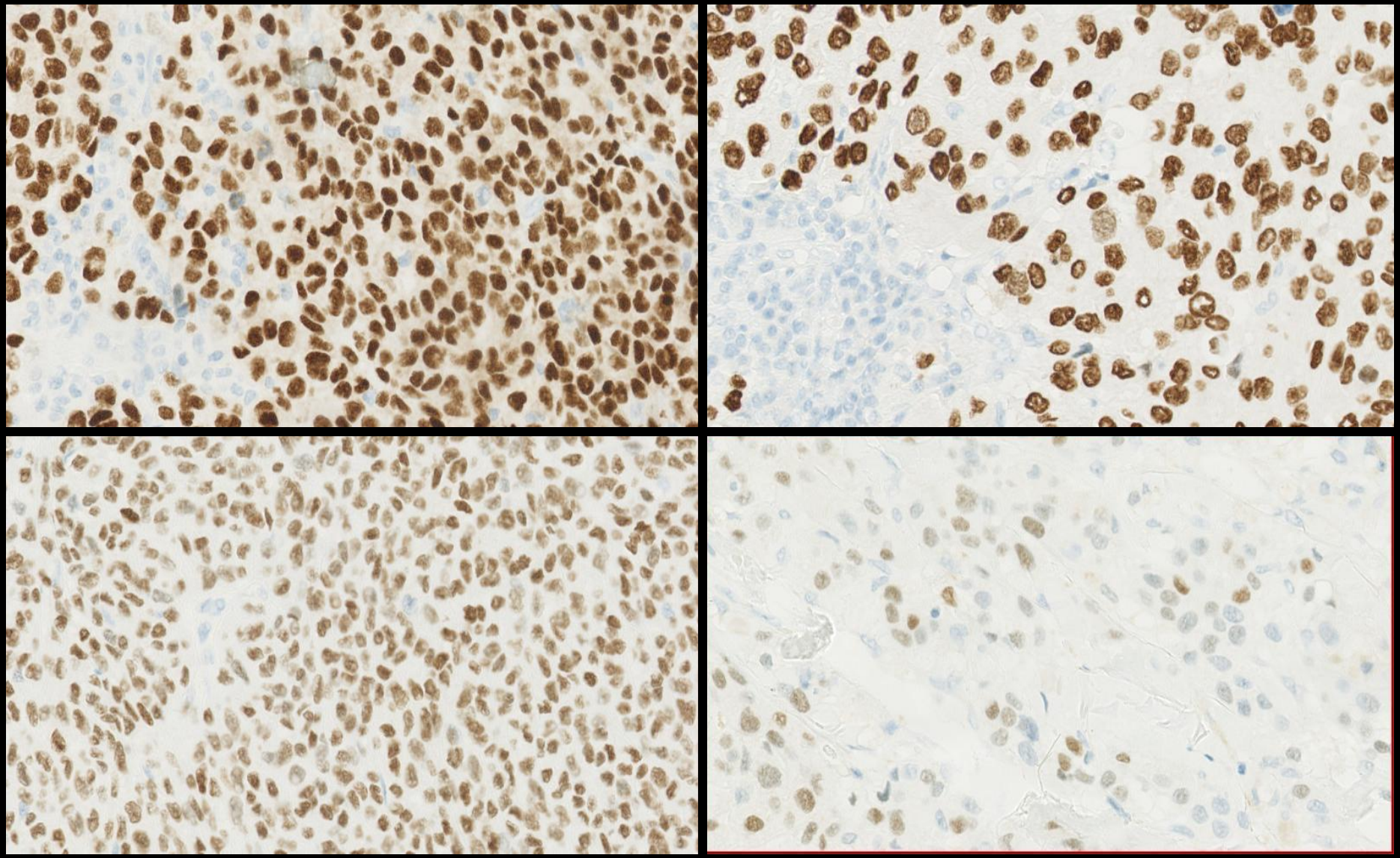

Melanomas x 200 – SOX 10

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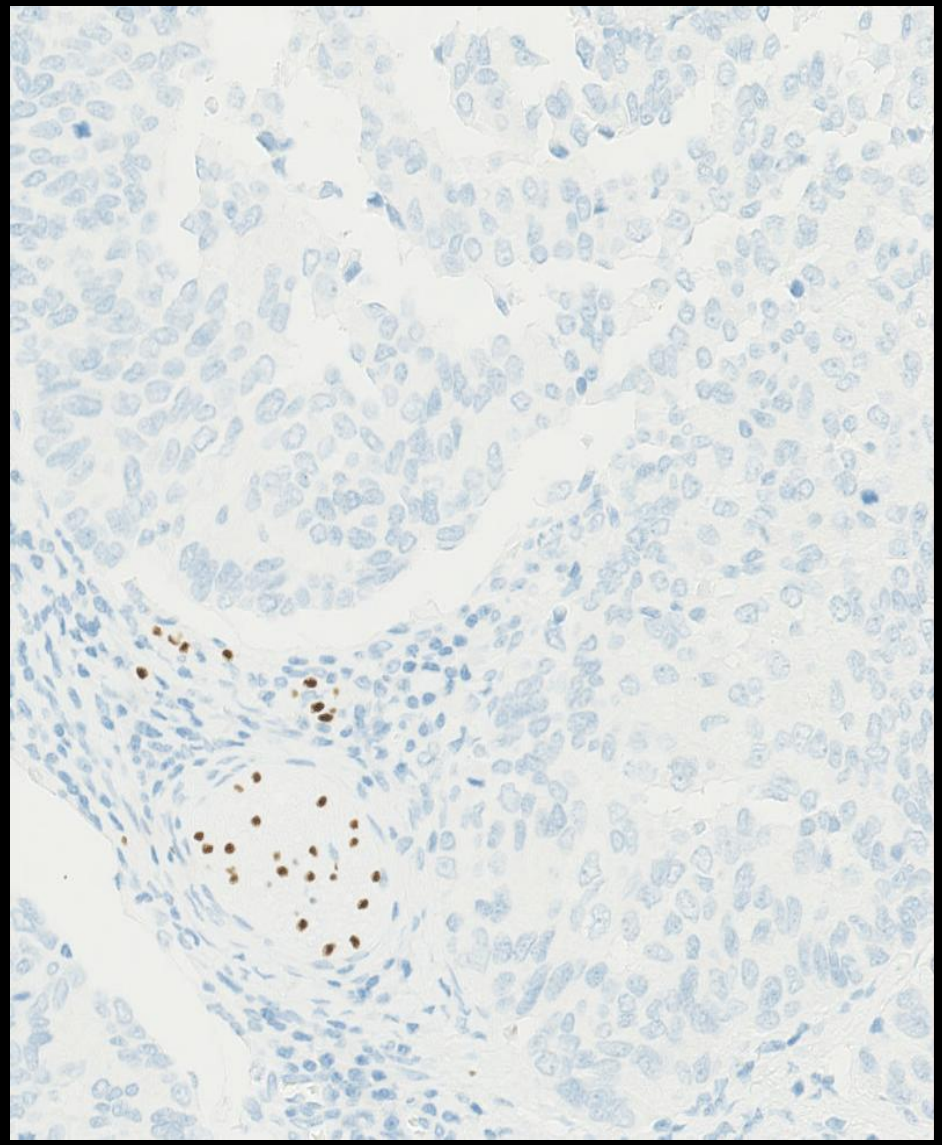
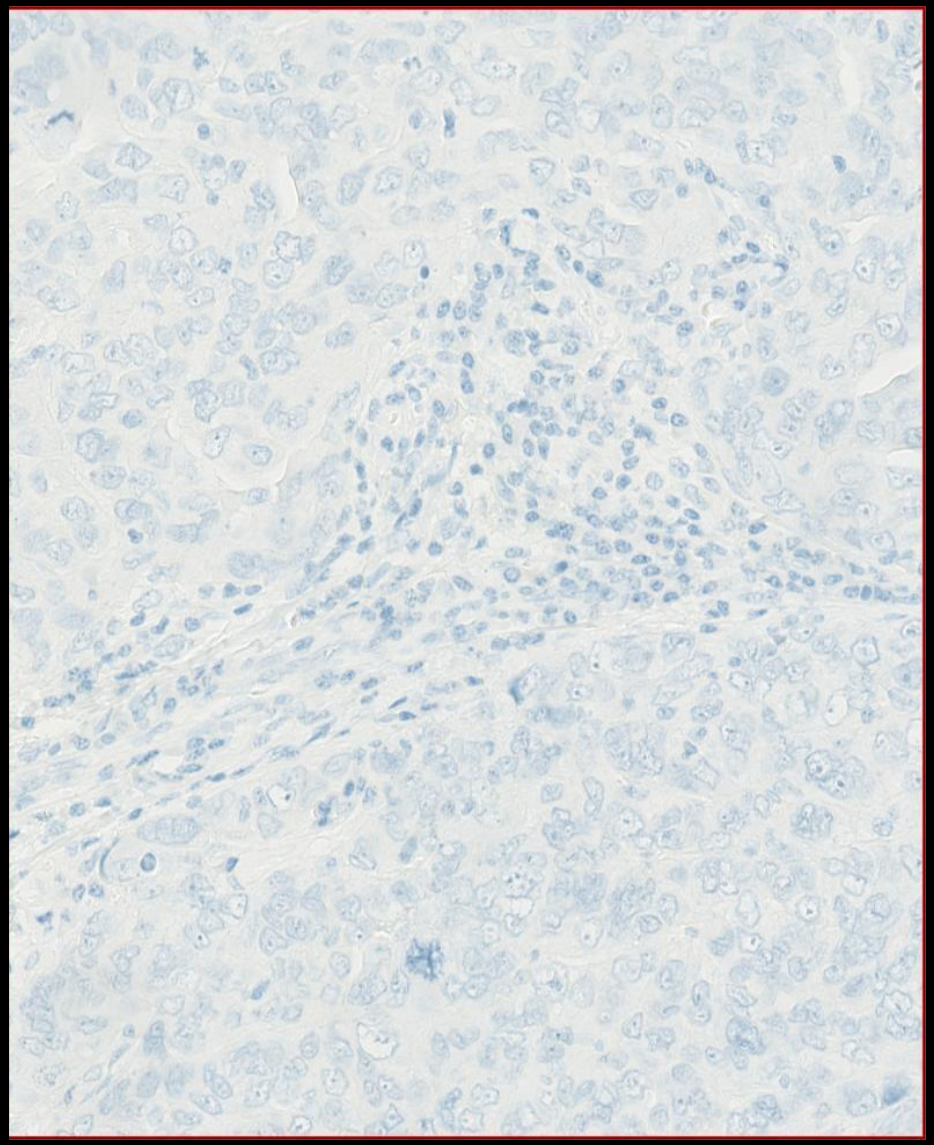


Cerv. uteri, ad. carc. x 200 – SOX 10



Ovarian ser. carc. x 200 – SOX 10

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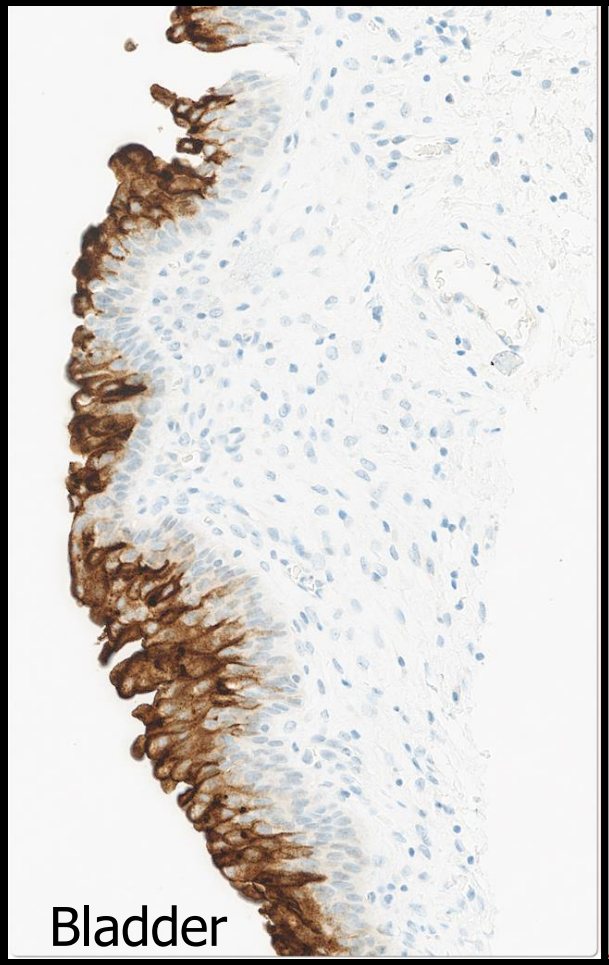
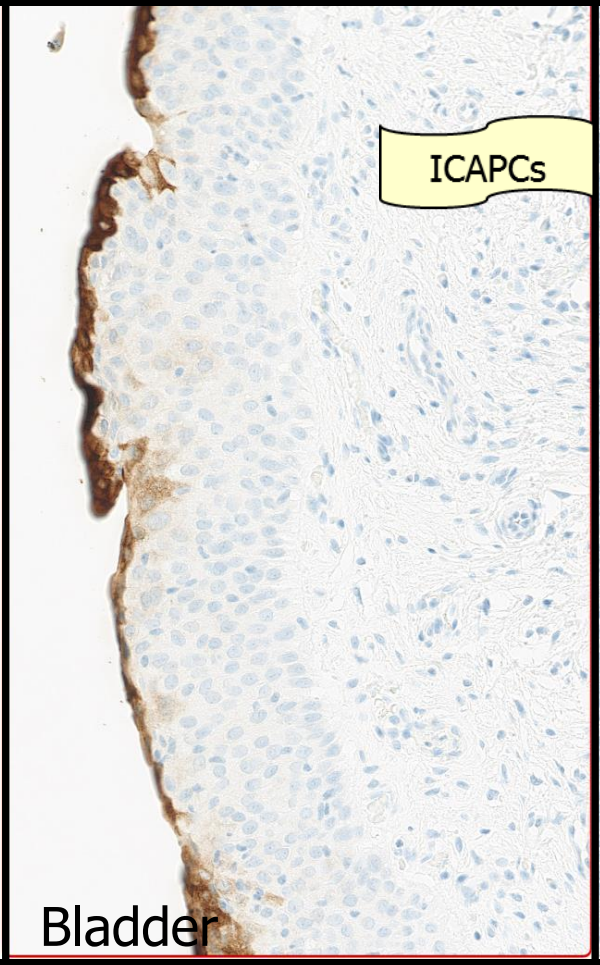
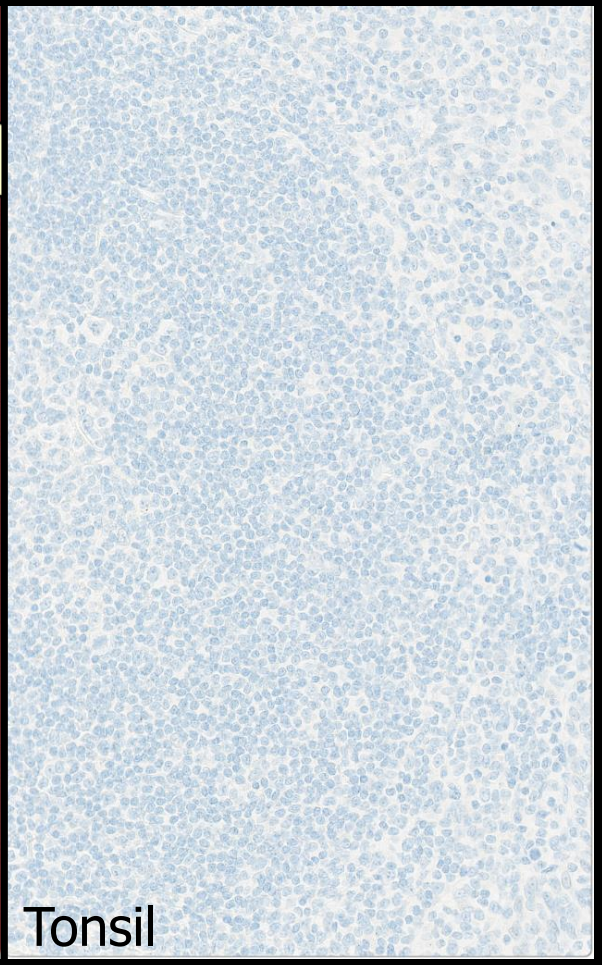
Uroplakin II reaction pattern



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.

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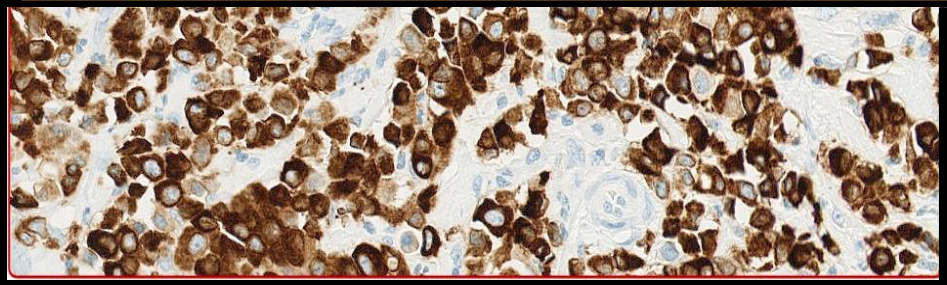
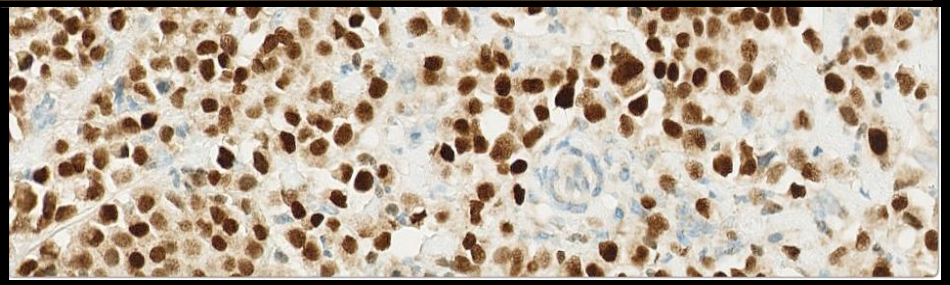
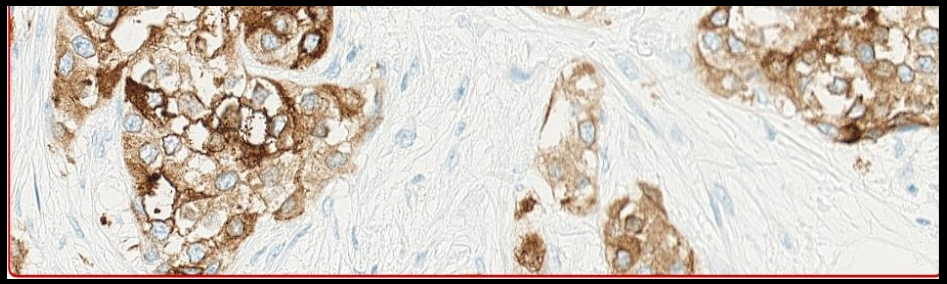
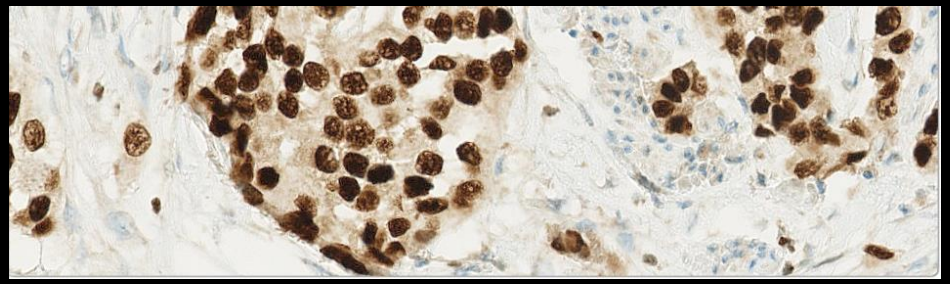
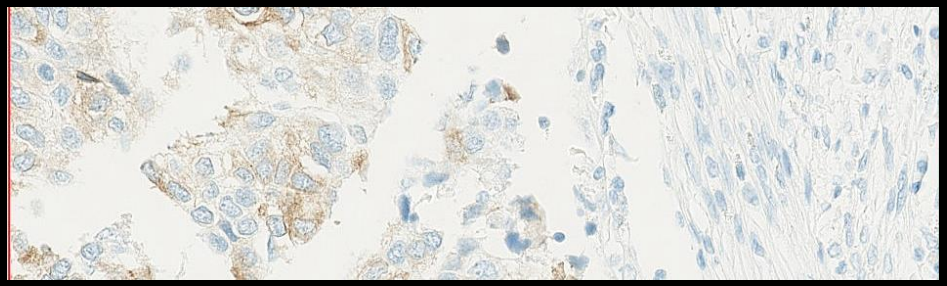
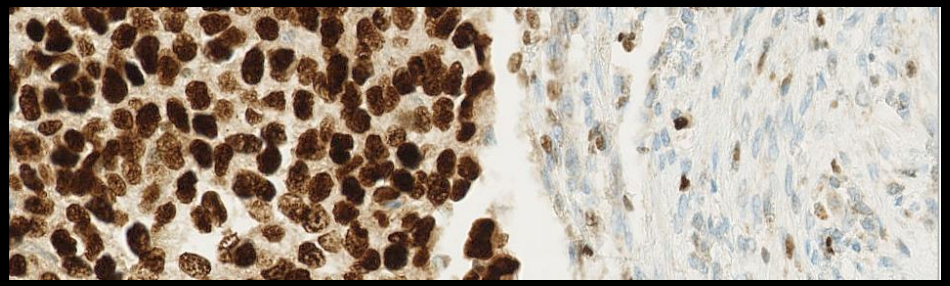
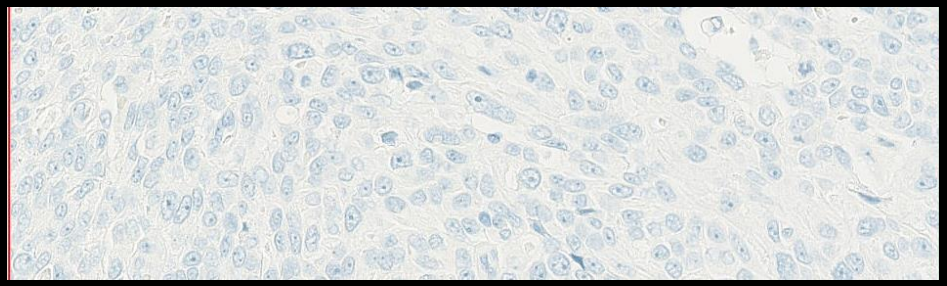
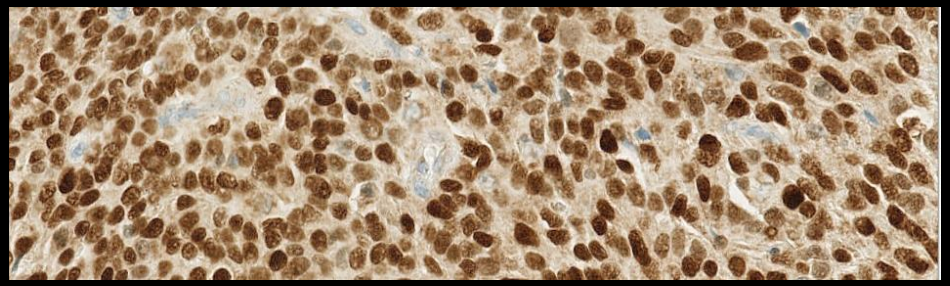

Uroth. carc. x 200 – Uroplakin II

Uroth. carc. x 200 – GATA3

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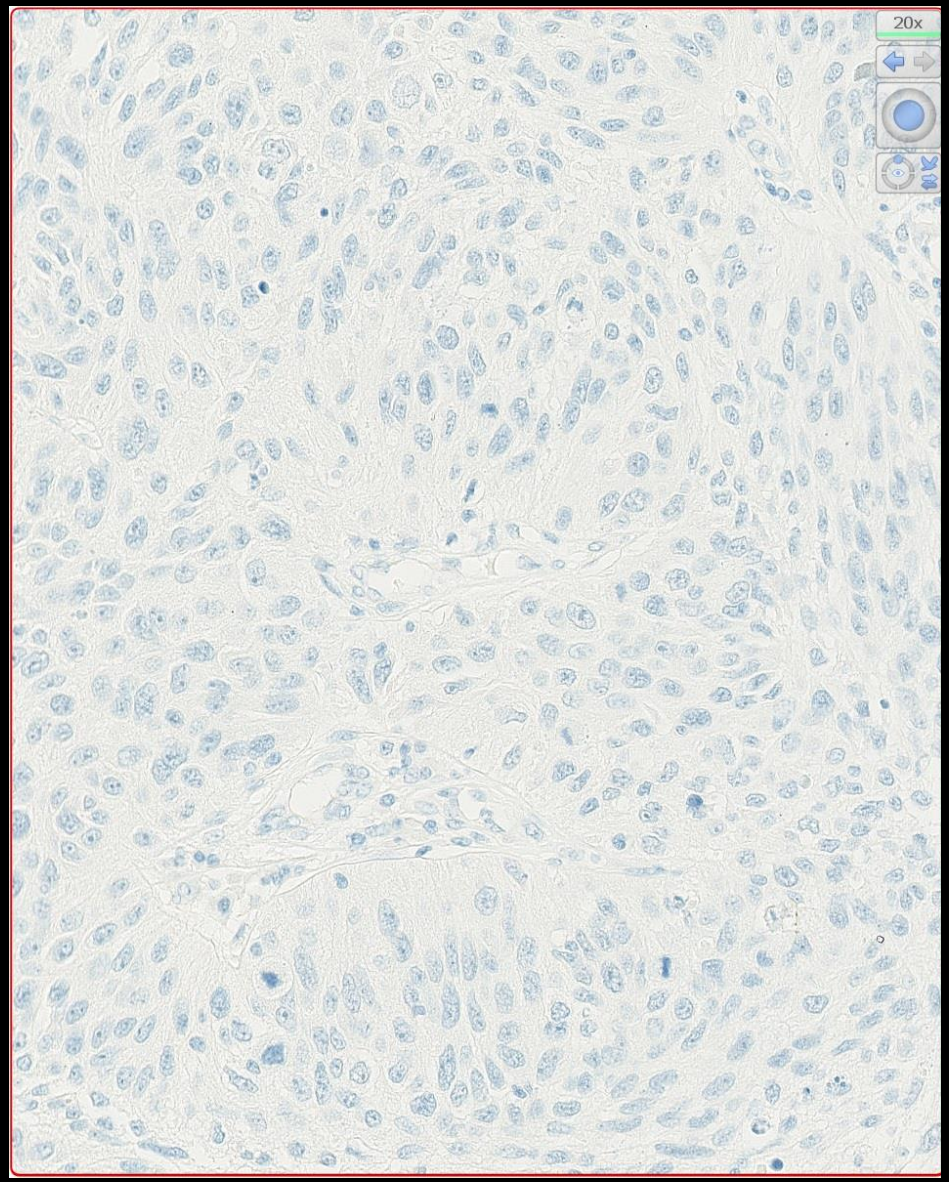
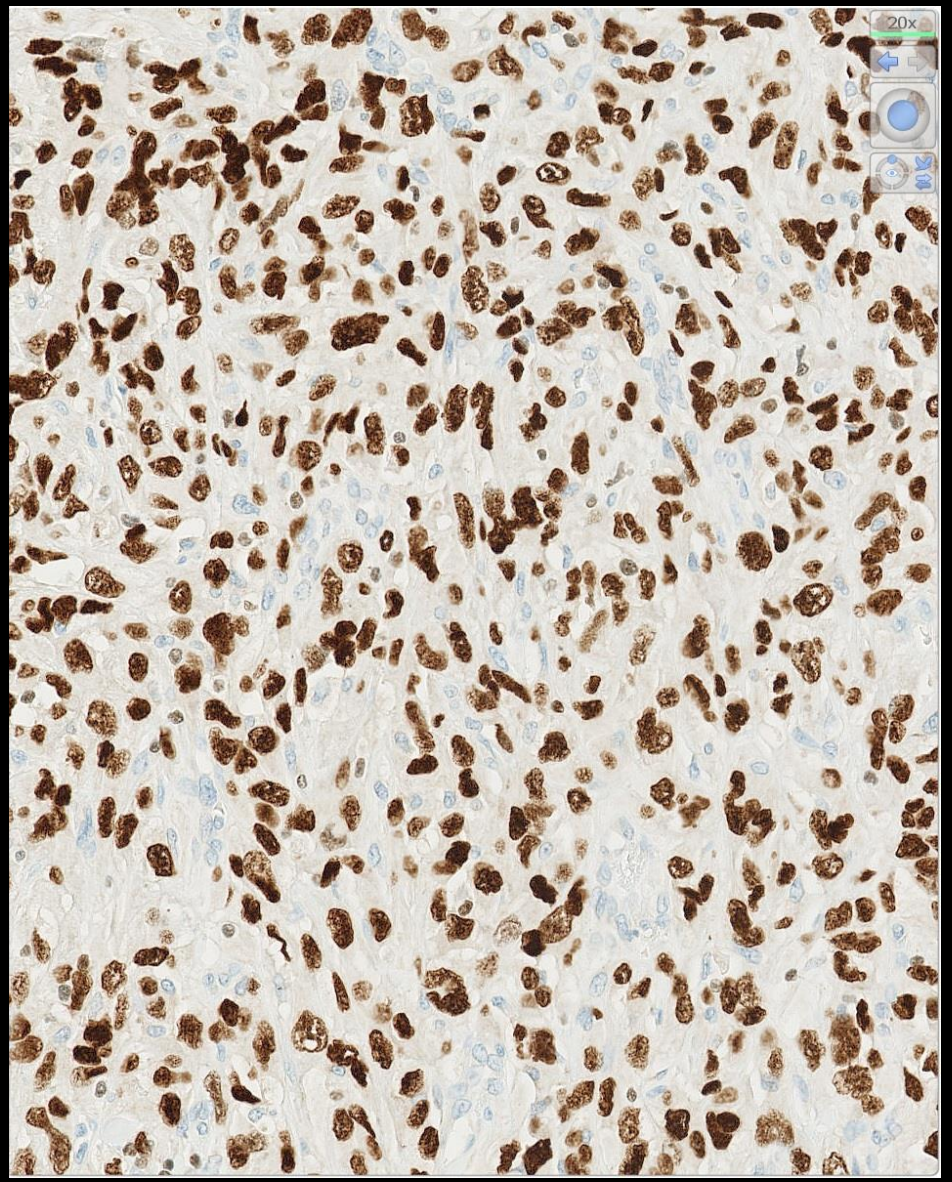


Lung. sq. carc. x 200 – Uroplakin II



Lung. sq. carc. x 200 – GATA3

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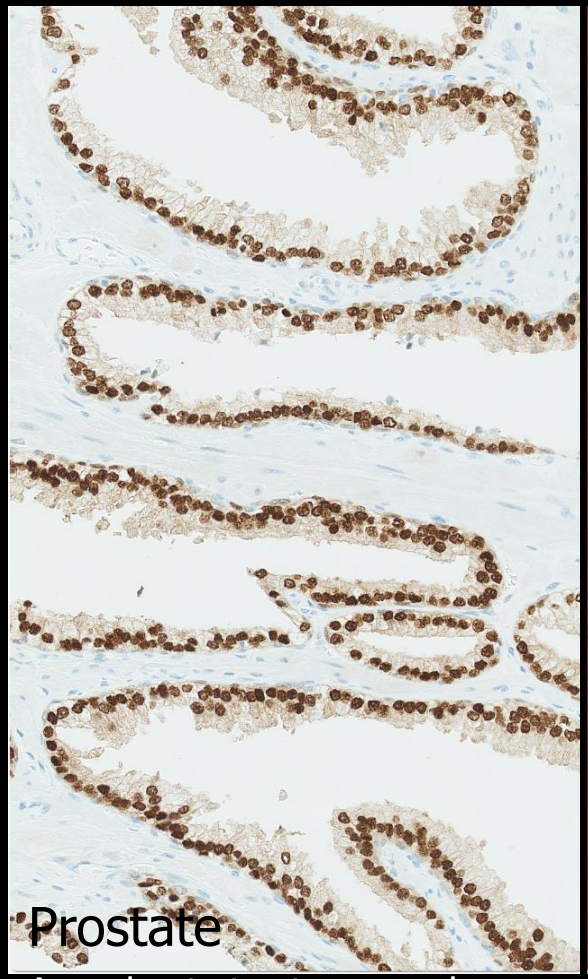
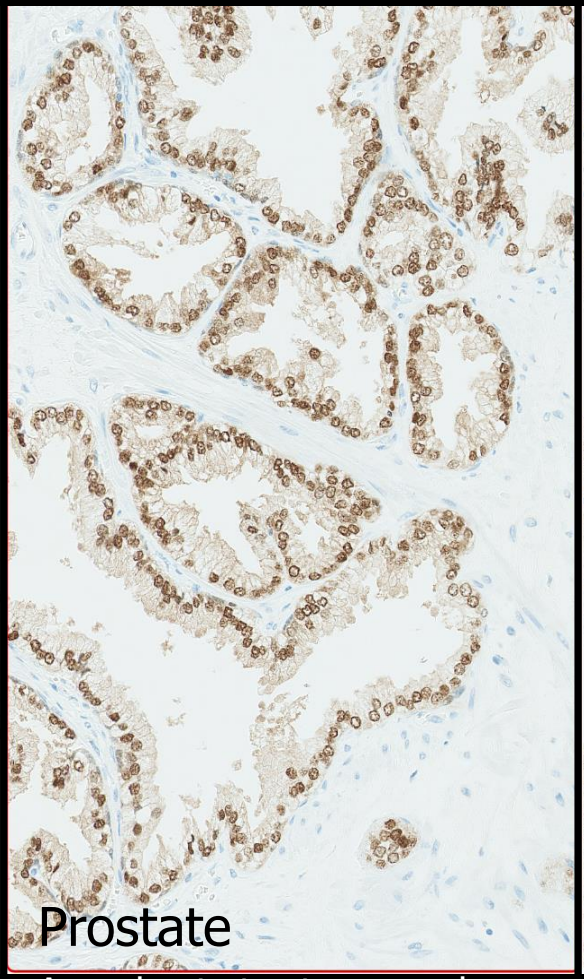
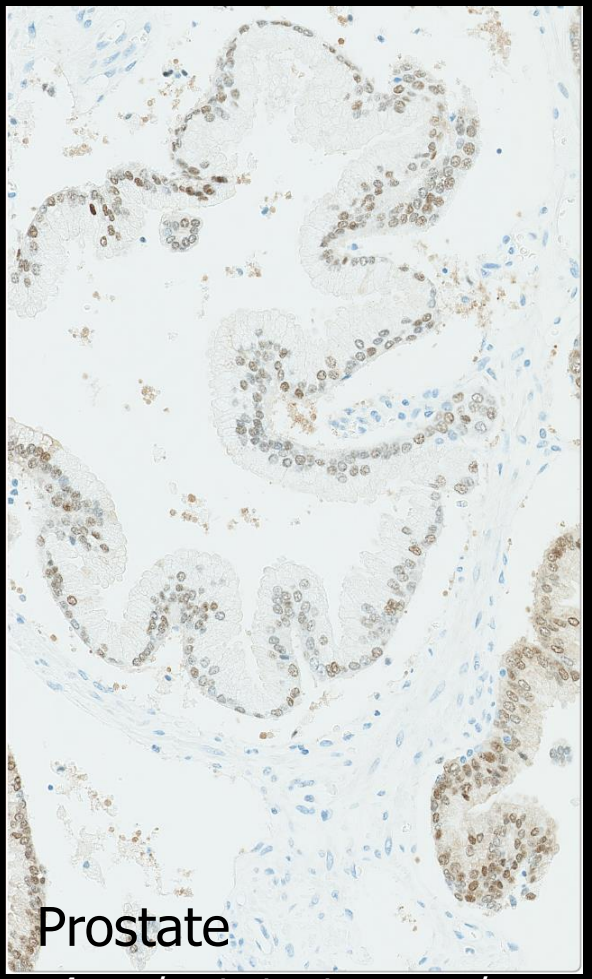
NKX3.1 reaction pattern



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.

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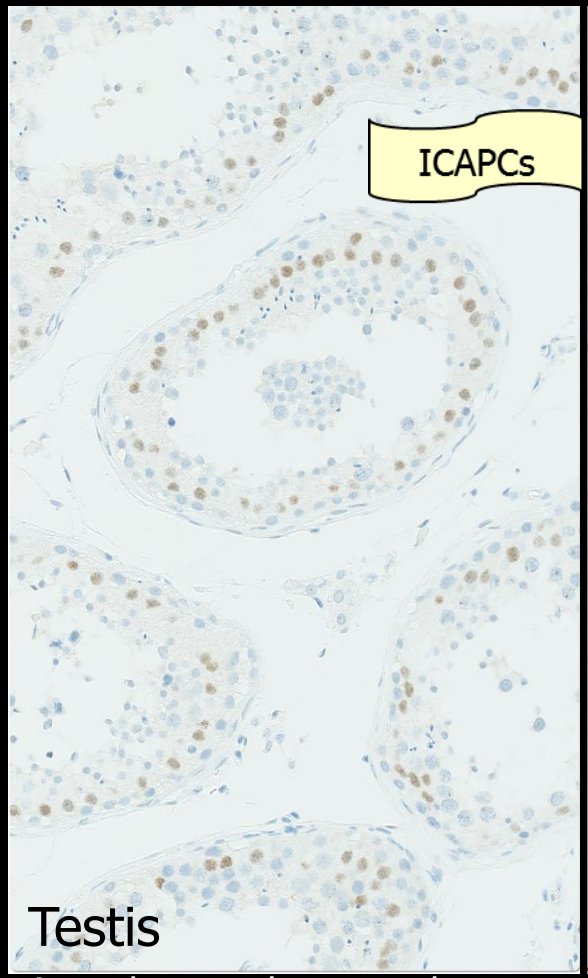
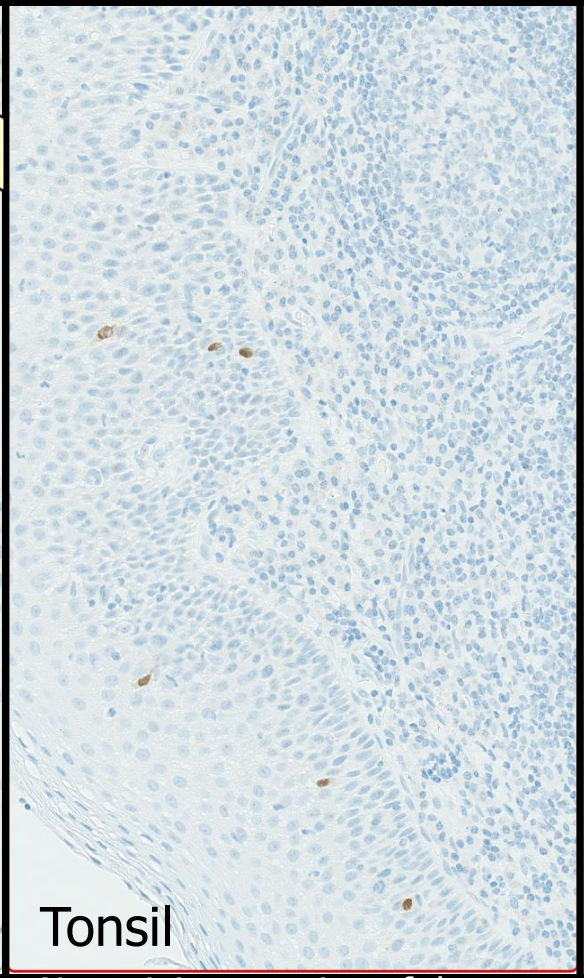
NKX3.1 reaction pattern



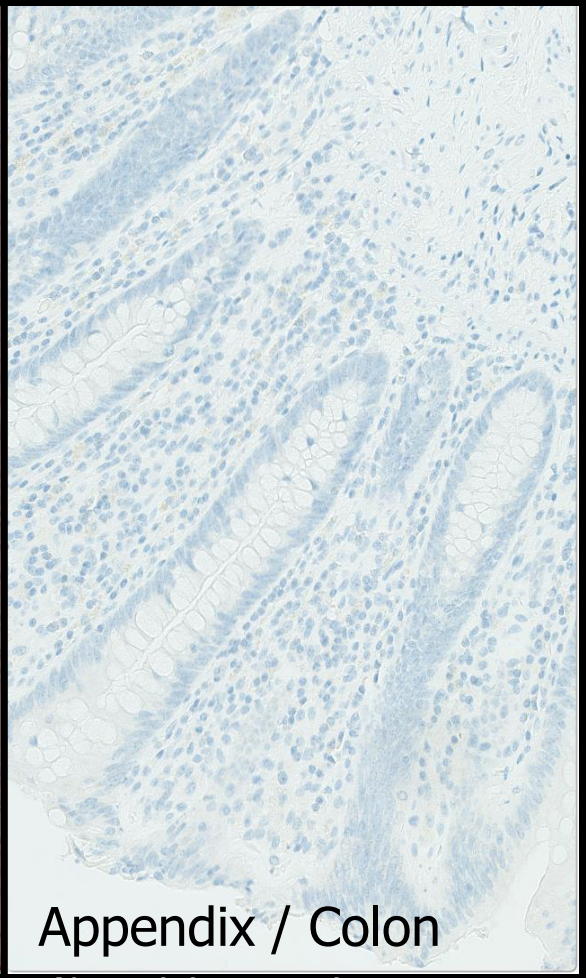
Testis

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.

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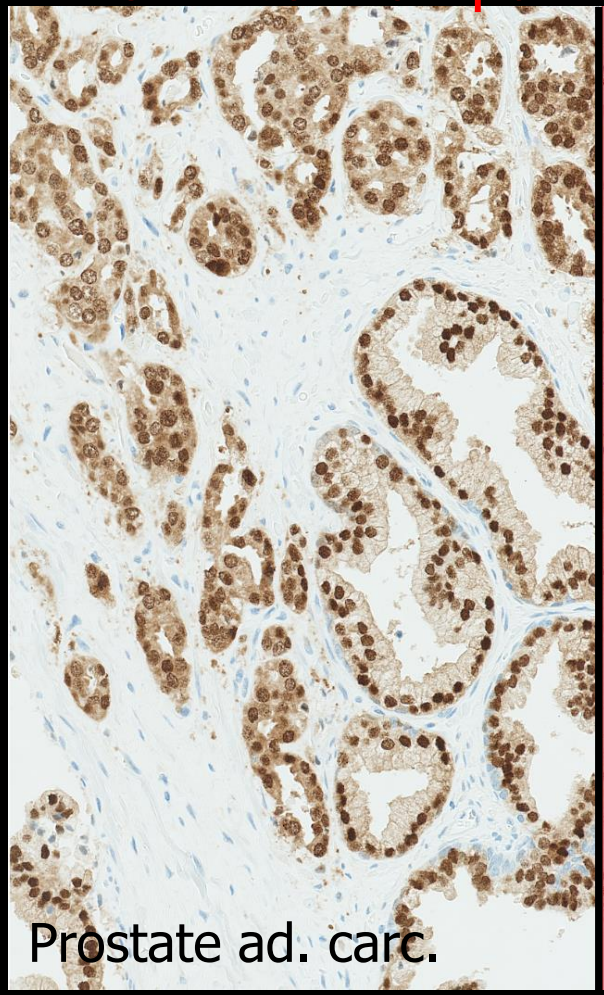
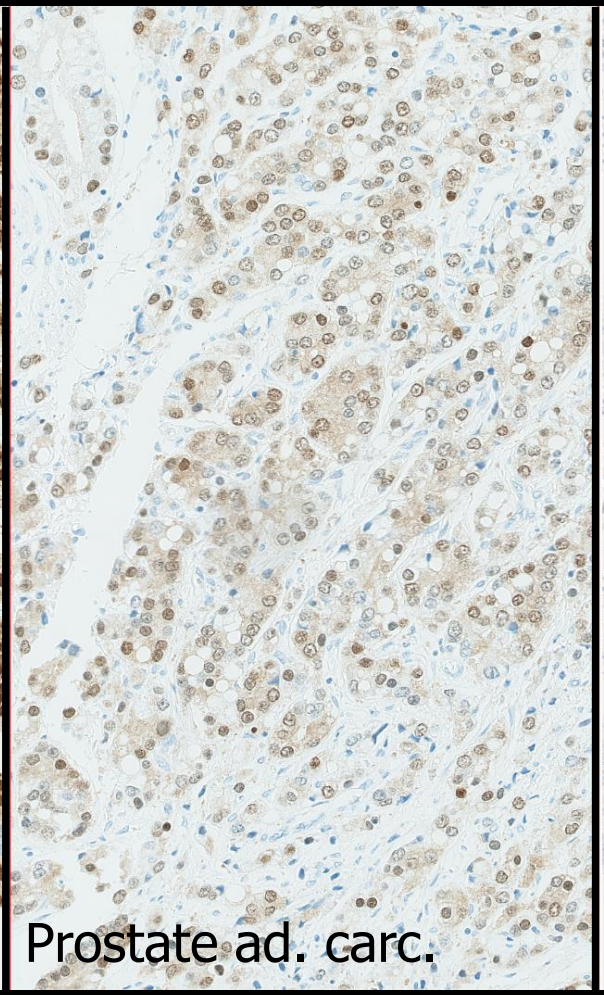
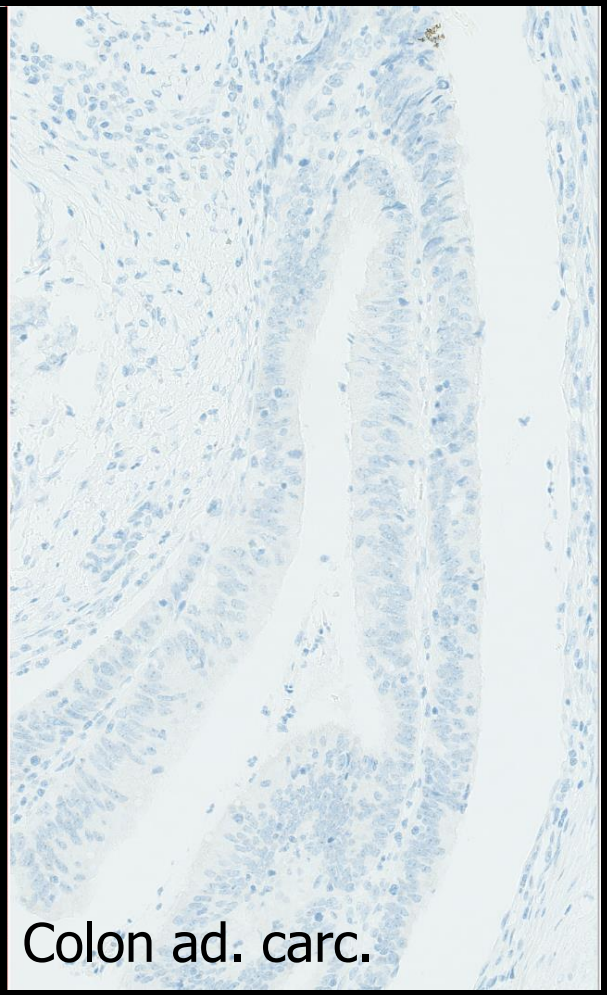
NKX3.1 reaction pattern


Prostate ad. carc.


Prostate ad. carc.


Colon ad. carc.

Internal studies:

+ 18 of 18 prostate adenocarcinomas
10% cut-off

- in 39 of 39 other neoplasias

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UPT II: SOX10

	Retrieval	Titre	Detection	RTU	Detection
mAb BC34	HIER High	1:25-200	3-step	-	-
rmAb EP268, SP267*	HIER High	1:30-200	2- & 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	-	-

UPT II: NKX3.1*

	Retrieval	Titre	Detection	RTU	Detection
rmAb EP356*	HIER High	1:50-100	3-step	-	-

**In-house data for best technical result*

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