

The unknown primary tumour: IHC Classification, antibody selection, protocol optimization, controls and EQA (part II)

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Secondary panels for **carcinoma** identification/subclassification

- Cell adhesion molecules
 - EpCAM
 - Claudin 4
- **Cytokeratin subtypes**
- Oncofetal proteins
- Transcription factors
- Neuroendocrine proteins
- Hormone receptors
- Secretory proteins
- . . .

- "GI-markers"
- "Fem.gen.tract markers"
- "Liver cell markers"
- Neuroendocrine cell markers
- "Breast markers"
- "Lung markers"
- "Urinary tract markers"
- Prostate markers
- Squamous cell markers
- "Mesothelial markers"
- "Adrenal cortical markers"
- Germinal cell markers

General epithelial markers:

Epithelial cell adhesion molecule (Ep-CAM)

Claudin 4

Ep-CAM

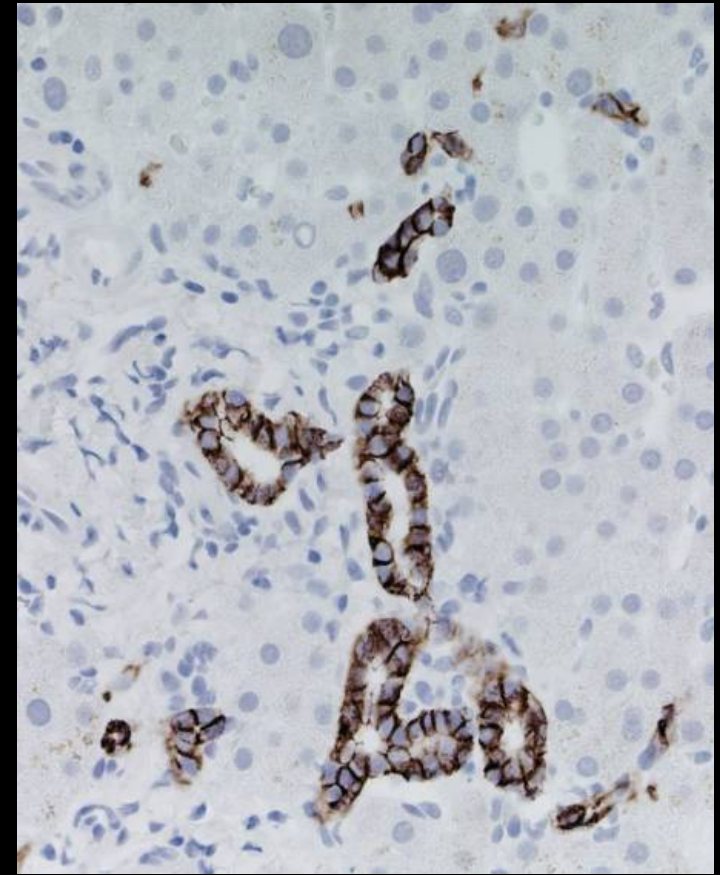
“Epithelial specific antigen”: Glycoproteins located on the cell membrane surface (preferentially basolaterally) and in the cytoplasm of **virtually all epithelial cells** with the exception of

- squamous epithelia (mucosae and reactive focal pos.)
- hepatocytes (pos. in reactive)
- renal proximal tubular cells
- gastric parietal cells
- some endocrine cells

Also negative

- mesothelial cells (pos. in reactive)
- mesenchymal cells
- germ cells
- neural crest cells
(except. olfactory neurons)

Liver



Ep-CAM

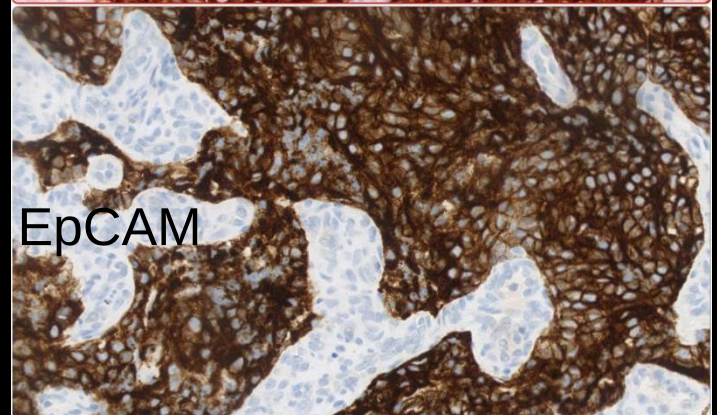
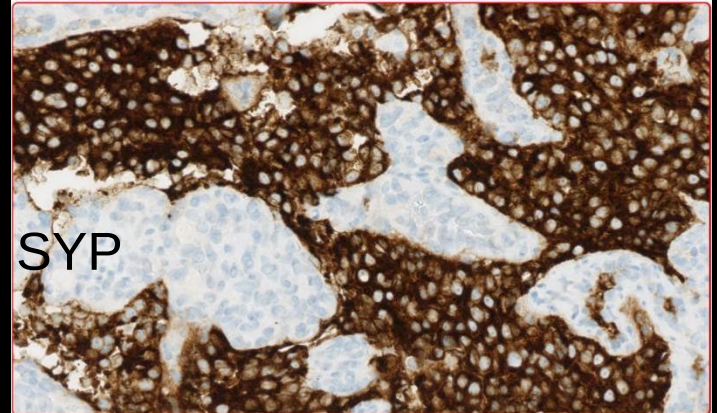
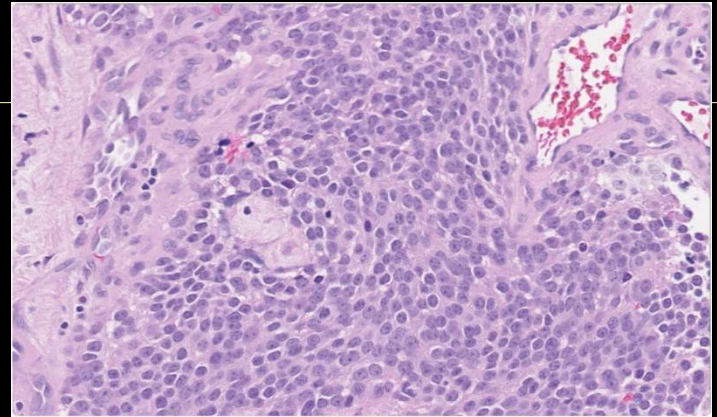
Often upgraded in malignancies

+(-)

- Adenocarcinomas (of most types)
- Neuroendocrine neoplasms

+/-

- Germinal cell tumours
 - (seminoma weak)
- Synovial sarcoma
- Brenner tumour
- Desmoplastic small cell tumour
- Olfactory neuroblastoma $\implies$

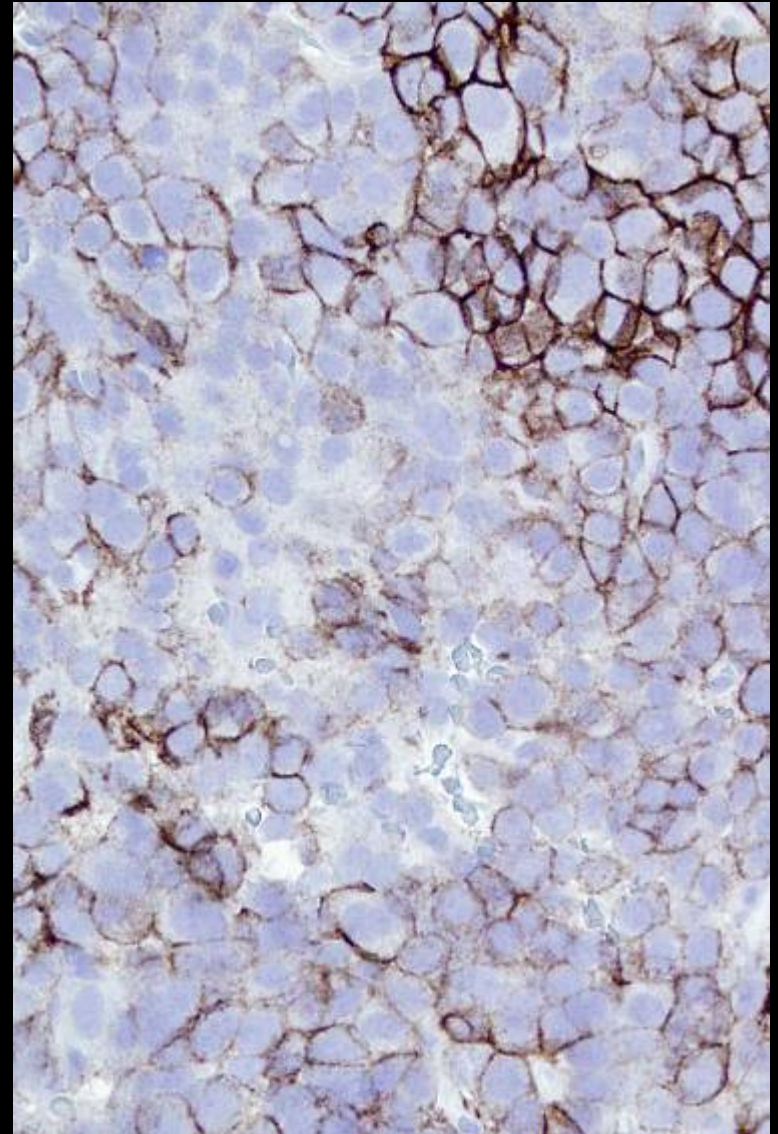


Olfactory neuroblastoma

Ep-CAM

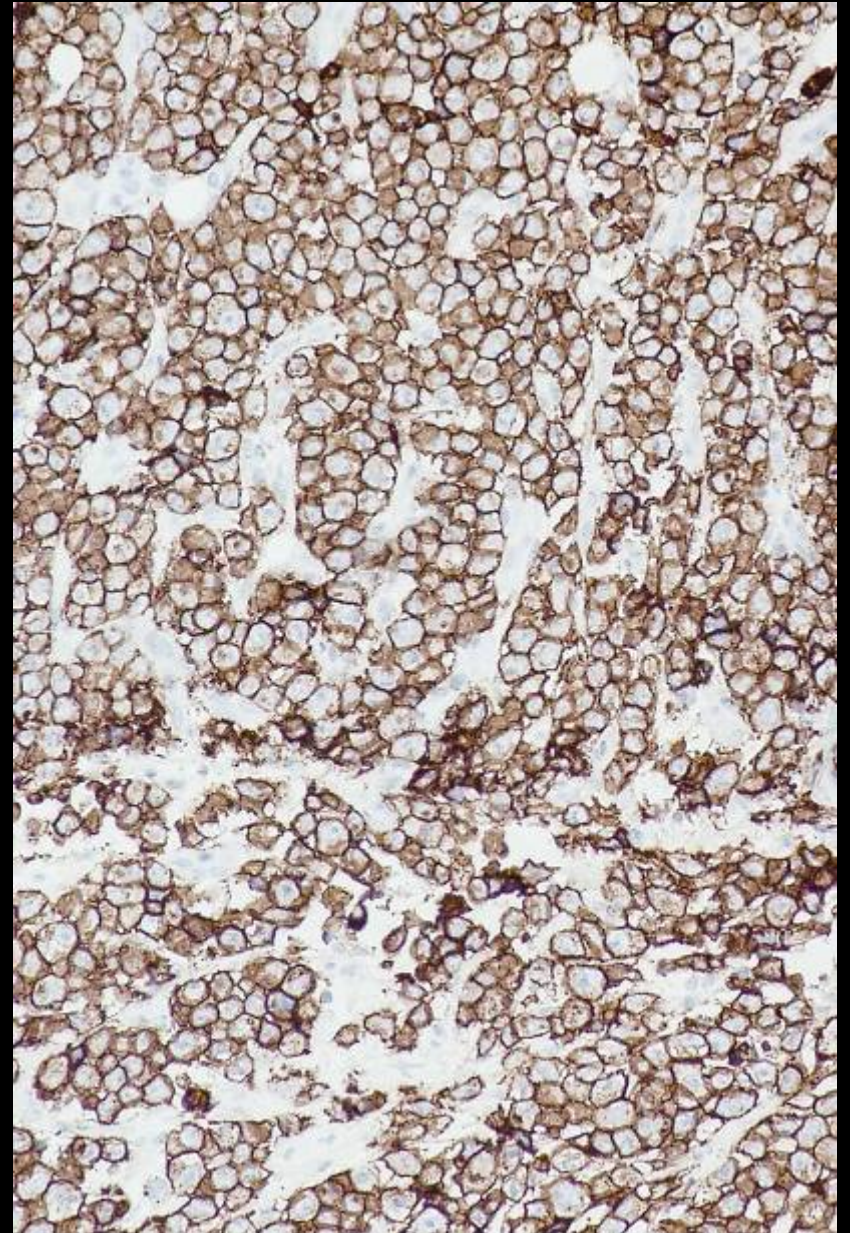
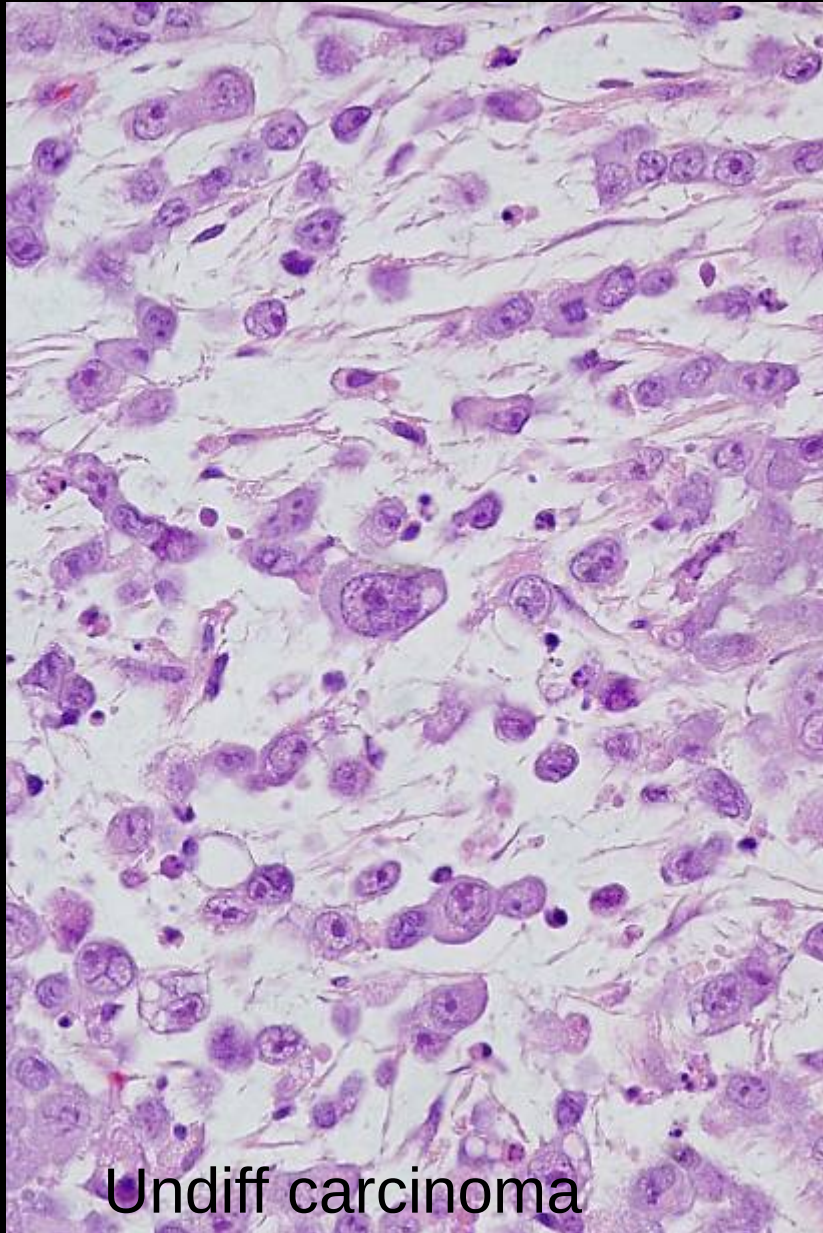
-/+

- Lobular breast carcinoma
- Hepatocellular carcinoma
- Squamous cell carcinoma
- Renal cell carcinoma
- Malignant mesothelioma
-
- Adrenal cortical carcinoma
- Choroid plexus carcinoma
- Sarcomas (apart from synovial)
- Lymphomas



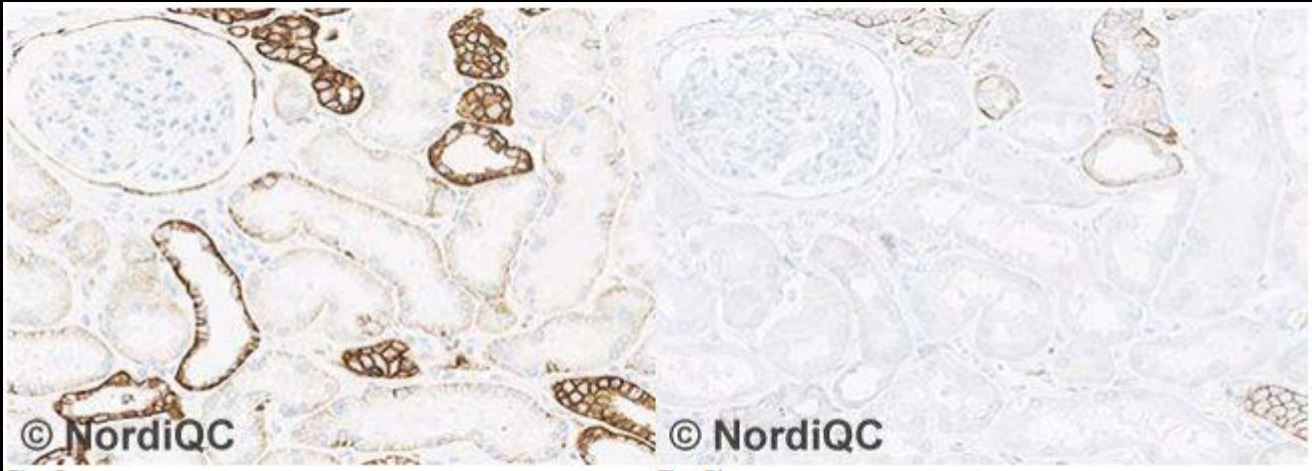
HCC

Ep-CAM



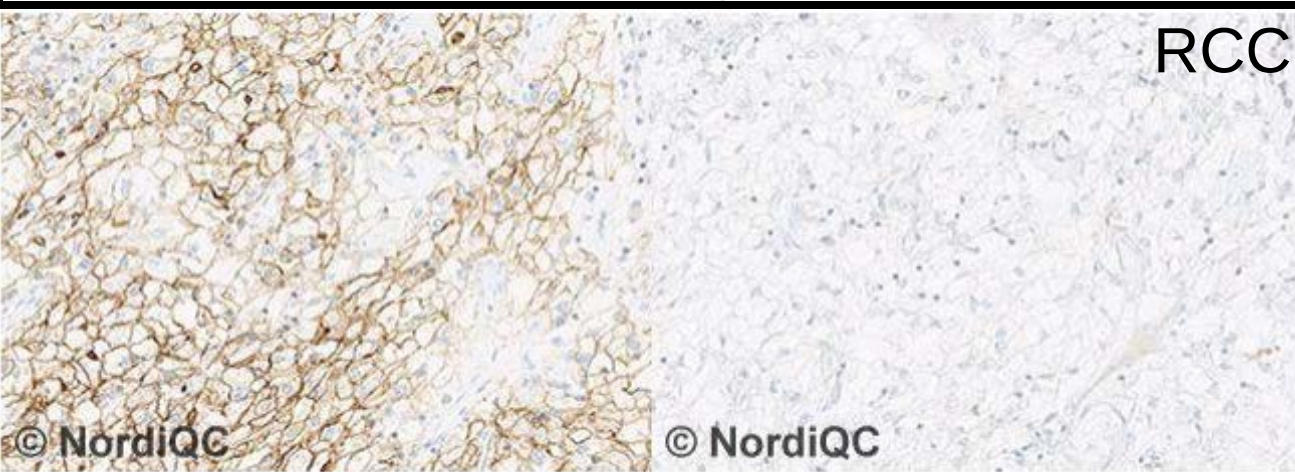
Ep-CAM run 45 2015

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 Ber-Ep4	77	Dako	9	16	38	18	31%	89%
	2	Diagnostic BioSystems						
	2	Thermo/NeoMarkers						
mAb clone MOC-31	19	Dako	9	6	6	3	63%	100%
	3	Leica/Novocastra						
	1	Cell Marque						
	1	Monosan						
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%

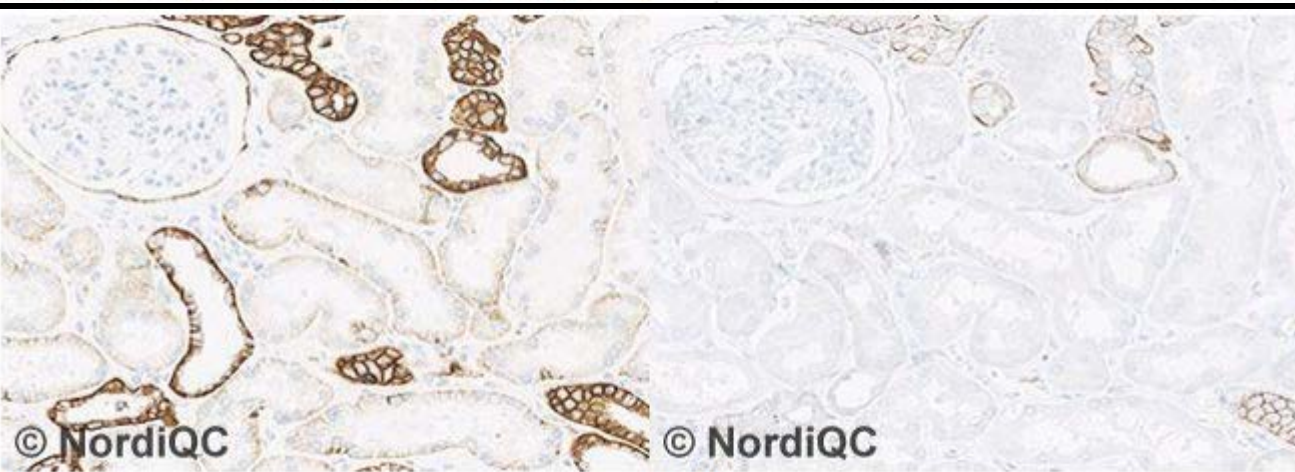


DAKO
TRS Low
3-step
polymer

Ventana
CC1
2-step
multimer



RCC



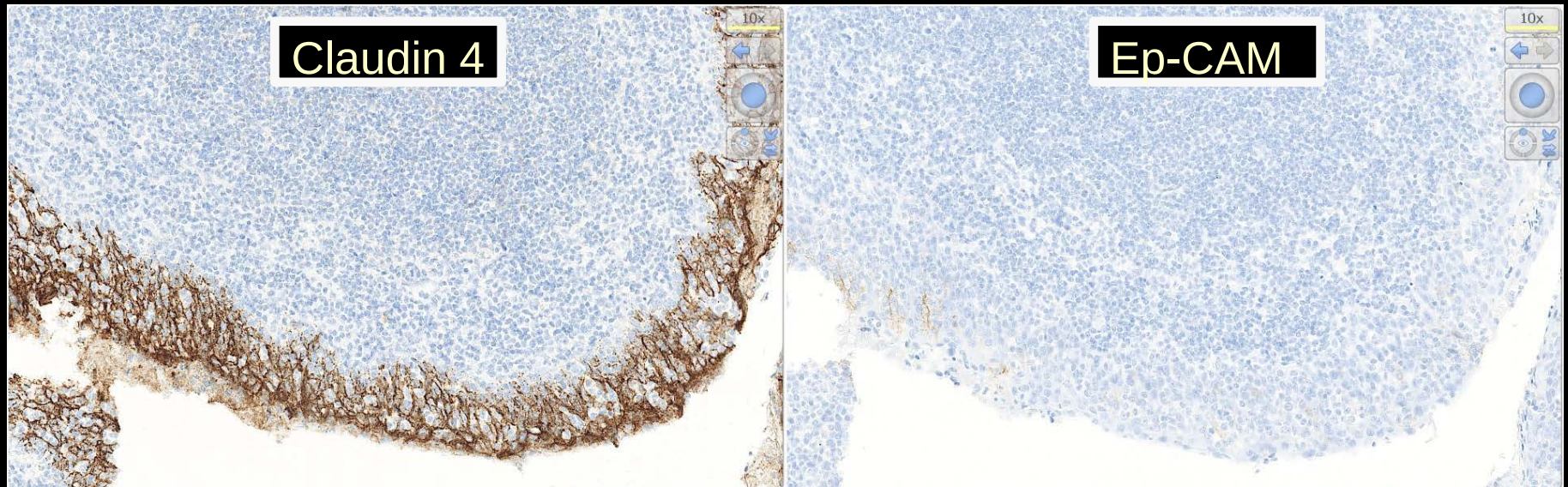
Ventana
CC1
2-step
multimer

Diva2
pH6.2
Thermo

DAKO
TRS Low
3-step
polymer

Claudin 4

Integral membrane protein, which belongs to the claudin family. The protein is a component of **tight junction** strands and may play a role in internal organ development and function.



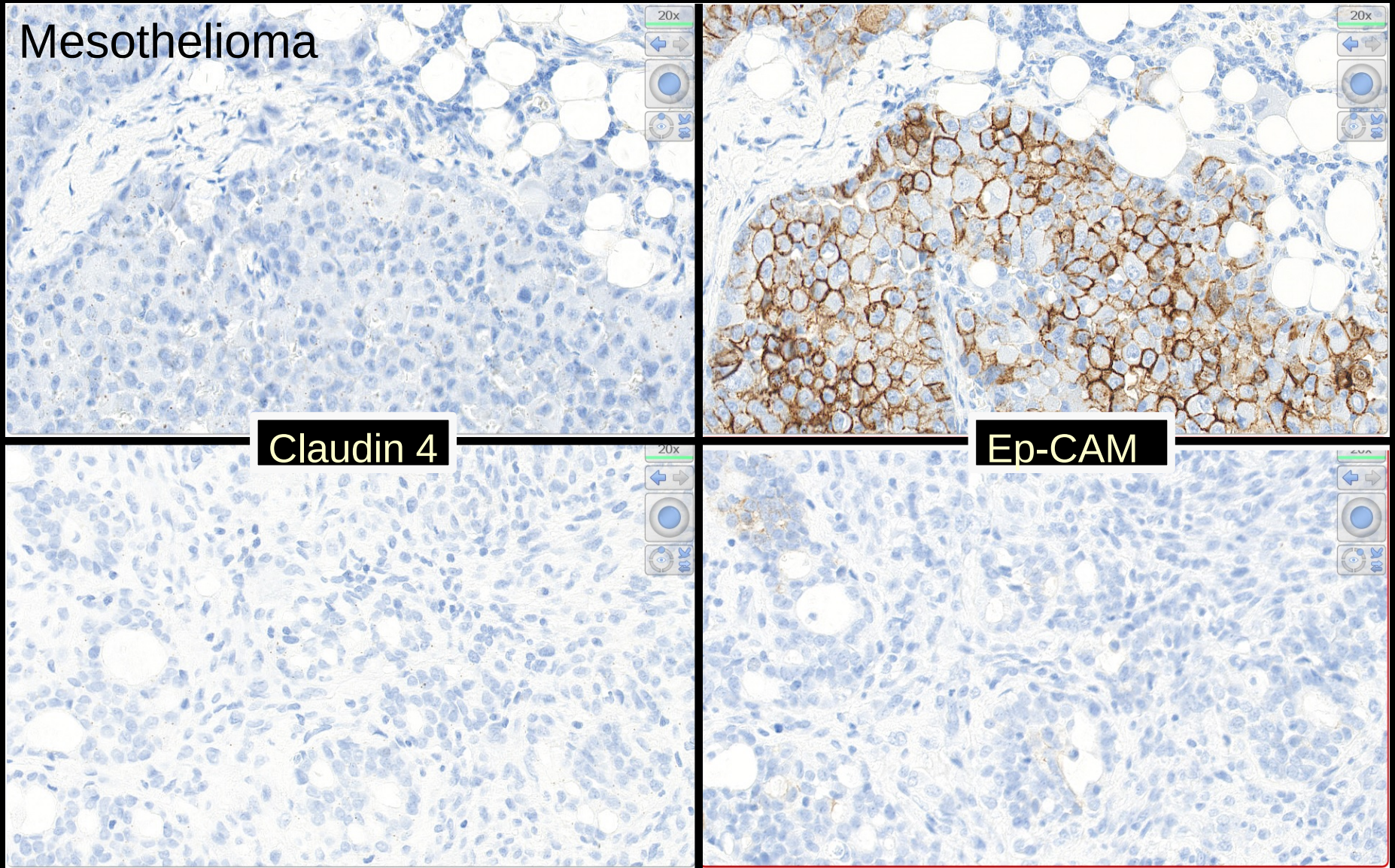
Tonsil

Claudin 4 vs. Ep-CAM

Mesothelioma

Claudin 4

Ep-CAM



Cytokeratins – a multigene family

- Highly complex family of intermediate filaments
- > 50 distinct types (excl. trichocytic keratins)
- Types CK1-20 diagnostically most relevant:

Class I (type A - Acidic):

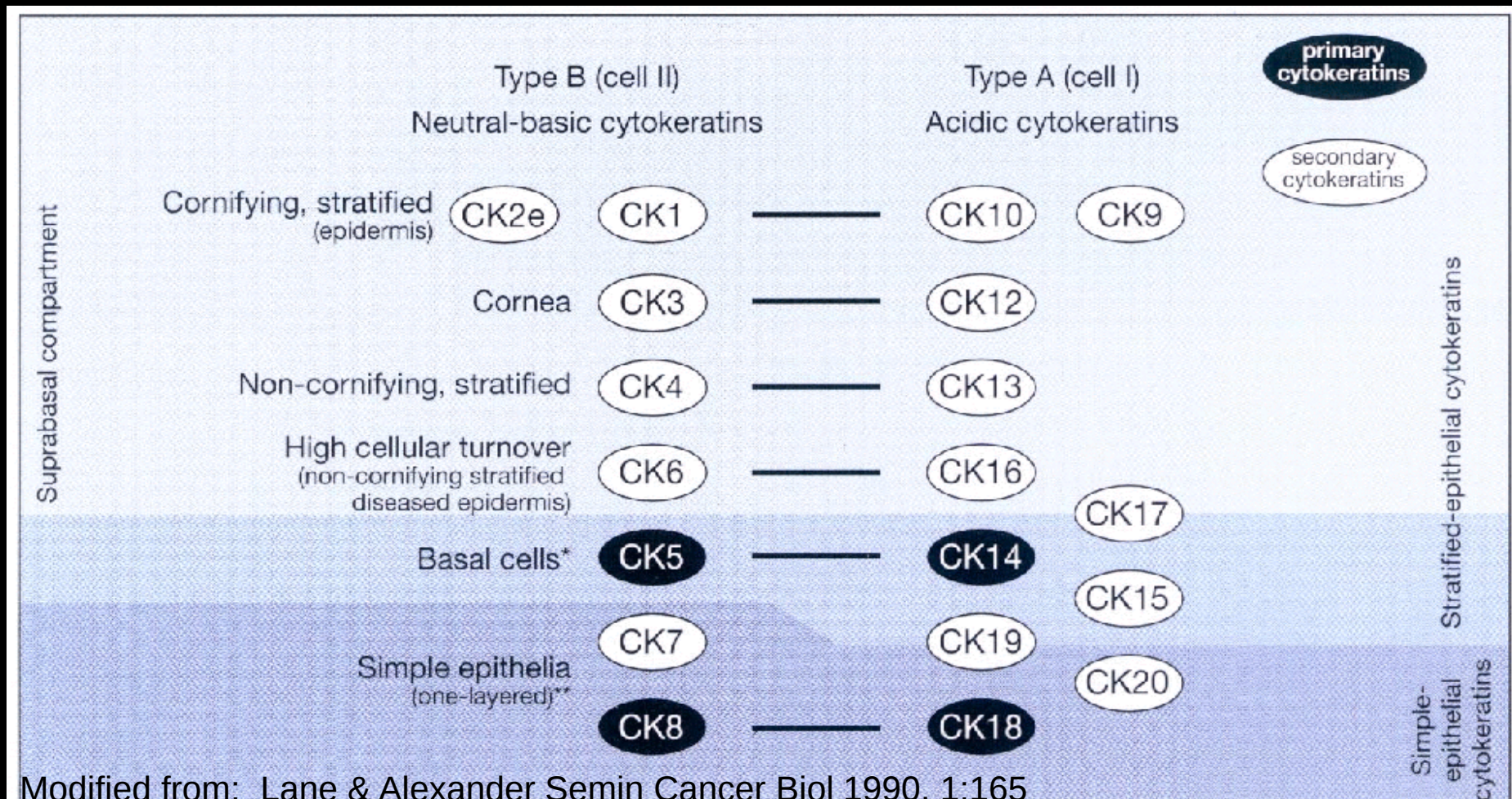
- CK9 (64 kDa) - CK20 (40 kDa)

Class II (type B - Basic/neutral):

- CK1 (68 kDa) - CK8 (52.5 kDa)

Pairing of cytokeratins

- One CK class I and one CK class II 'always' paired
- CK class I in a pair ~ 8 kDa smaller than class II



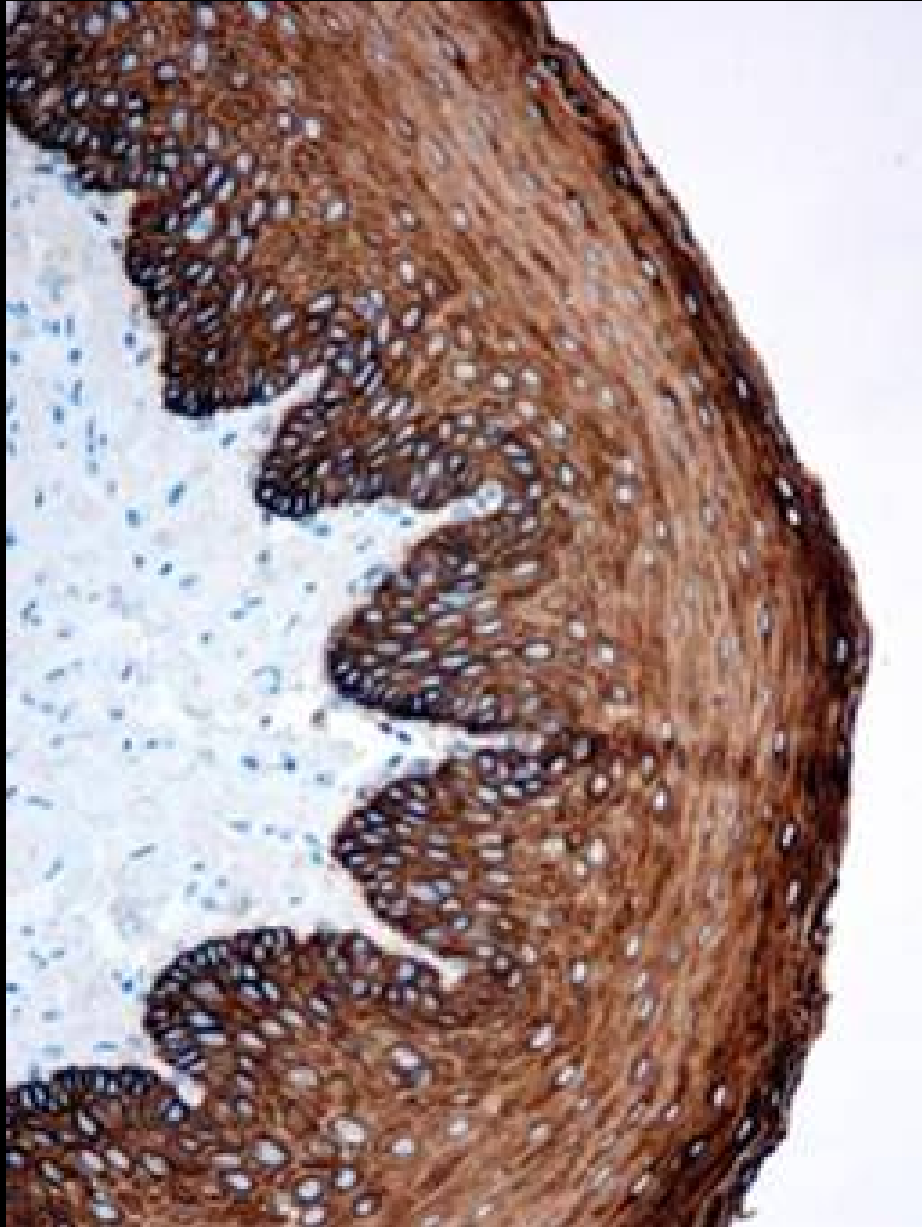
Cytokeratin types and cell types

Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	(+)	-	-	-	-
- suprabasal, non-keratinizing	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (¹ tonsil, ² mucosa)	-	-	-	+++	+++	(+) ¹	(++) ¹²	(+) ¹	-	(+) ¹
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermed. / ³ basal cells	-	(+) ³	+++	(+++) ³	-	(++) ³	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
⁴ Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+) ⁴	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (⁵ Merkel, ⁶ thyr.)	-	-	-	-	-	-	(+++) ⁵	(++) ⁶	(+++) ⁵	+++
Smooth muscle (vasc., myom.), myofibrobl., ⁷ sm.ves.endothelia	-	-	-	-	-	-	+	(++) ⁷	-	++

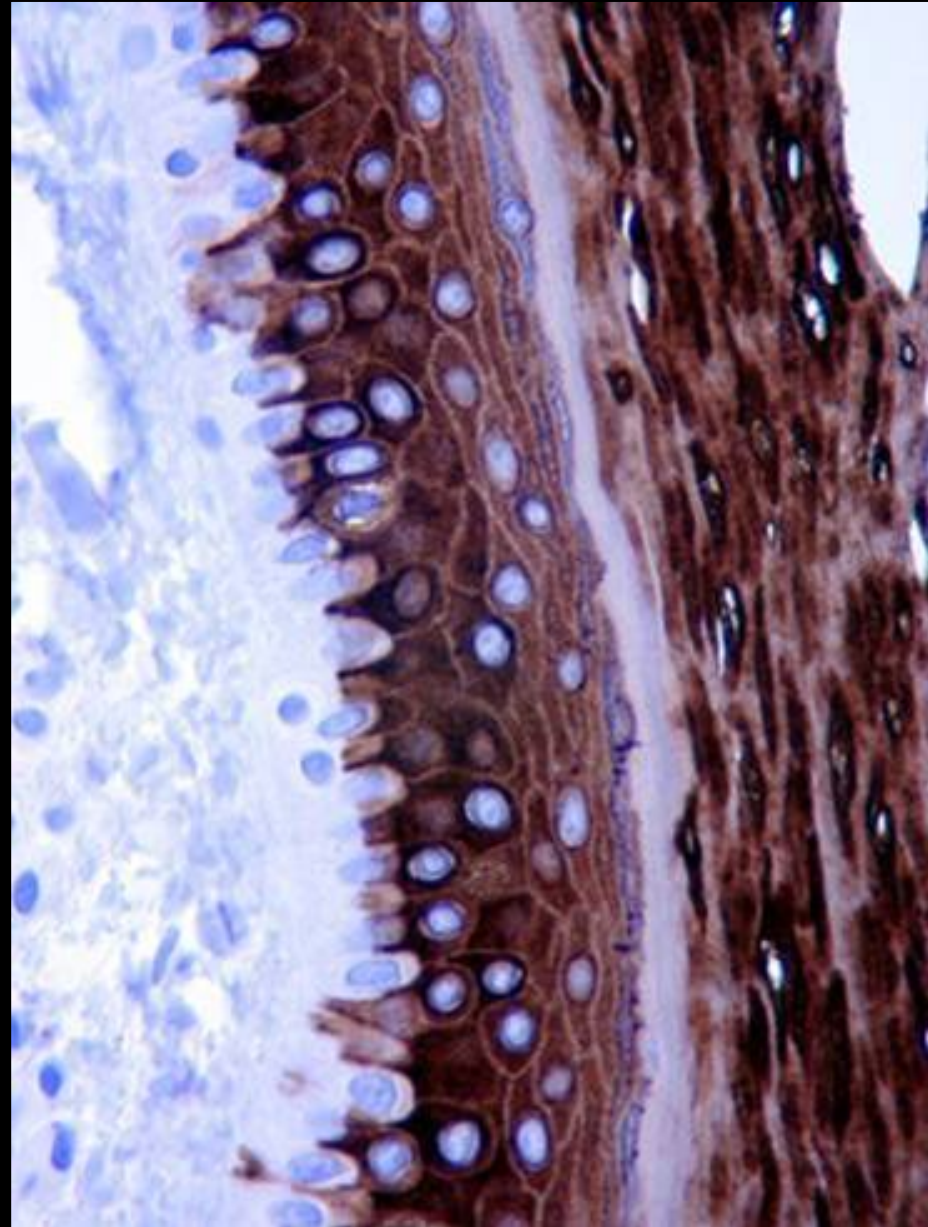
HMW Cytokeratin types - squamous epithelia

Neutral/Basic (B, class II)	1	4		5		17	19	7		8
Acidic (A, class I)	10		13		14				20	18
Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	(+)	-	-	-	-
- suprabasal, non-keratinizing	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (¹ tonsil, ² mucosa)	-	-	-	+++	+++	(+) ¹	(++) ¹²	(+) ¹	-	(+) ¹
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermed. / ³ basal cells	-	(+) ³	+++	(+++) ³	-	(++) ³	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
⁴ Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+) ⁴	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (⁵ Merkel, ⁶ thyr.)	-	-	-	-	-	-	(+++) ⁵	(++) ⁶	(+++) ⁵	+++
Smooth muscle (vasc., myom.), myofibrobl., ⁷ sm.ves.endothelia	-	-	-	-	-	-	+	(++) ⁷	-	++

HMW Cytokeratin types - squamous epithelia



CK5: esophagus

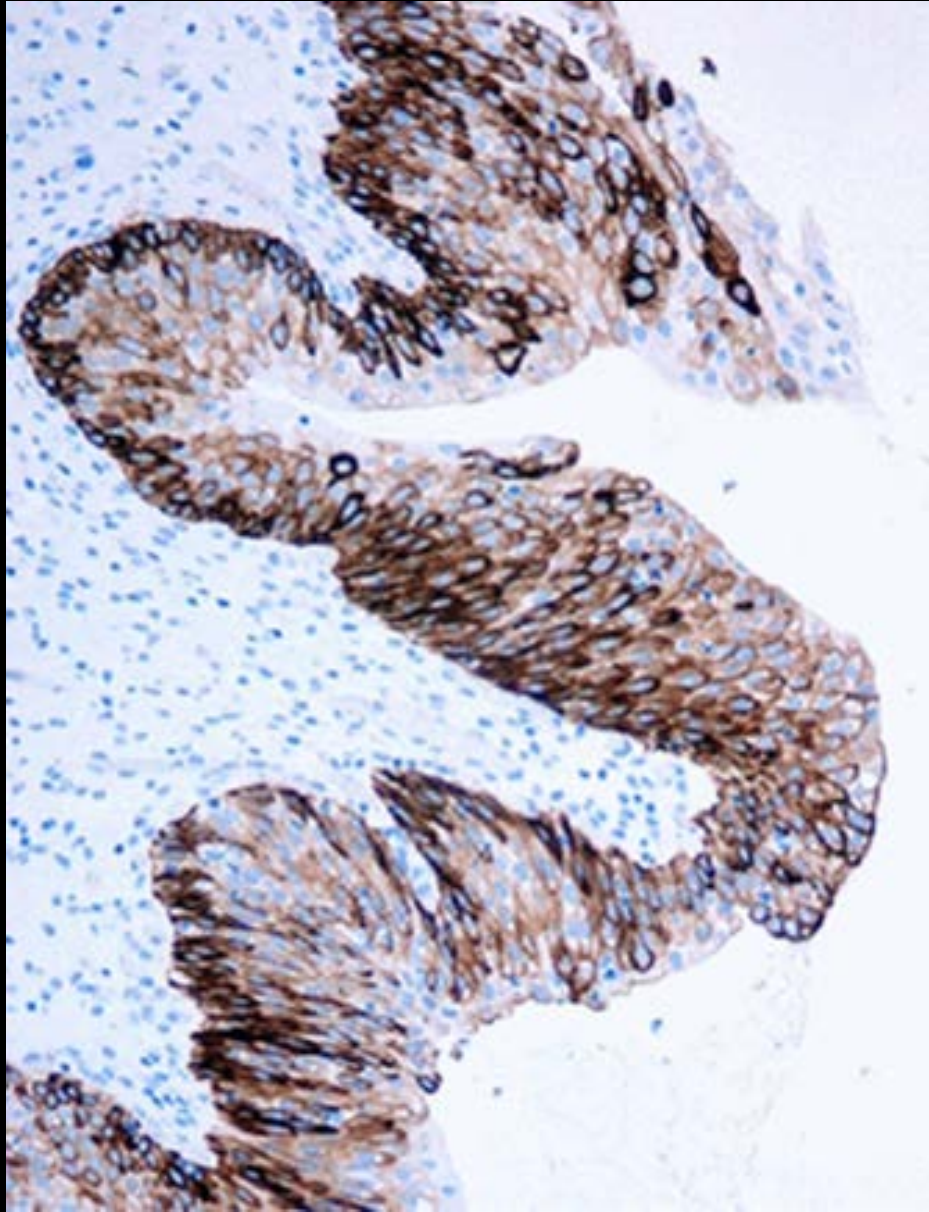


CK4: esophagus

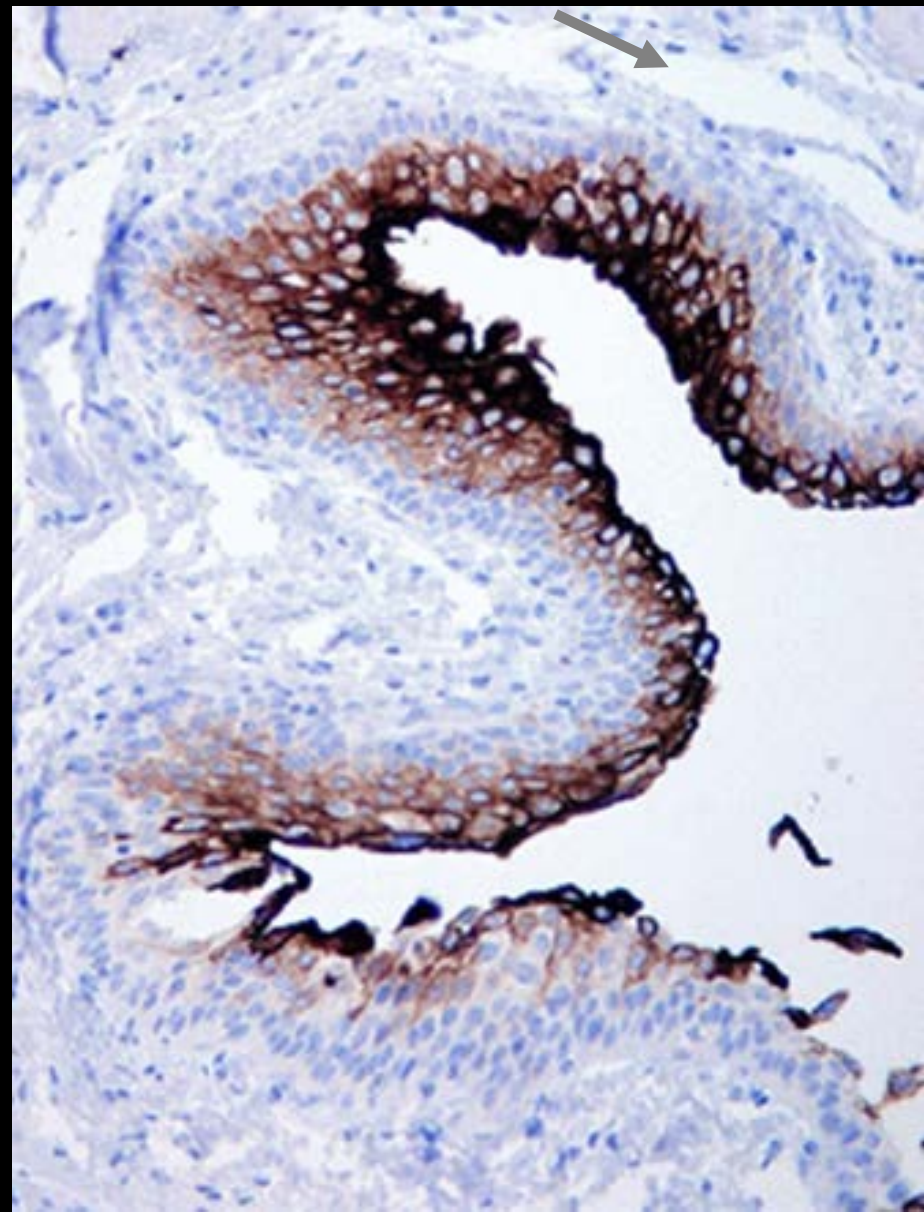
Cytokeratin types - urothelium

Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	(+)	-	-	-	-
- suprabasal, non-keratinizing	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (¹ tonsil, ² mucosa)	-	-	-	+++	+++	(+) ¹	(++) ¹²	(+) ¹	-	(+) ¹
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermed. / ³basal cells	-	(+) ³	+++	(+++) ³	-	(++) ³	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
⁴ Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+) ⁴	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (⁵ Merkel, ⁶ thyr.)	-	-	-	-	-	-	(+++) ⁵	(++) ⁶	(+++) ⁵	+++
Smooth muscle (vasc., myom.), myofibrobl., ⁷ sm.ves.endothelia	-	-	-	-	-	-	+	(++) ⁷	-	++

Cytokeratin types - urothelium



CK5 (+CK13)

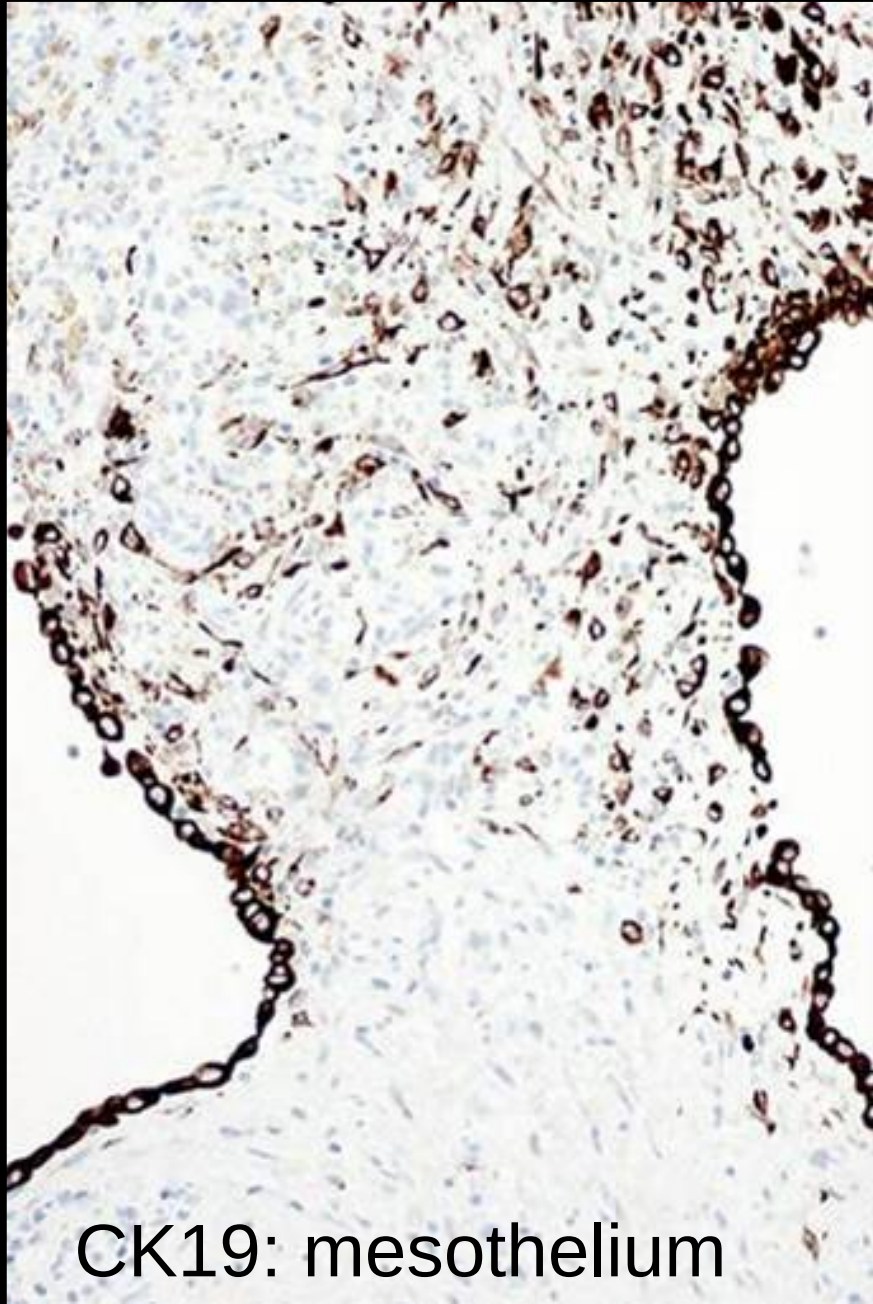


CK20

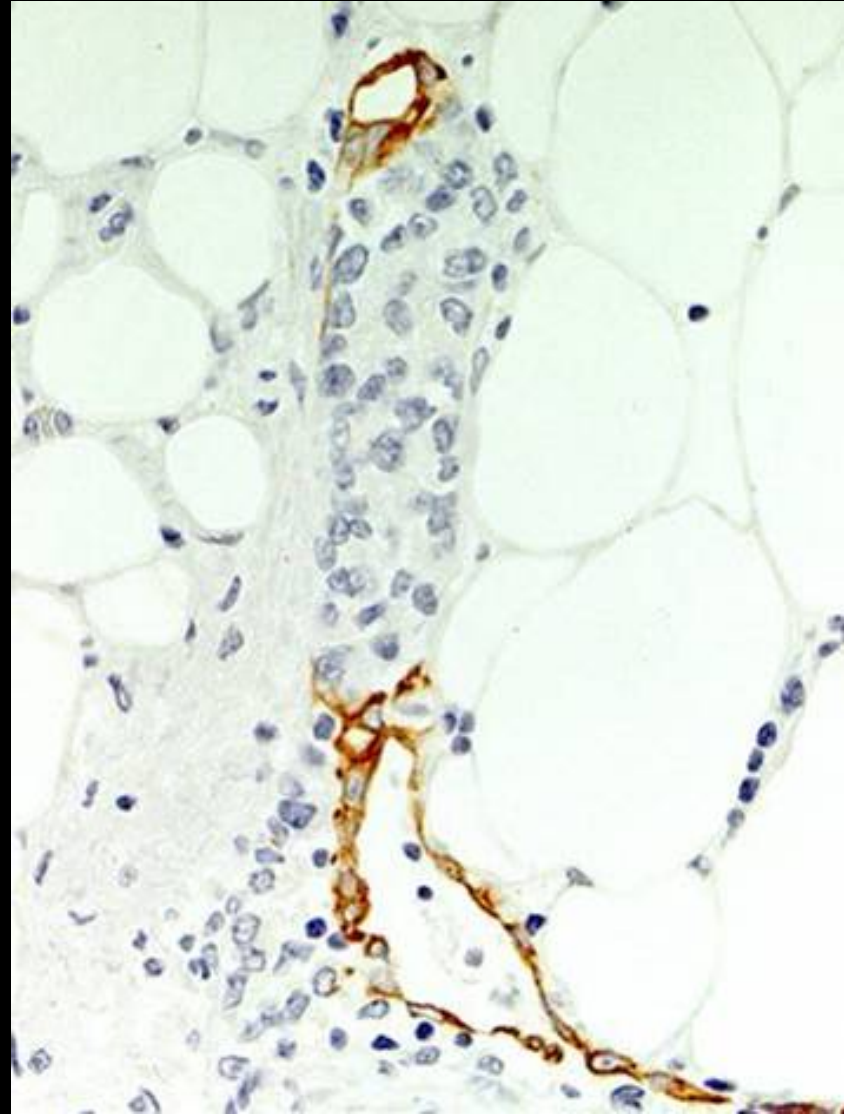
Cytokeratin types - mesothelium

Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	(+)	-	-	-	-
- suprabasal, non-keratiniz.ep.	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (¹ tonsil, ² mucosa)	-	-	-	+++	+++	(+) ¹	(++) ¹²	(+) ¹	-	(+) ¹
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermed. / ³ basal cells	-	(+) ³	+++	(+++) ³	-	(++) ³	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
⁴ Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+) ⁴	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (⁵ Merkel, ⁶ thyr.)	-	-	-	-	-	-	(+++) ⁵	(++) ⁶	(+++) ⁵	+++
Smooth muscle (vasc., myom.), myofibrobl., ⁷ sm.ves.endothelia	-	-	-	-	-	-	+	(++) ⁷	-	++

Cytokeratin types - mesothelium



CK19: mesothelium

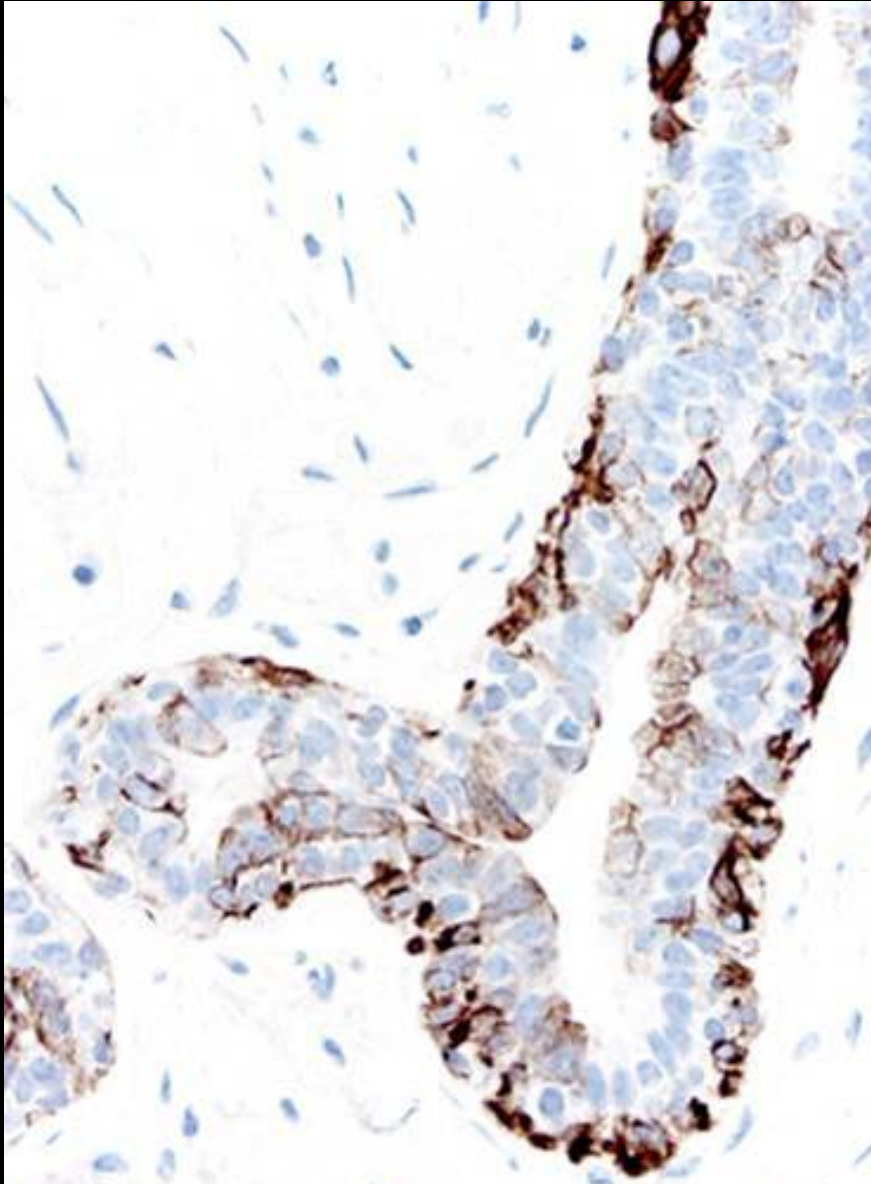


CK5: mesothelium
(adenocarcinoma neg.)

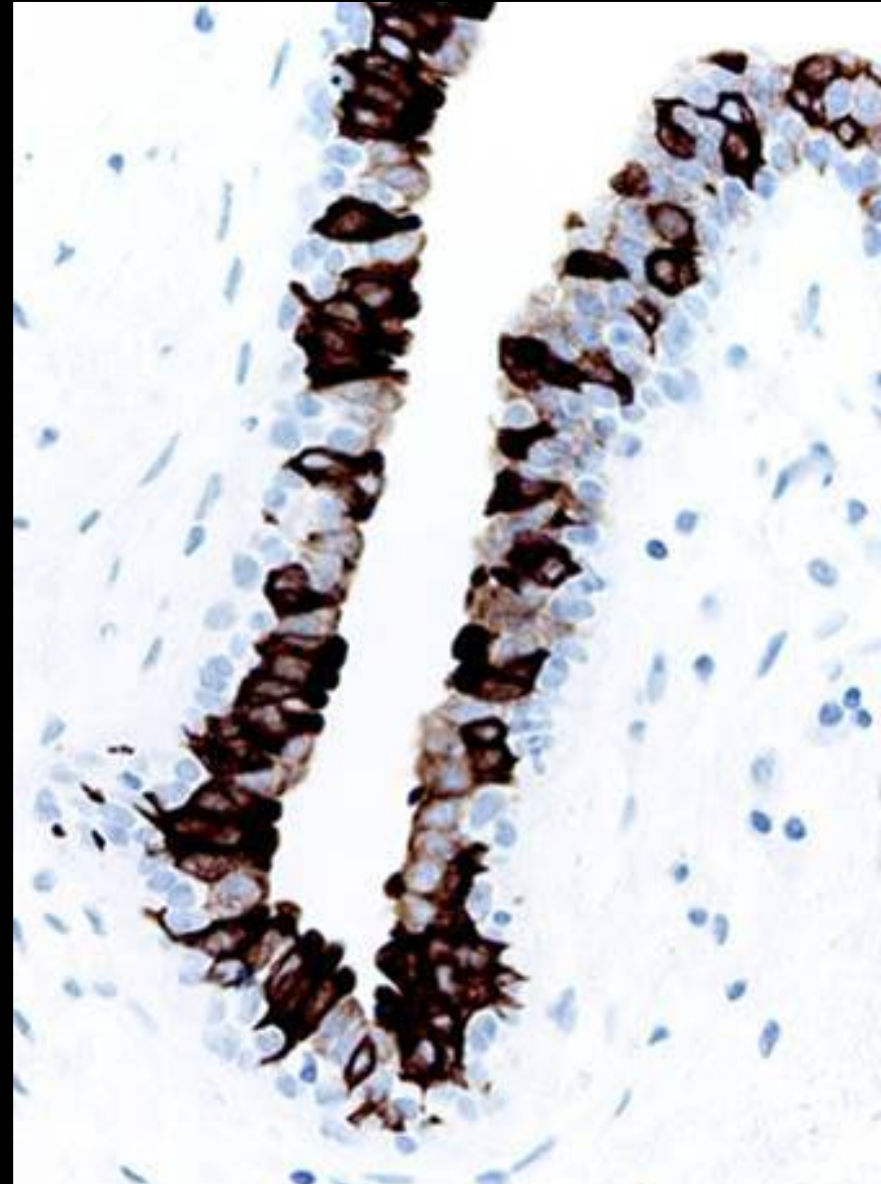
Cytokeratin types - complex epithelia

Neutral/Basic (B, class II) Acidic (A, class I)	1 10	4	13	5	14	17	19	7	20	8 18
Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	(+)	-	-	-	-
- suprabasal, non-keratinizing	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (¹ tonsil, ² mucosa)	-	-	-	-	+++	(+) ¹	(++) ¹²	(+) ¹	-	(+) ¹
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermed. / ³ basal cells	-	(+) ³	+++	(+++) ³	-	(++) ³	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
⁴ Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+) ⁴	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (⁵ Merkel, ⁶ thyr.)	-	-	-	-	-	-	(+++) ⁵	(++) ⁶	(+++) ⁵	+++
Smooth muscle (vasc., myom.), myofibrobl., ⁷ sm.ves.endothelia	-	-	-	-	-	-	+	(++) ⁷	-	++

Cytokeratin types - complex epithelia



CK5 (14,17): breast

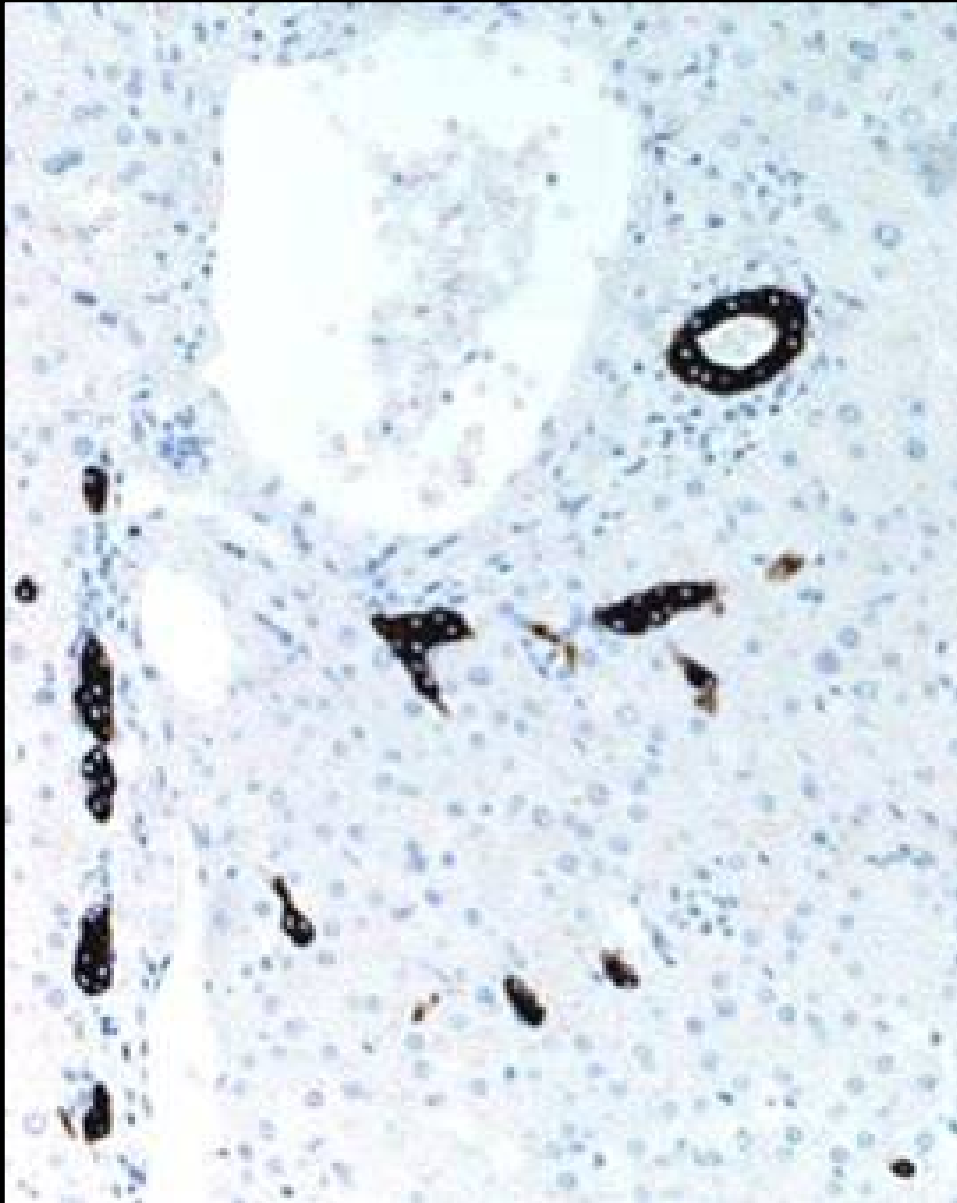


CK18 (8,7): breast

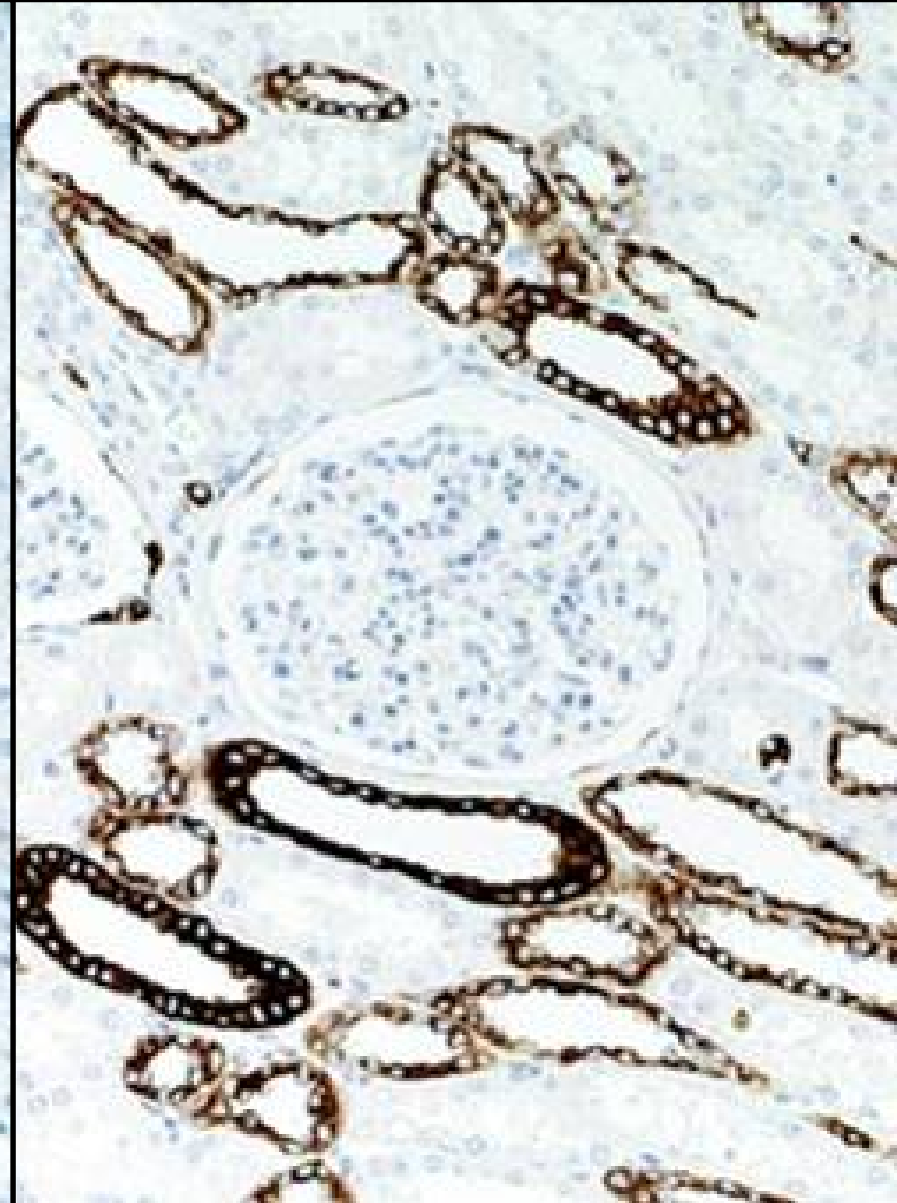
Cytokeratin types - simple epithelia

Neutral/Basic (B, class II) Acidic (A, class I)	1 10	4	13	5	14	17	19	7	20	8 18
Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	-(+)	-	-	-	-
- suprabasal, non-keratinizing	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (¹ tonsil, ² mucosa)	-	-	-	-	+++	-(+) ¹	(++) ¹²	-(+) ¹	-	-(+) ¹
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermed. / ³ basal cells	-	(+) ³	+++	(+++) ³	-	(++) ³	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
⁴ Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+) ⁴	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (⁵ Merkel, ⁶ thyr.)	-	-	-	-	-	-	(+++) ⁵	(++) ⁶	(+++) ⁵	+++
Smooth muscle (vasc., myom.), myofibrobl., ⁷ sm.ves.endothelia	-	-	-	-	-	-	+	(++) ⁷	-	++

Cytokeratin types - simple epithelia (liver, kidney)



CK7(19): bile ducts

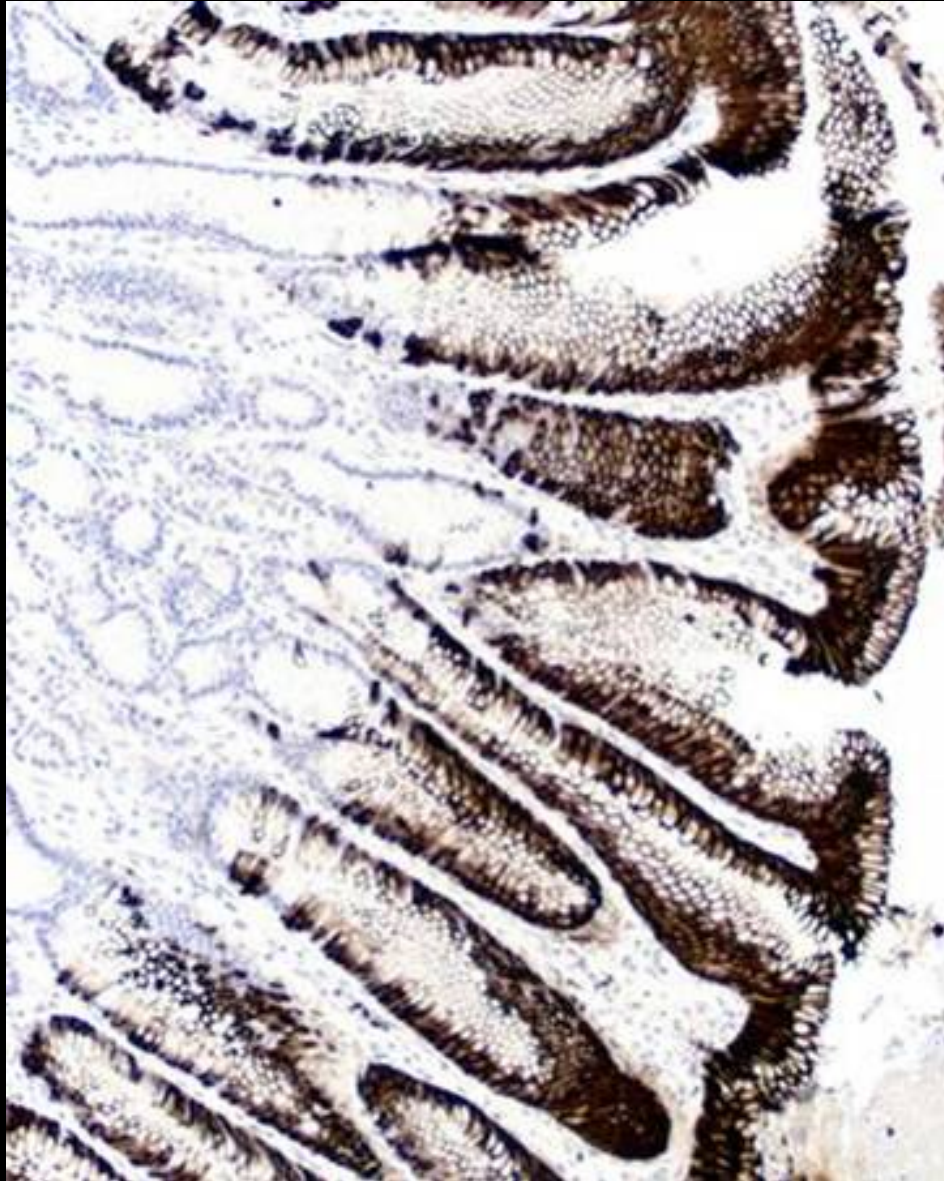


CK7(19): renal col.

Cytokeratin types - simple epithelia

Neutral/Basic (B, class II) Acidic (A, class I)	1 10	4	13	5	14	17	19	7	20	8 18
Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	-(+)	-	-	-	-
- suprabasal, non-keratinizing	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (¹ tonsil, ² mucosa)	-	-	-	-	+++	-(+) ¹	(++) ¹²	-(+) ¹	-	-(+) ¹
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermed. / ³ basal cells	-	(+) ³	+++	(+++) ³	-	(++) ³	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
⁴Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+) ⁴	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (⁵ Merkel, ⁶ thyr.)	-	-	-	-	-	-	(+++) ⁵	(++) ⁶	(+++) ⁵	+++
Smooth muscle (vasc., myom.), myofibrobl., ⁷ sm.ves.endothelia	-	-	-	-	-	-	+	(++) ⁷	-	++

Cytokeratin types - simple epithelia



CK20: gastric foveolae

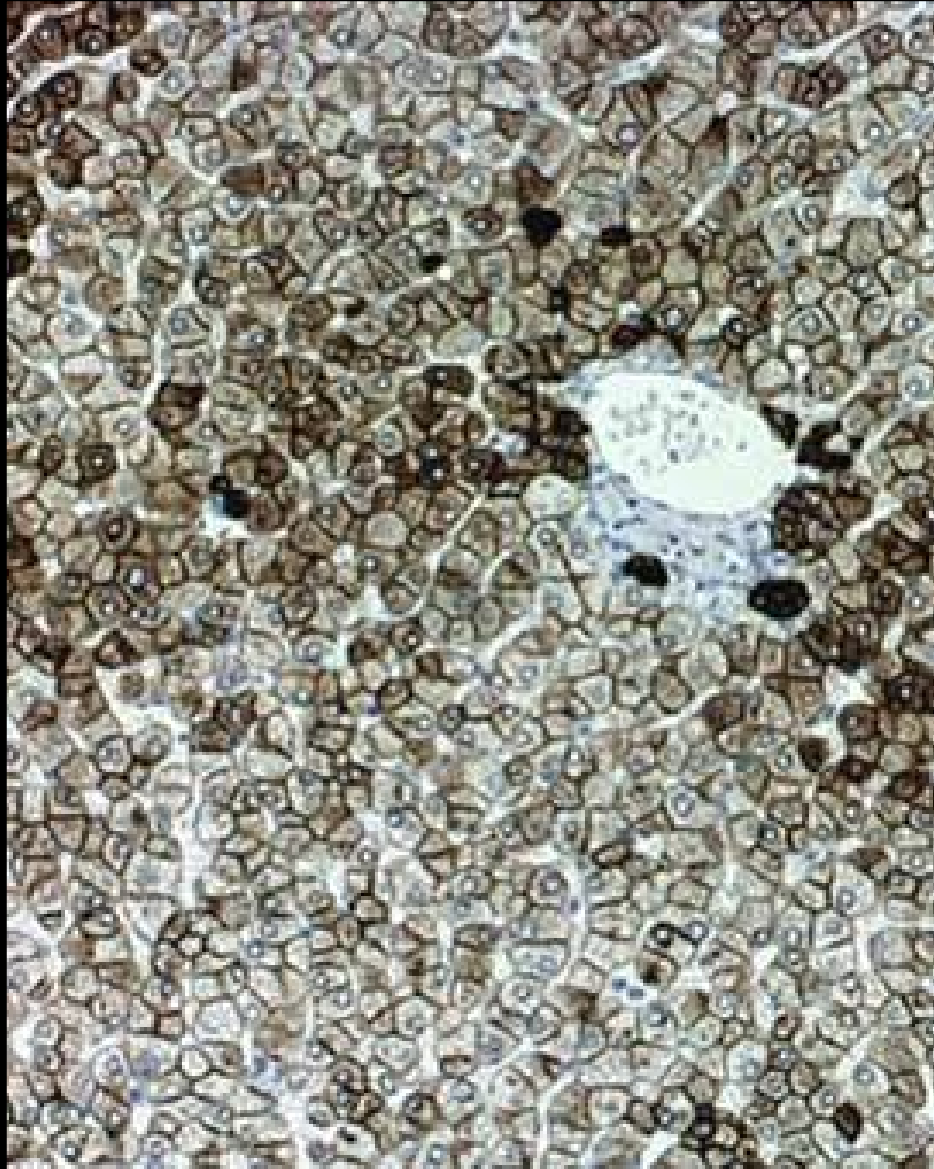


CK20: colon

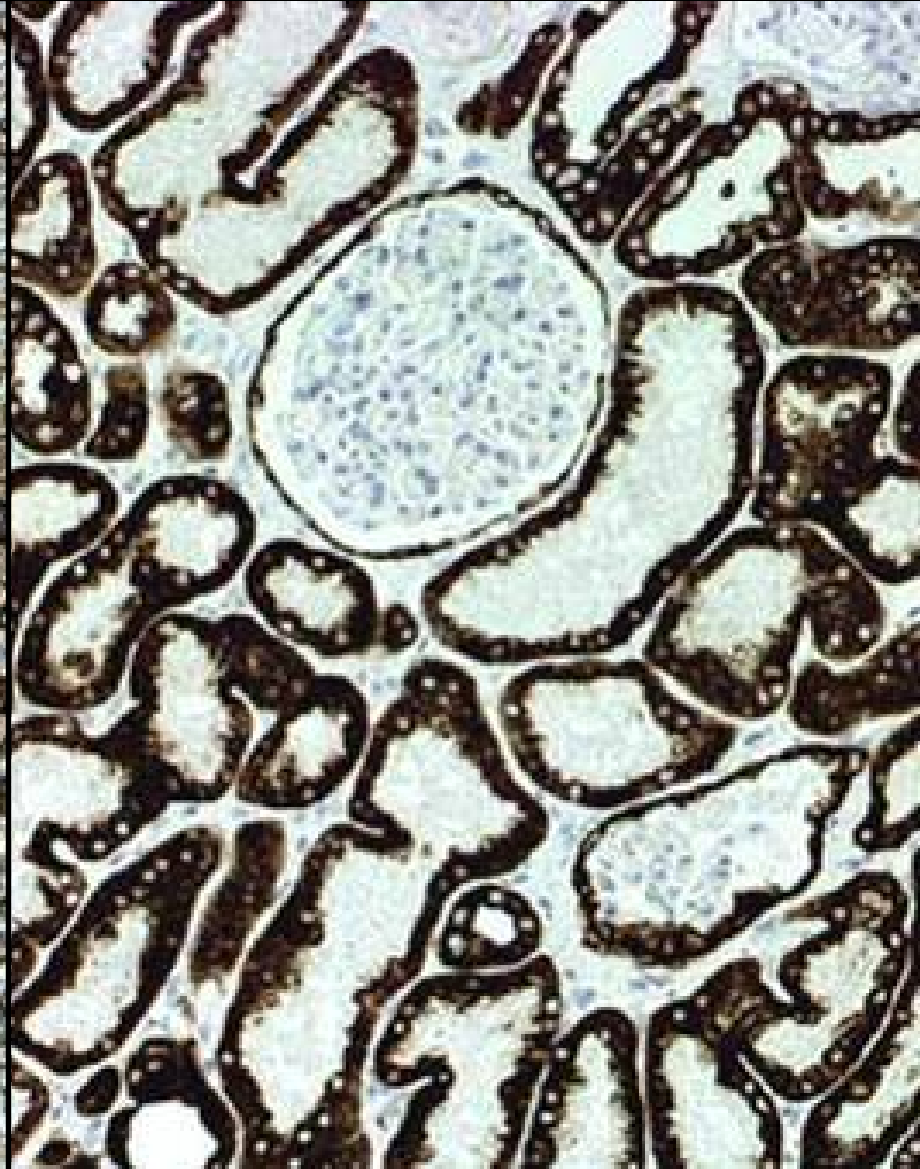
Cytokeratin types - simple epithelia

Neutral/Basic (B, class II) Acidic (A, class I)	1 10	4	13	5	14	17	19	7	20	8 18
Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	-(+)	-	-	-	-
- suprabasal, non-keratinizing	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (¹ tonsil, ² mucosa)	-	-	-	-	+++	-(+) ¹	(++) ¹²	-(+) ¹	-	-(+) ¹
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermed. / ³ basal cells	-	(+) ³	+++	(+++) ³	-	(++) ³	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
⁴ Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+) ⁴	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (⁵ Merkel, ⁶ thyr.)	-	-	-	-	-	-	(+++) ⁵	(++) ⁶	(+++) ⁵	+++
Smooth muscle (vasc., myom.), myofibrobl., ⁷ sm.ves.endothelia	-	-	-	-	-	-	+	(++) ⁷	-	++

Cytokeratin types - simple epithelia



CK8: liver

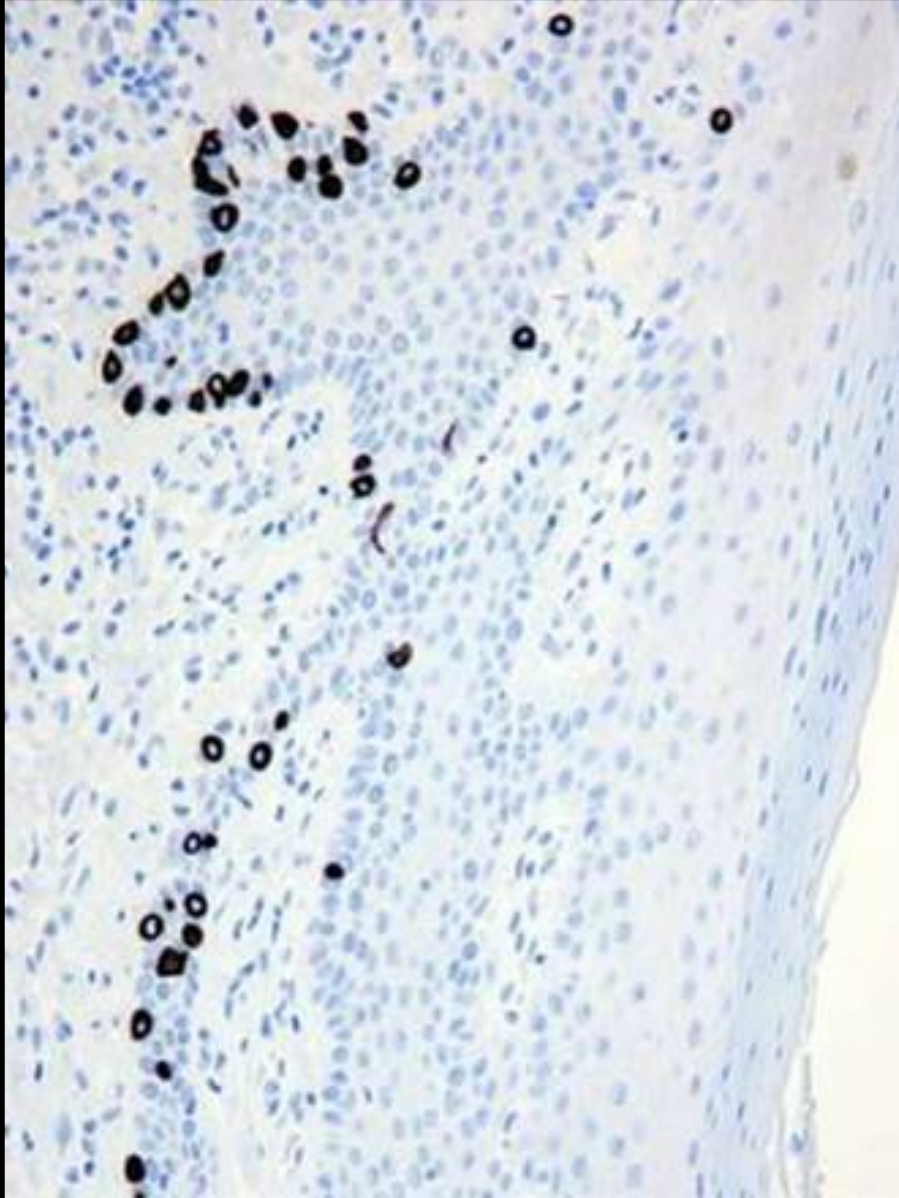


CK8: kidney

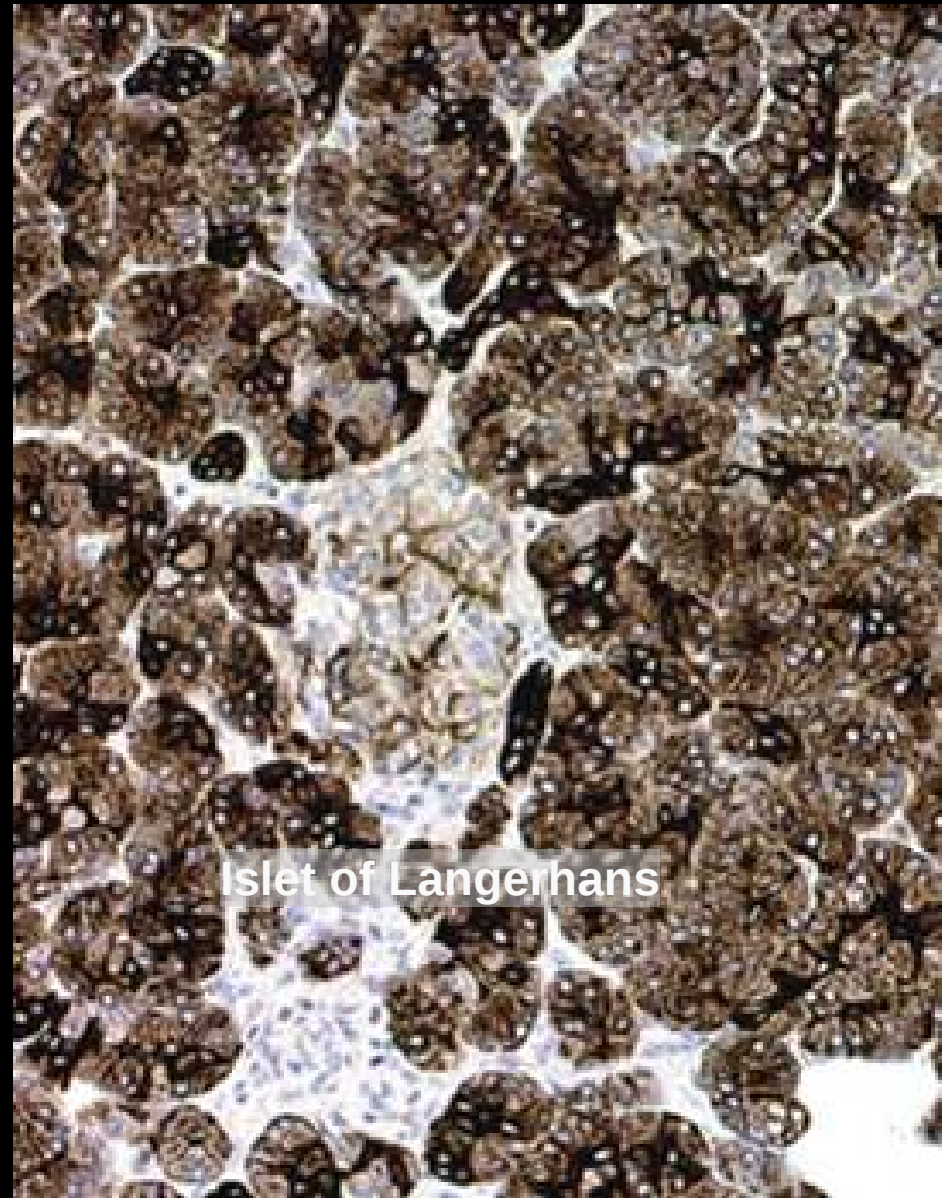
Cytokeratin types - simple epithelia

Neutral/Basic (B, class II) Acidic (A, class I)	1 10	4	13	5	14	17	19	7	20	8 18
Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	-(+)	-	-	-	-
- suprabasal, non-keratinizing	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (¹ tonsil, ² mucosa)	-	-	-	-	+++	-(+) ¹	(++) ¹²	-(+) ¹	-	-(+) ¹
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermed. / ³ basal cells	-	(+) ³	+++	(+++) ³	-	(++) ³	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
⁴ Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+) ⁴	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (⁵Merkel, ⁶thyr.)	-	-	-	-	-	-	(+++) ⁵	(++) ⁶	(+++) ⁵	+++
Smooth muscle (vasc., myom.), myofibrobl., ⁷ sm.ves.endothelia	-	-	-	-	-	-	+	(++) ⁷	-	++

Cytokeratin types - simple epithelia



CK20: Merkel cells

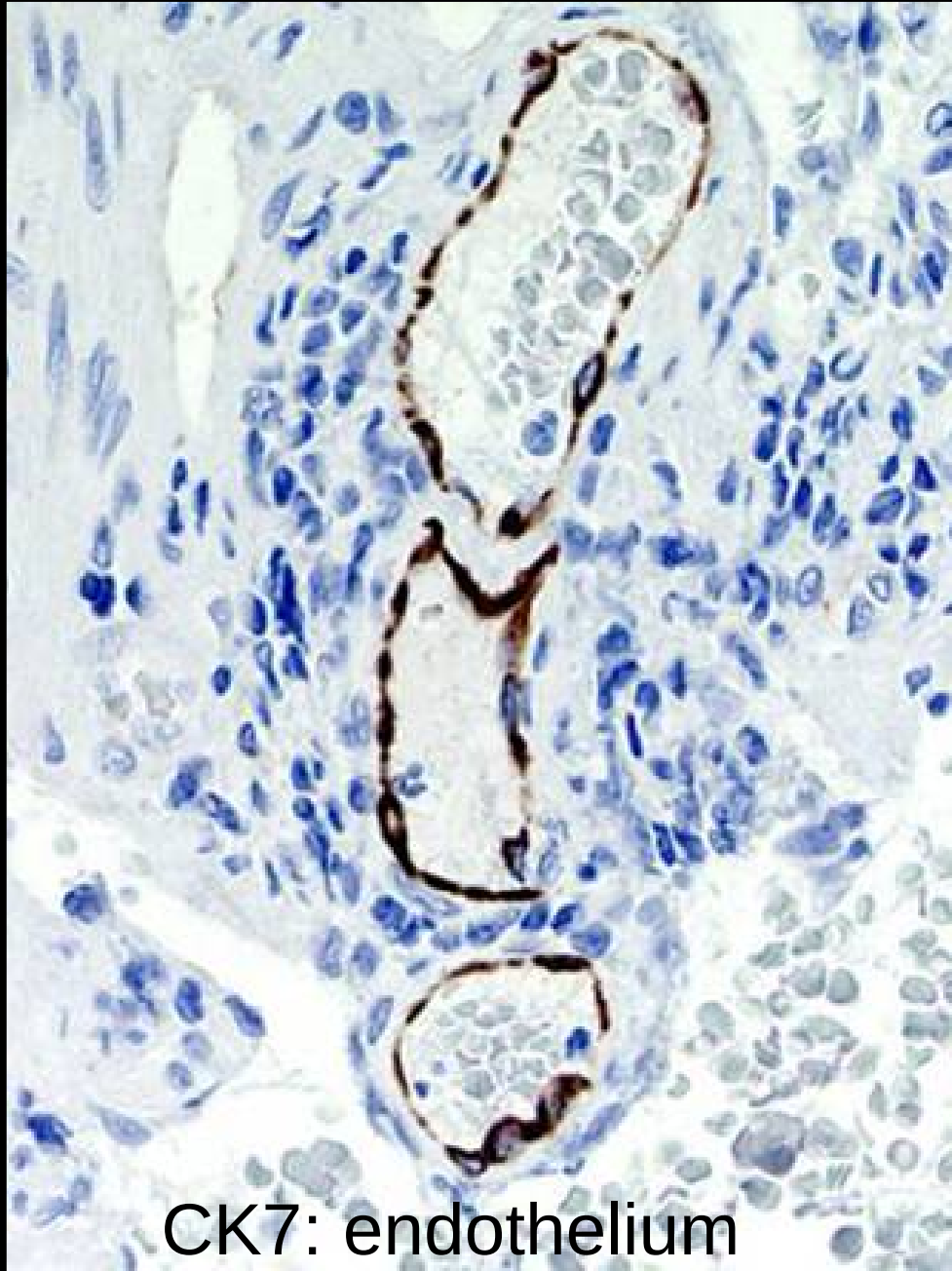


CK8: Pancreas

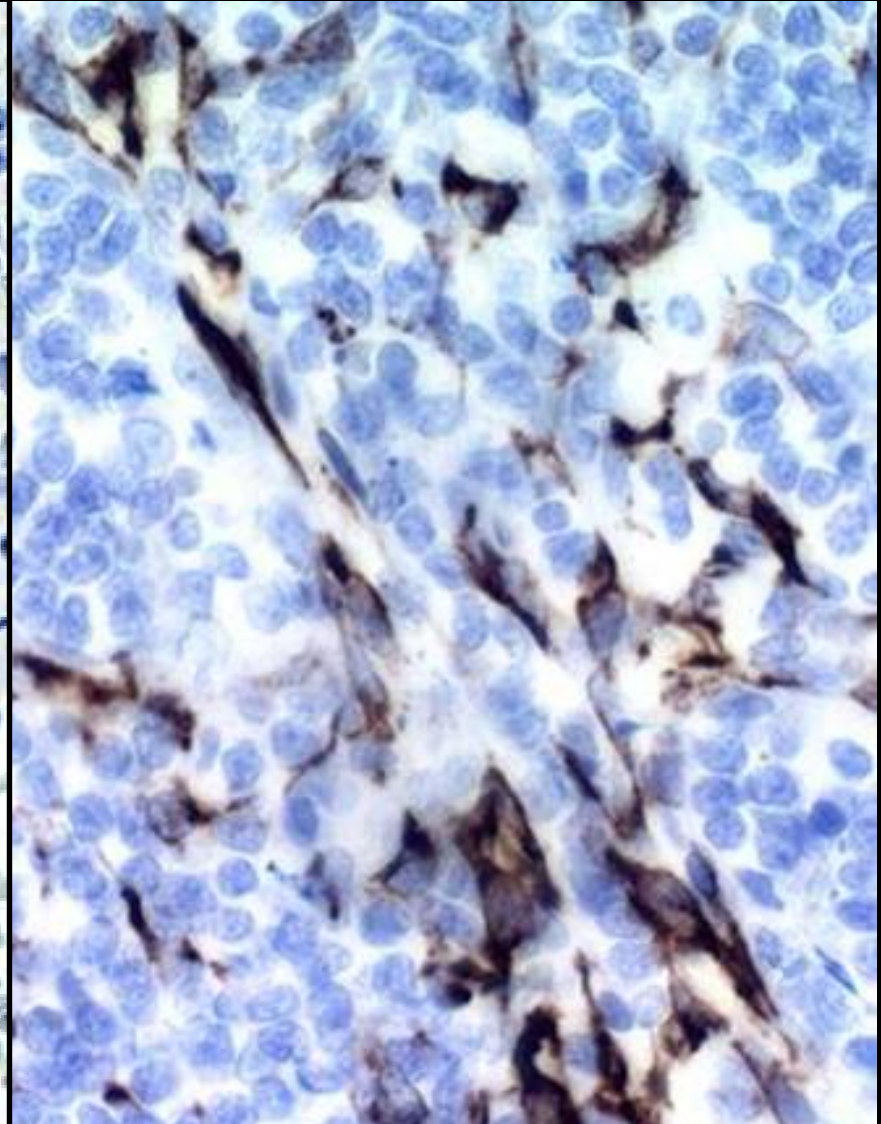
Cytokeratin types - mesenchymal cells

Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous epithelia:										
- suprabasal, keratinizing	+++	-	-	+	++	-(+)	-	-	-	-
- suprabasal, non-keratinizing	+	+++	+++	+	++	(+)	-	-	-	-
- basal cells (¹ tonsil, ² mucosa)	-	-	-	-	+++	-(+) ¹	(++) ¹²	-(+) ¹	-	-(+) ¹
Transit. epith.: superficial cells	-	-	-	-	-	-	+++	+++	++	+++
- intermed. / ³ basal cells	-	(+) ³	+++	(+++) ³	-	(++) ³	+++	+++	(+)	+++
Mesothelium	-	-	-	++	++	+	+++	+++	-	+++
Bronchus, breast, prost., cerv.:										
- basal/myoepithelial cells	-	-	-	+++	++	+++	++	-	-	-
- luminal cells	-	-	-	+	+	+	+++	+++	-	+++
Biliary/pancr. ducts, lung alv., endometr., renal collect. ducts	-	-	-	-	-	-	+++	+++	-	+++
⁴ Stomach (foveola), intestine	-	-	-	-	-	-	+++	(+) ⁴	+++	+++
Hepatocytes, pancr. acini, prox. renal tubules	-	-	-	-	-	-	-	-	-	+++
Endocrine cells (⁵ Merkel, ⁶ thyr.)	-	-	-	-	-	-	(+++) ⁵	(++) ⁶	(+++) ⁵	+++
Smooth muscle (vasc., myom.), myofibrobl., ⁷sm.ves.endothelia	-	-	-	-	-	-	+	(++) ⁷	-	++

Cytokeratin types - mesenchymal cells



CK7: endothelium



CK8: lymph node
fibroblastic reticulum cells

Cytokeratins in epithelial neoplasias

Neutral/Basic (B, class II) Acidic (A, class I)	1 10	4	13	5	14	17	19	7	20	8 18
Squamous cell carcinoma	(+)	+	+	++	++	(+)	(+)	(+)	-	(+)
Transitional cell tumour	-	+	+	+	+	+	++	++	++	++
Malignant mesothelioma	-	-	-	++	++	+	++	+	-	++
Adenocarcinoma: complex epithelia (lung, breast)	-	-	-	(+)	(+)	(+)	++	++	-	++
Adenocarc.: biliary tract, pancr., endom., ovary	-	-	-	(+)	(+)	(+)	++	++	(+)	++
Adenocarc.: stomach	-	-	-	-	-	-	++	+	+	++
Adenocarc.: intestine	-	-	-	-	-	-	++	+	++	++
Hepatocellular carcinoma	-	-	-	-	-	-	-	-	-	-
Renal cell carcinoma	-	-	-	-	-	-	-	+	-	++
Endocrine tumours: carcinoids	-	-	-	-	-	-	+	+	-	++
- Merkel cell carcinoma	-	-	-	-	-	-	+	-	++	++
- Thyroid carcinoma	-	-	-	-	-	-	+	++	-	++

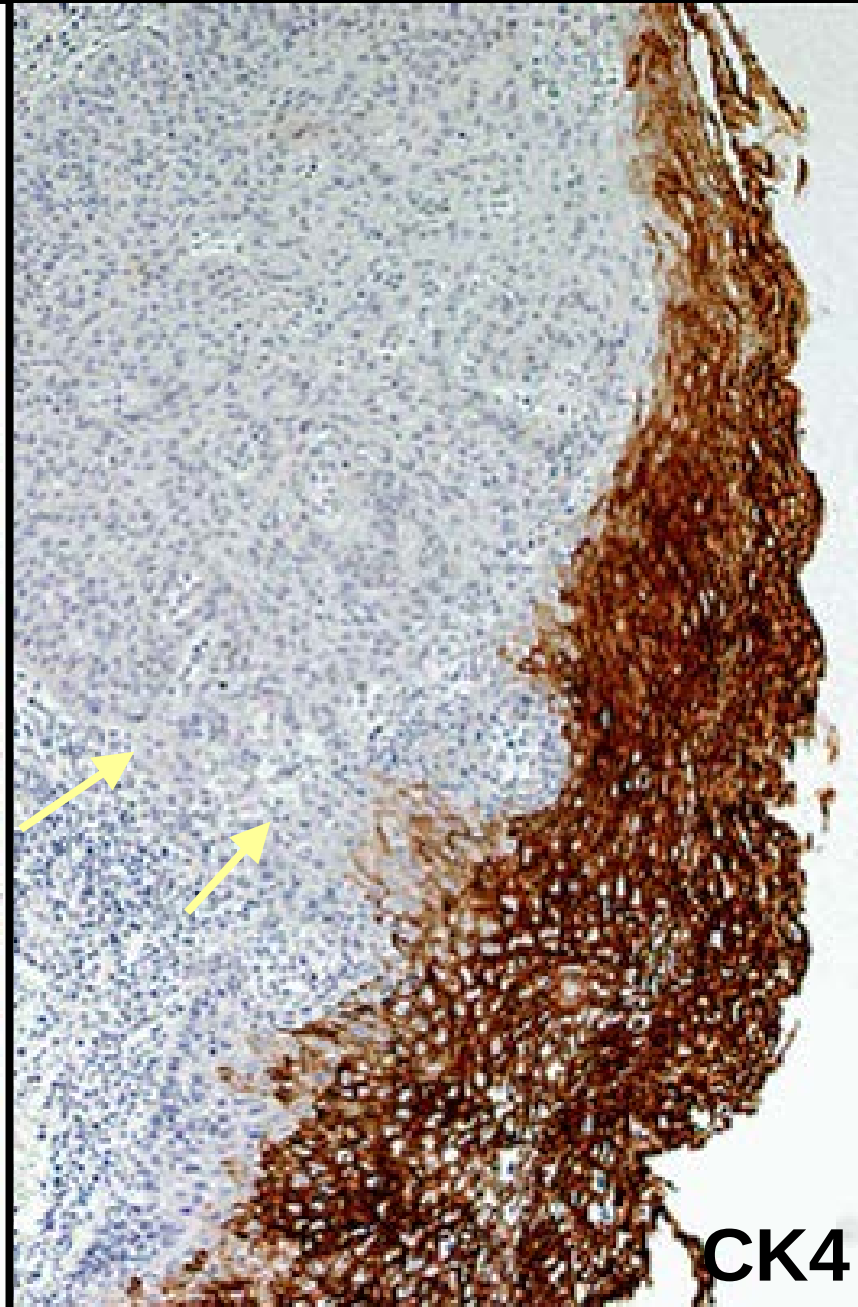
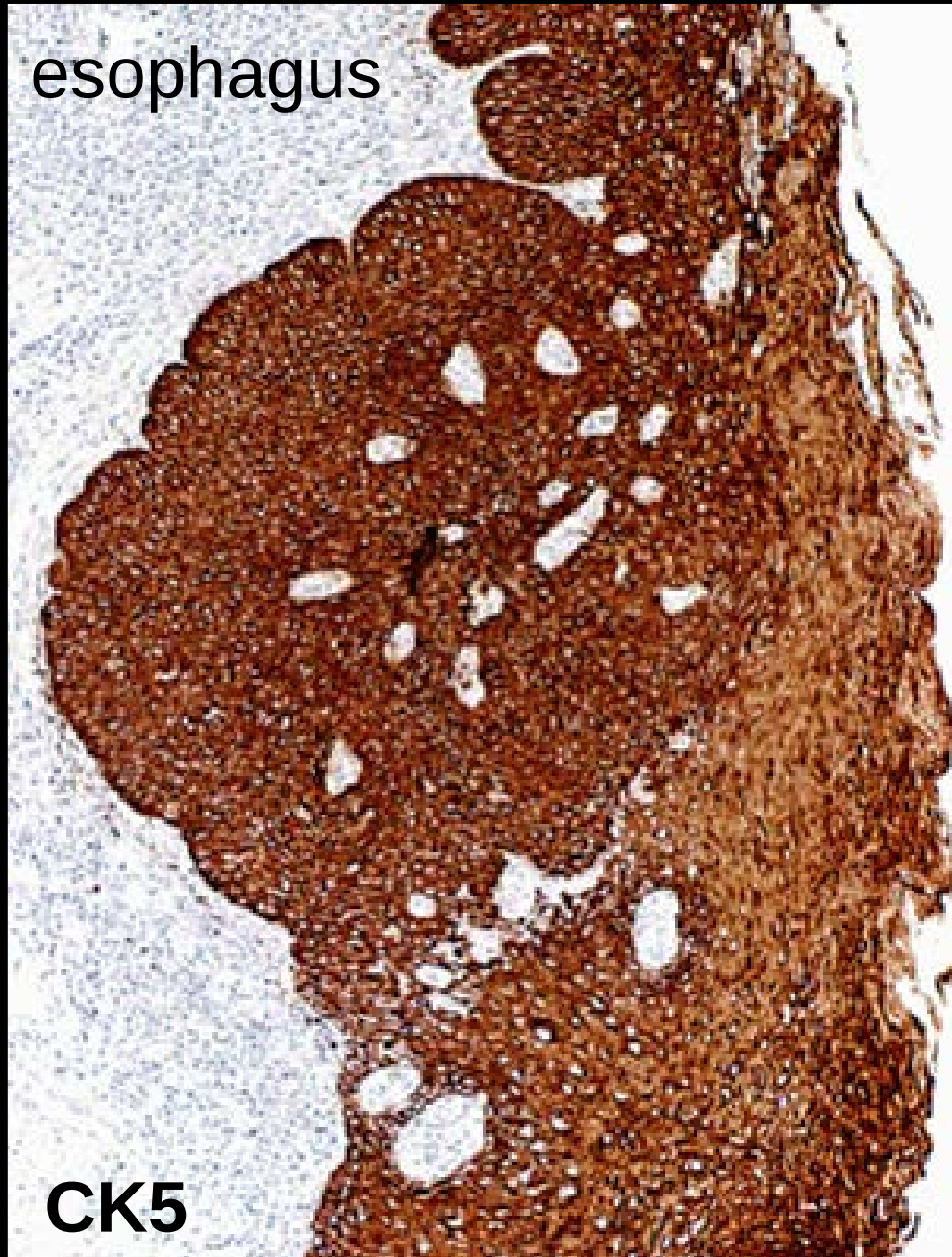
Cytokeratins in squamous cell carcinoma

Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous cell carcinoma	+	+	+	++	++	+	+	+	-	+
Transitional cell tumour	-	+	+	+	+	+	++	++	++	++
Malignant mesothelioma	-	-	-	++	++	+	++	+	-	++
Adenocarcinoma: complex epithelia (lung, breast)	-	-	-	(+)	(+)	(+)	++	++	-	++
Adenocarc.: biliary tract, pancr., endom., ovary	-	-	-	(+)	(+)	(+)	++	++	(+)	++
Adenocarc.: stomach	-	-	-	-	-	-	++	+	+	++
Adenocarc.: intestine	-	-	-	-	-	-	++	+	++	++
Hepatocellular carcinoma	-	-	-	-	-	-	-	+	-	++
Renal cell carcinoma	-	-	-	-	-	-	+	+	-	++
Endocrine tumours: carcinoids	-	-	-	-	-	-	+	+	-	++
- Merkel cell carcinoma	-	-	-	-	-	-	+	-	++	++
- Thyroid carcinoma	-	-	-	-	-	-	+	++	-	++

Cytokeratins in squamous cell carcinoma

esophagus

CK5

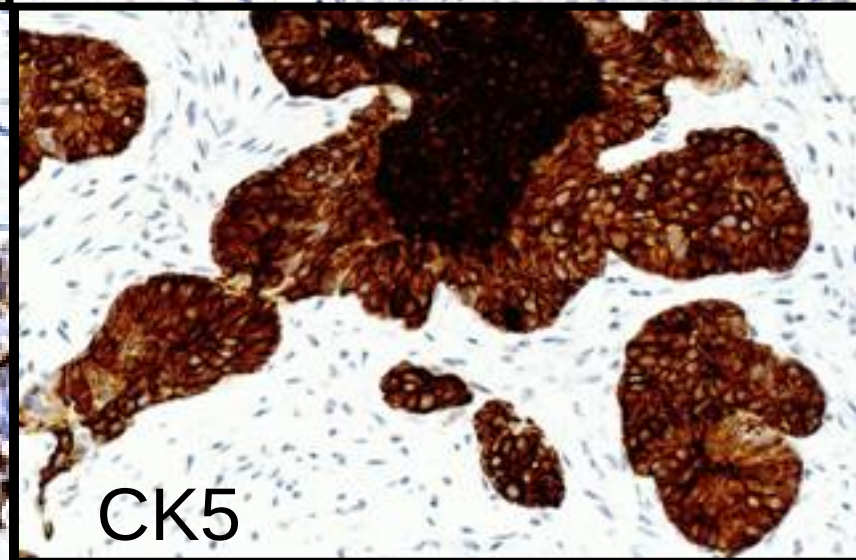
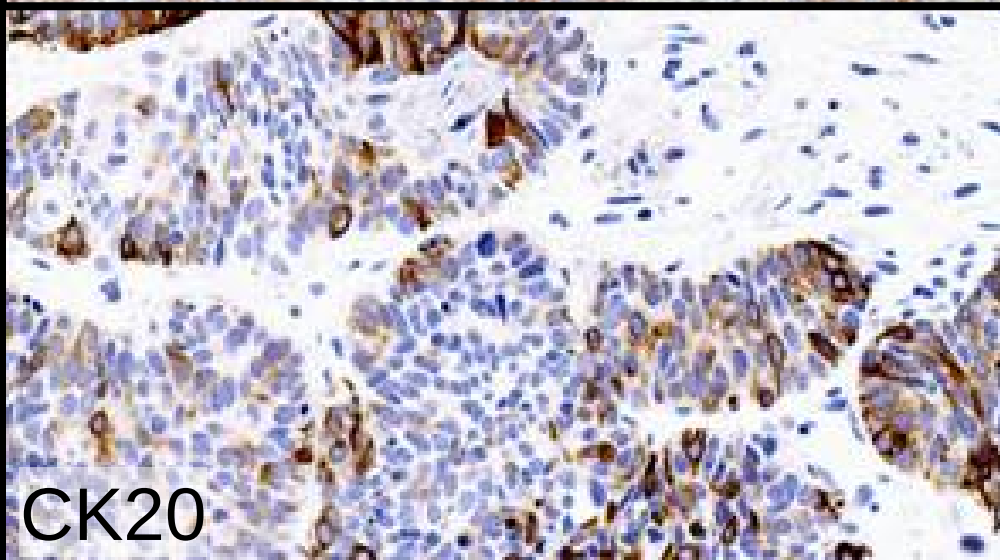
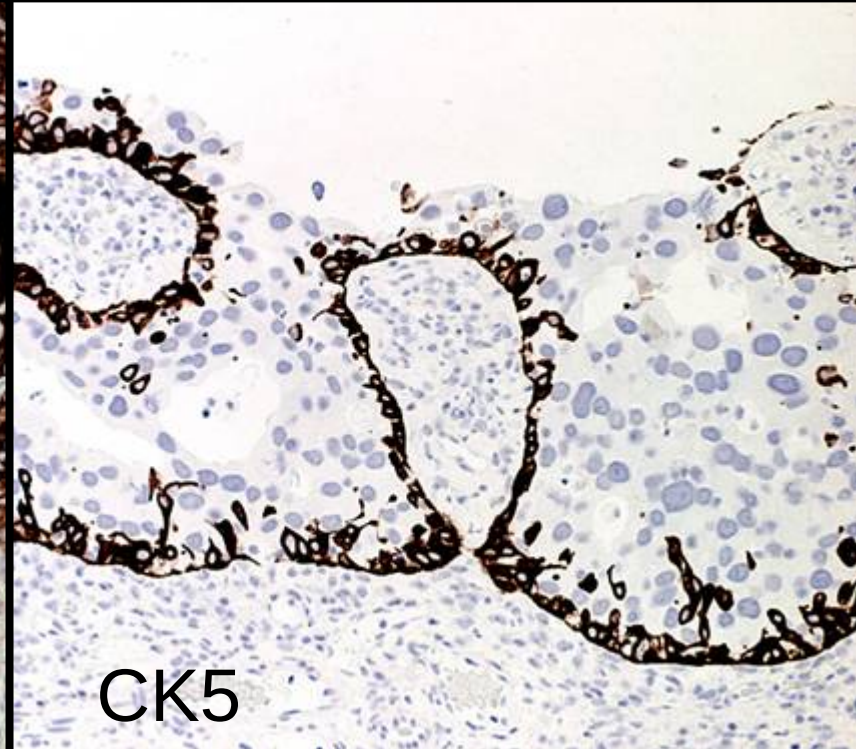
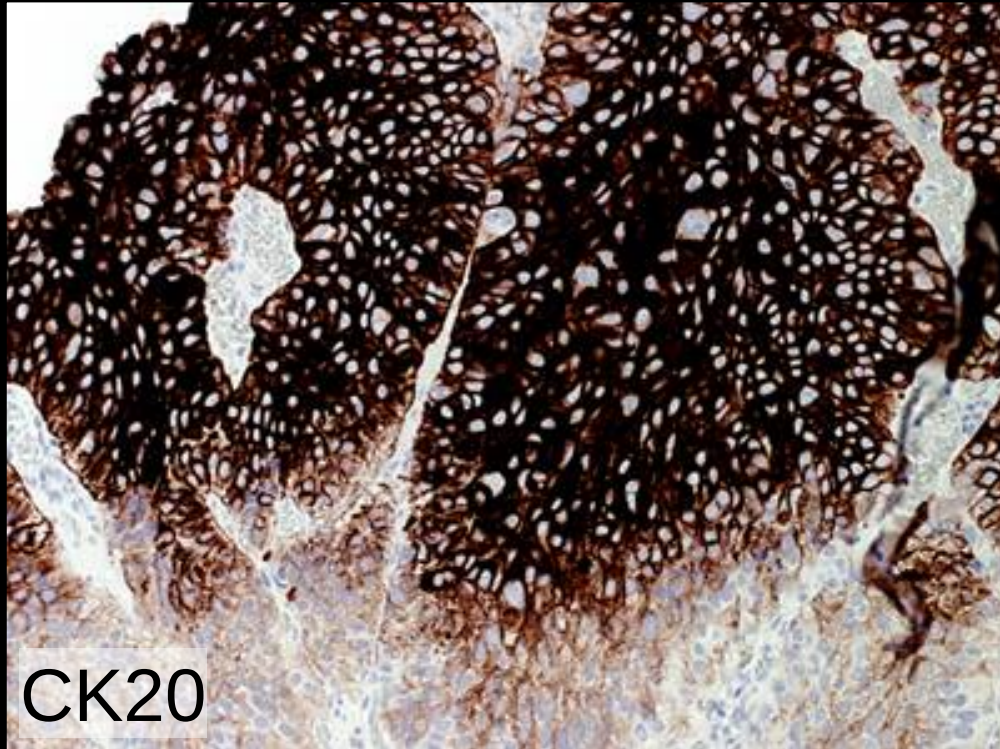


CK4

Cytokeratins in urothelial carcinoma

Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous cell carcinoma	+	+	+	++	++	+	+	+	-	+
Transitional cell tumour	-	+	+	++	+	+	++	++	++	++
Malignant mesothelioma	-	-	-	++	++	+	++	+	-	++
Adenocarcinoma: complex epithelia (lung, breast)	-	-	-	(+)	(+)	(+)	++	++	-	++
Adenocarc.: biliary tract, pancr., endom., ovary	-	-	-	(+)	(+)	(+)	++	++	(+)	++
Adenocarc.: stomach	-	-	-	-	-	-	++	+	+	++
Adenocarc.: intestine	-	-	-	-	-	-	++	+	++	++
Hepatocellular carcinoma										
Renal cell carcinoma	-	-	-	-	-	-	-	+	-	++
Endocrine tumours: carcinoids	-	-	-	-	-	-	+	+	-	++
- Merkel cell carcinoma	-	-	-	-	-	-	+	-	++	++
- Thyroid carcinoma	-	-	-	-	-	-	+	++	-	++

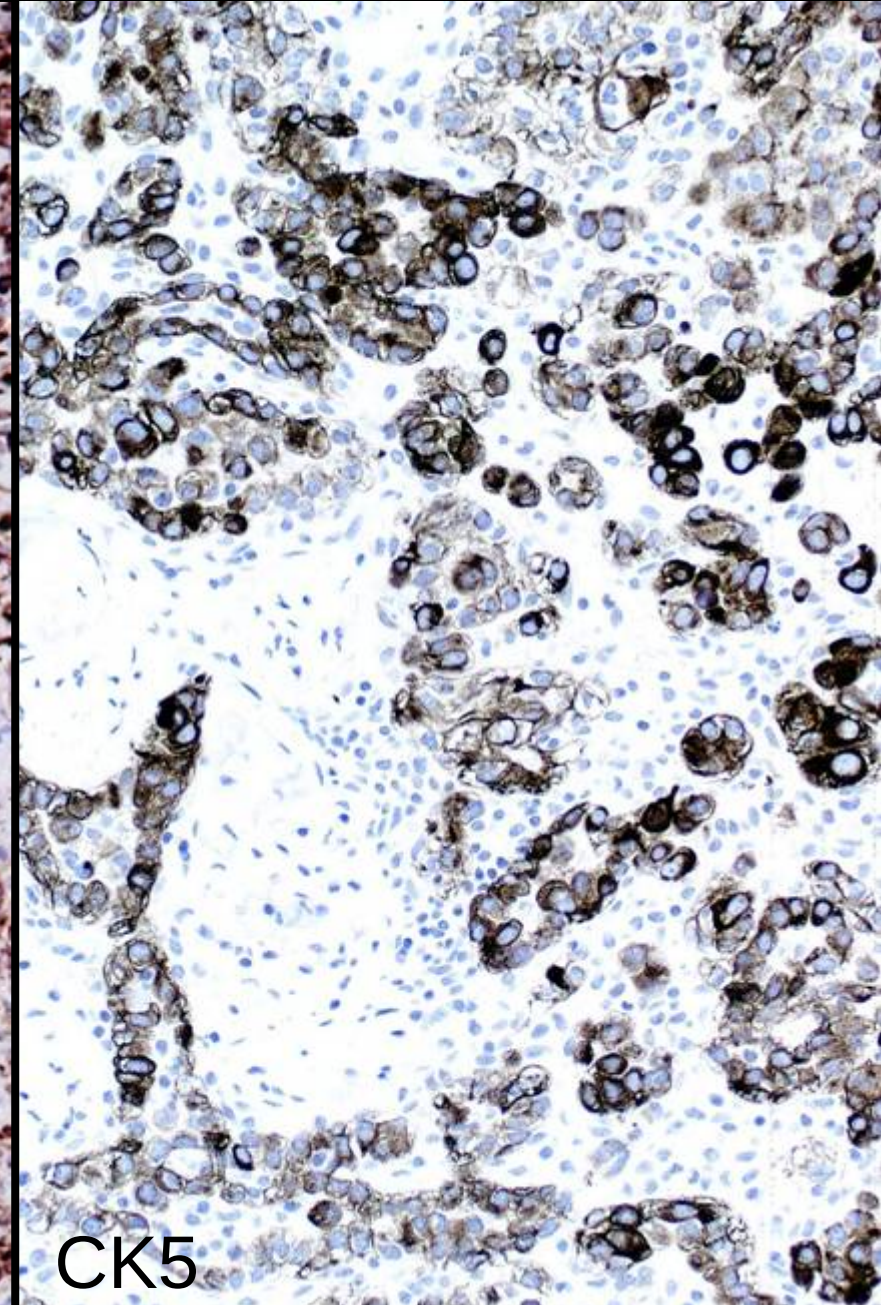
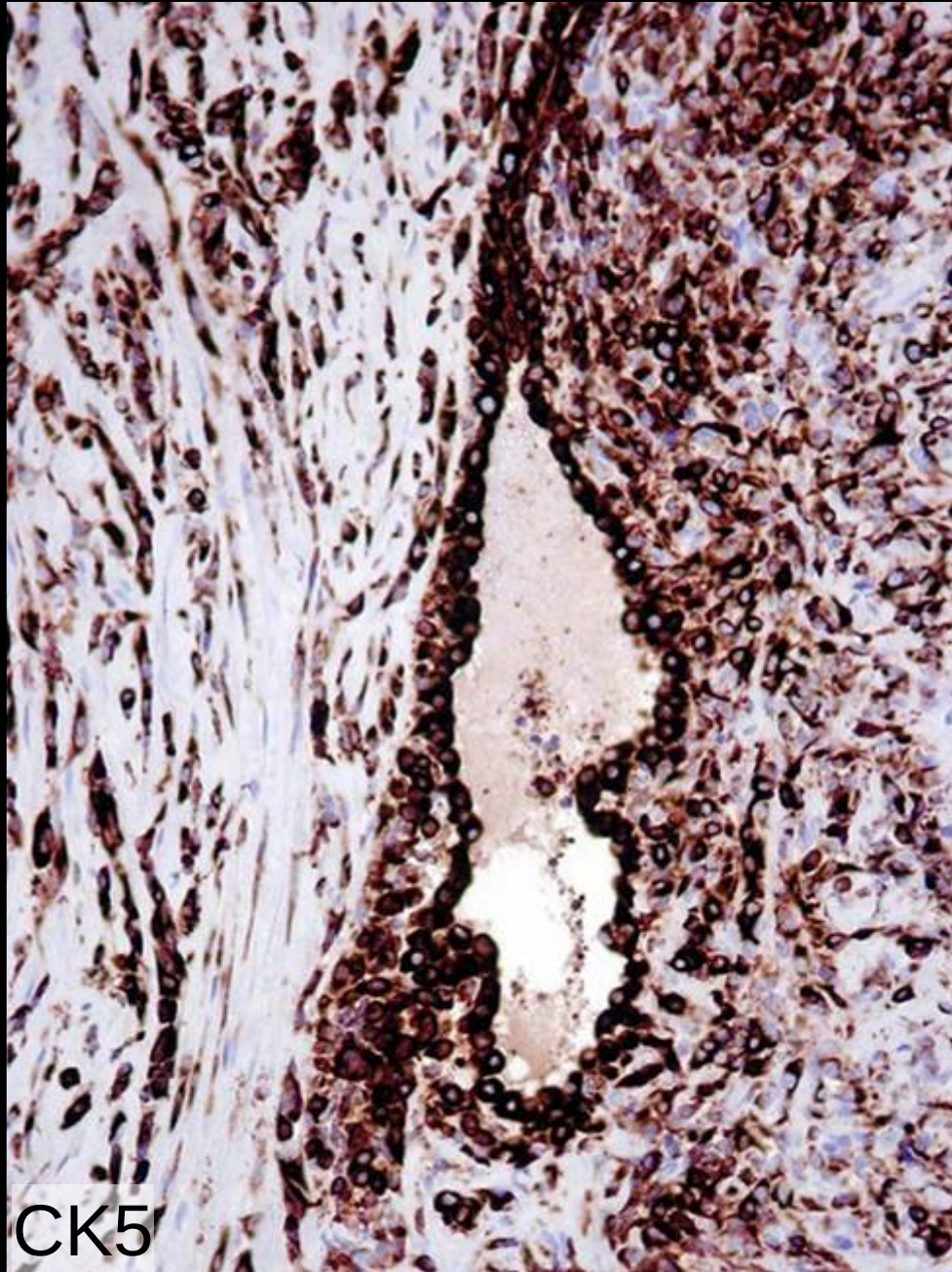
Cytokeratins in urothelial carcinoma



Cytokeratins in malignant mesothelioma

Neutral/Basic (B, class II) Acidic (A, class I)	1 10	4	13	5	14	17	19	7	20	8 18
Squamous cell carcinoma	(+)	+	+	++	++	(+)	(+)	(+)	-	(+)
Transitional cell tumour	-	+	+	+	+	+	++	++	++	++
Malignant mesothelioma	-	-	-	++	++	+	++	+	-	++
Adenocarcinoma: complex epithelia (lung, breast)	-	-	-	(+)	(+)	(+)	++	++	-	++
Adenocarc.: biliary tract, pancr., endom., ovary	-	-	-	(+)	(+)	(+)	++	++	(+)	++
Adenocarc.: stomach	-	-	-	-	-	-	++	+	+	++
Adenocarc.: intestine	-	-	-	-	-	-	++	+	++	++
Hepatocellular carcinoma	-	-	-	-	-	-	-	+	-	++
Renal cell carcinoma	-	-	-	-	-	-	-	+	-	++
Endocrine tumours: carcinoids	-	-	-	-	-	-	+	+	-	++
- Merkel cell carcinoma	-	-	-	-	-	-	+	-	++	++
- Thyroid carcinoma	-	-	-	-	-	-	+	++	-	++

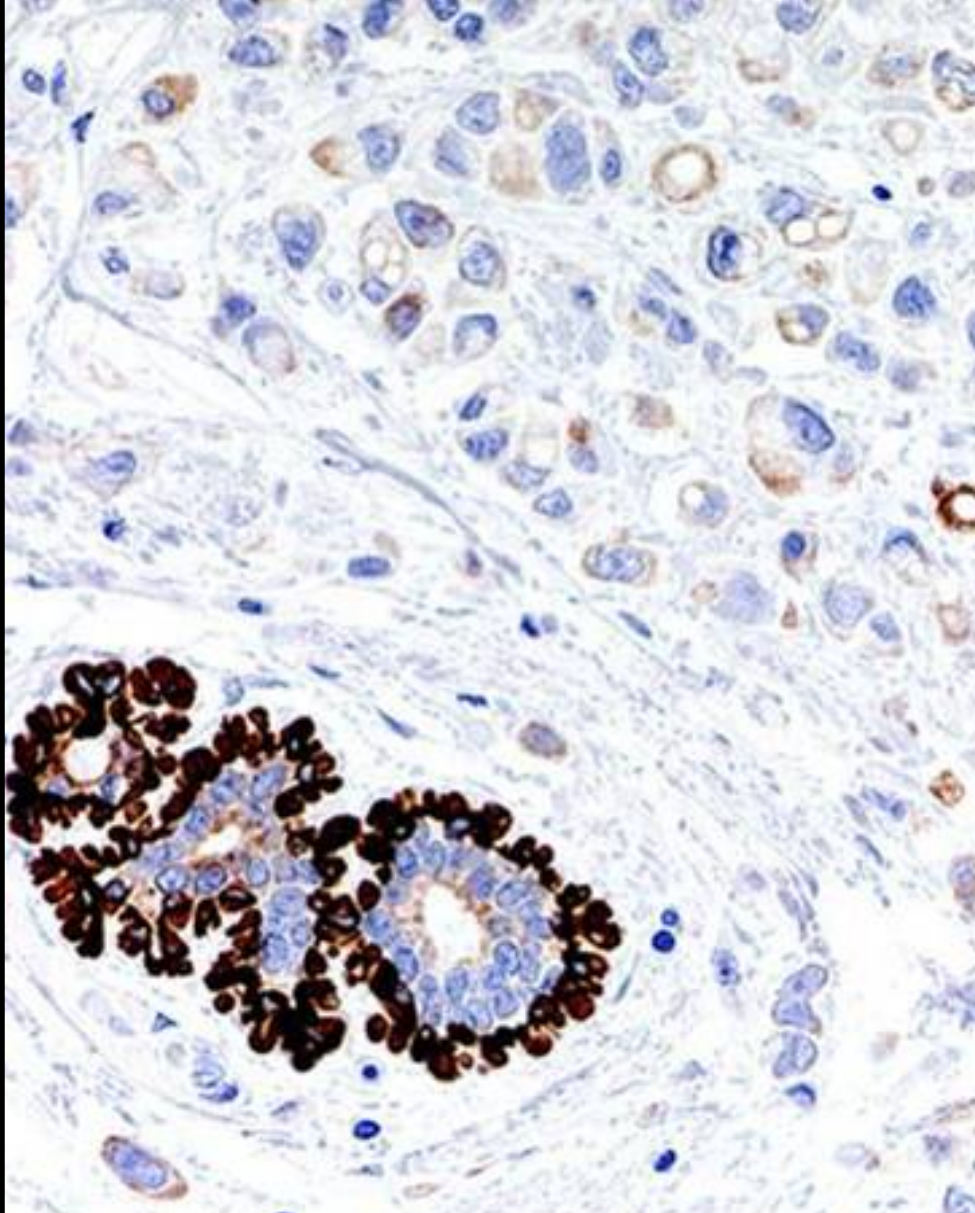
Cytokeratins in malignant mesothelioma



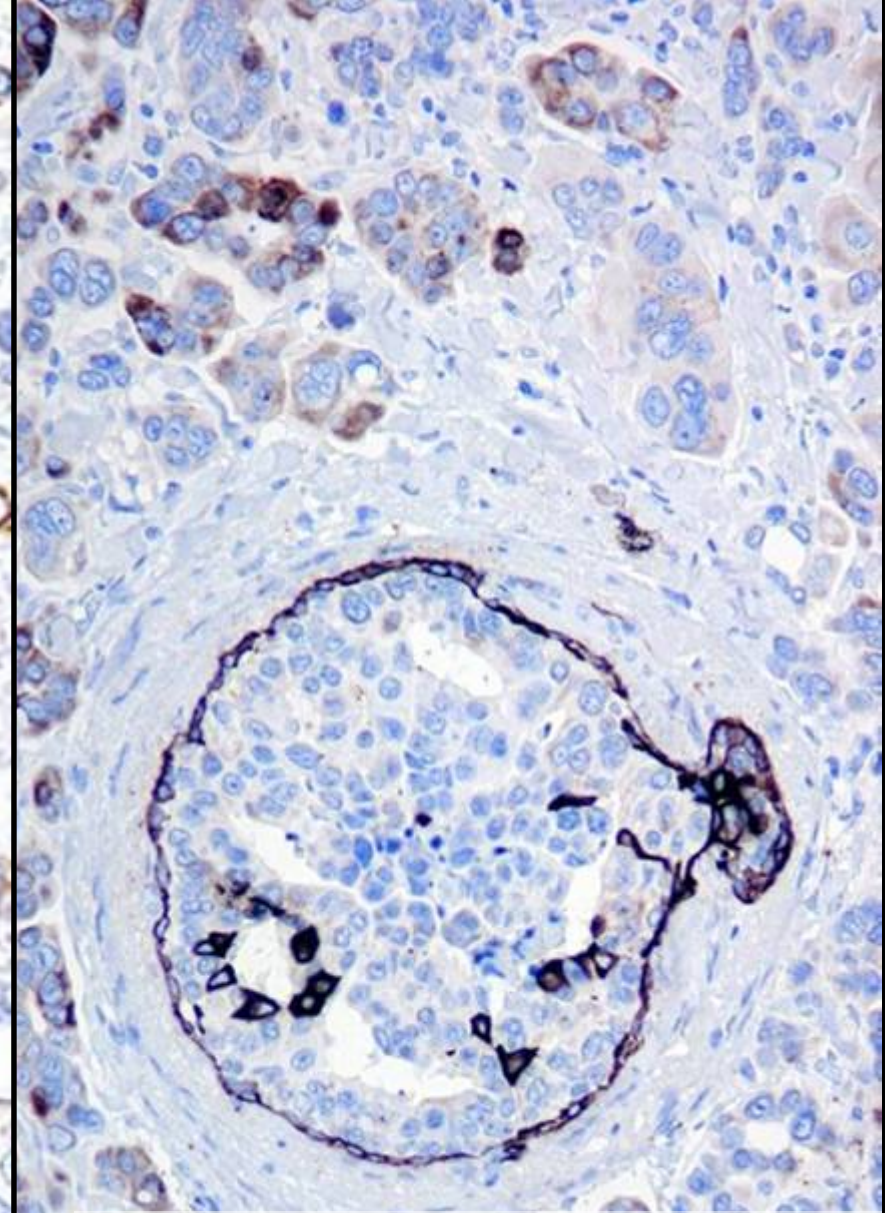
Cytokeratins in adenocarcinomas

Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous cell carcinoma	(+)	+	+	++	++	(+)	(+)	(+)	-	(+)
Transitional cell tumour	-	+	+	+	+	+	++	++	++	++
Malignant mesothelioma	-	-	-	++	++	+	++	+	-	++
Adenocarcinoma: complex epith. (lung, breast)	-	-	-	(+)	(+)	(+)	++	++	-	++
Adenocarc.: biliary tract, pancr., endom., ovary	-	-	-	(+)	(+)	(+)	++	++	(+)	++
Adenocarc.: stomach	-	-	-	-	-	-	++	+	+	++
Adenocarc.: intestine	-	-	-	-	-	-	++	+	++	++
Hepatocellular carcinoma										
Renal cell carcinoma	-	-	-	-	-	-	-	+	-	++
Endocrine tumours: carcinoids	-	-	-	-	-	-	+	+	-	++
- Merkel cell carcinoma	-	-	-	-	-	-	+	-	++	++
- Thyroid carcinoma	-	-	-	-	-	-	+	++	-	++

Cytokeratins in adenocarcinomas



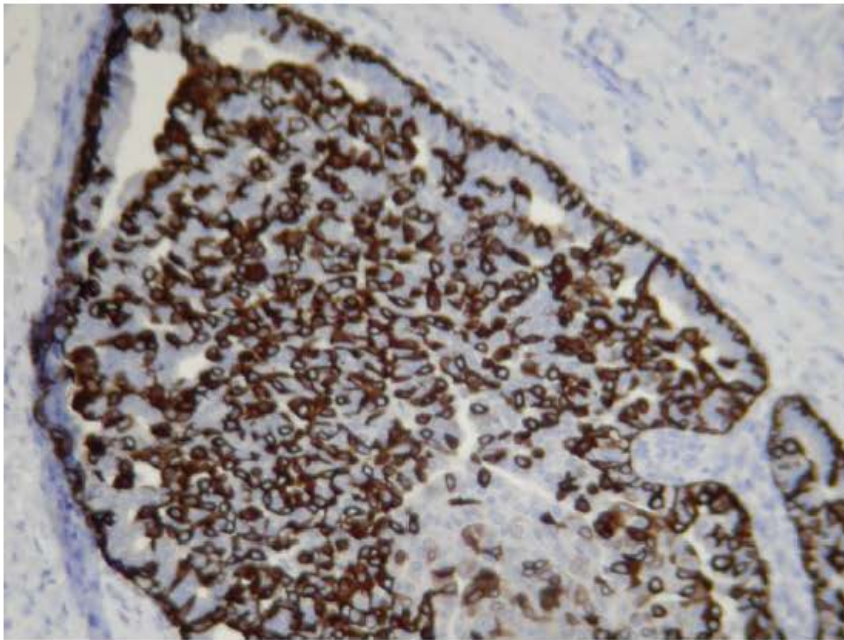
CK5: Breast lob. carcinoma



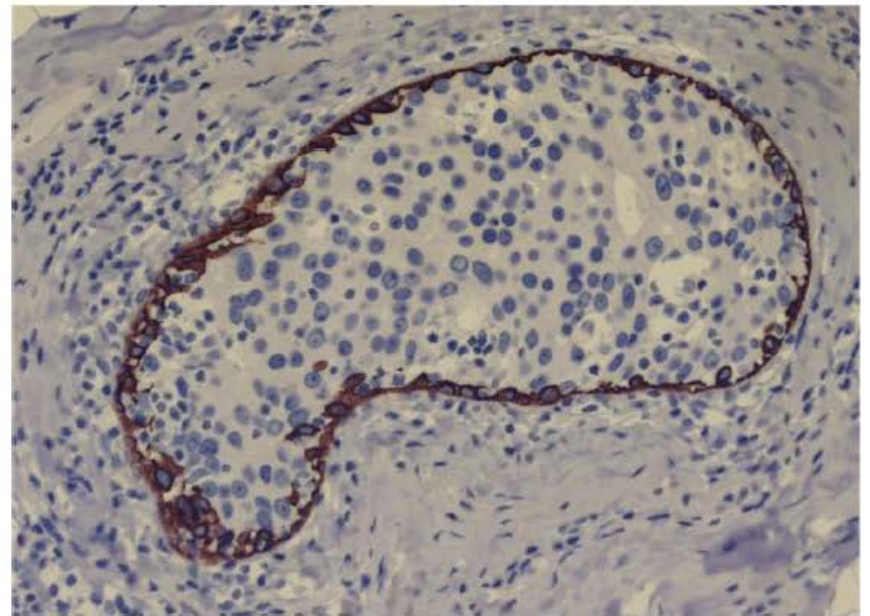
CK5: Breast duct. carc.

Ductal Carcinoma In Situ

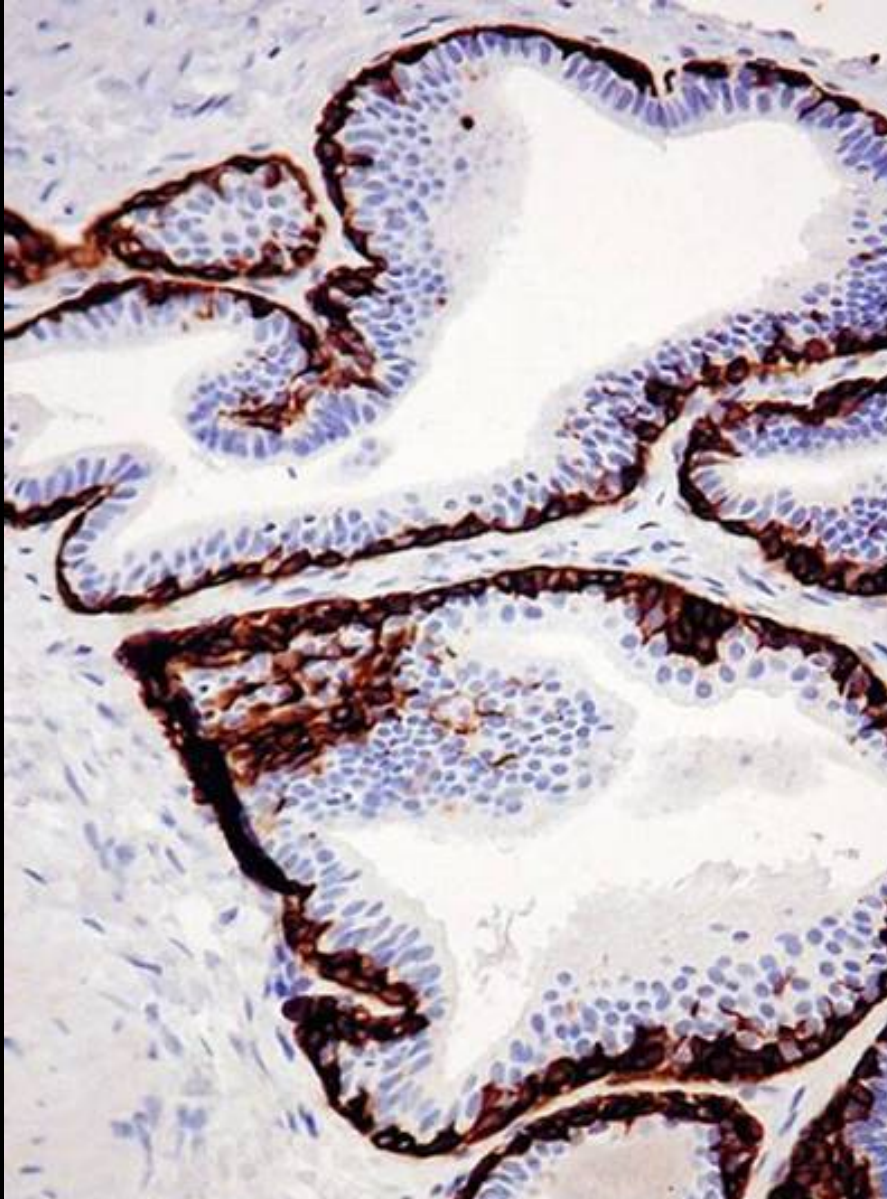
CK14 Ductal Hyperplasia (UDH)



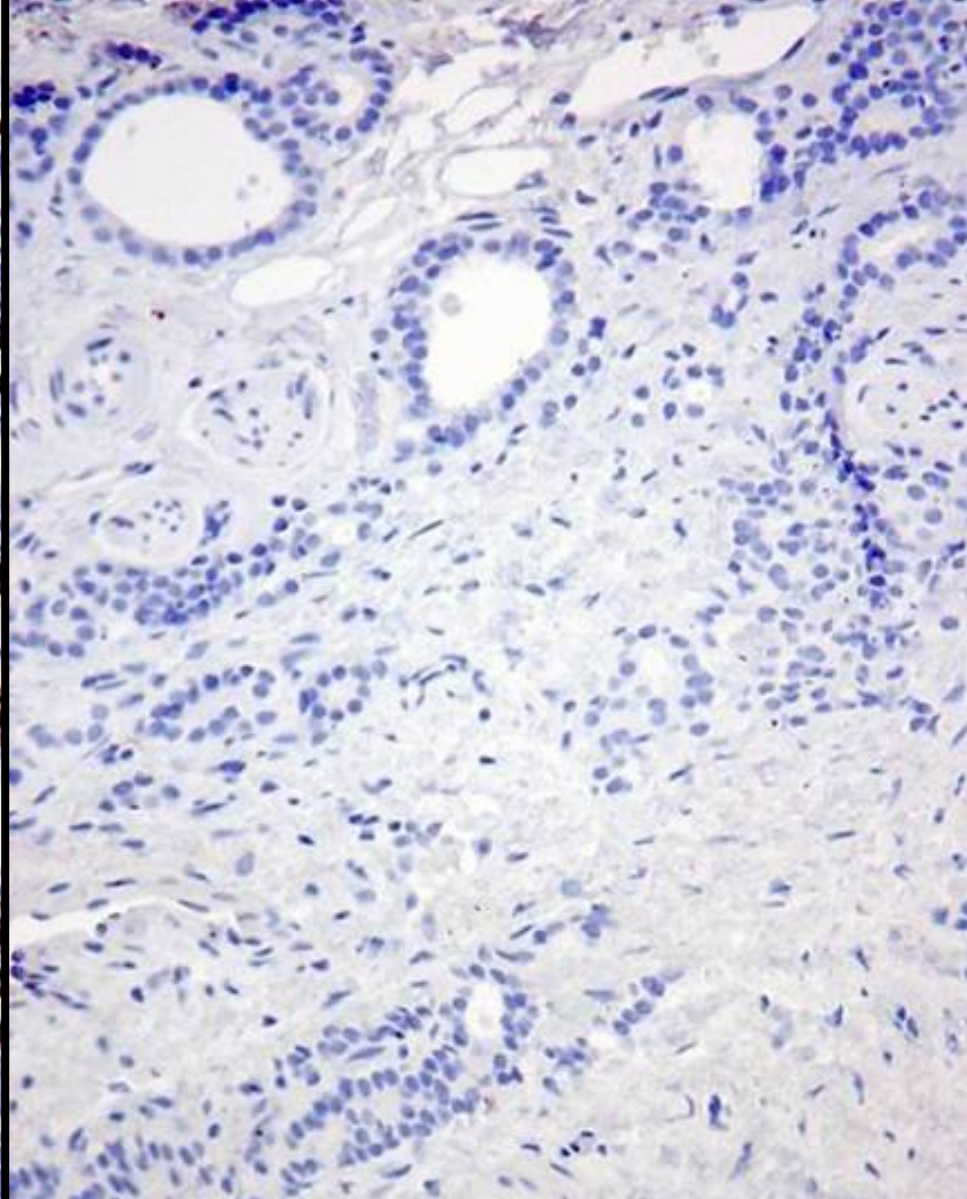
CK14 Ductal Carcinoma In Situ



Cytokeratins in adenocarcinomas



CK5: Prostate hyperplasia

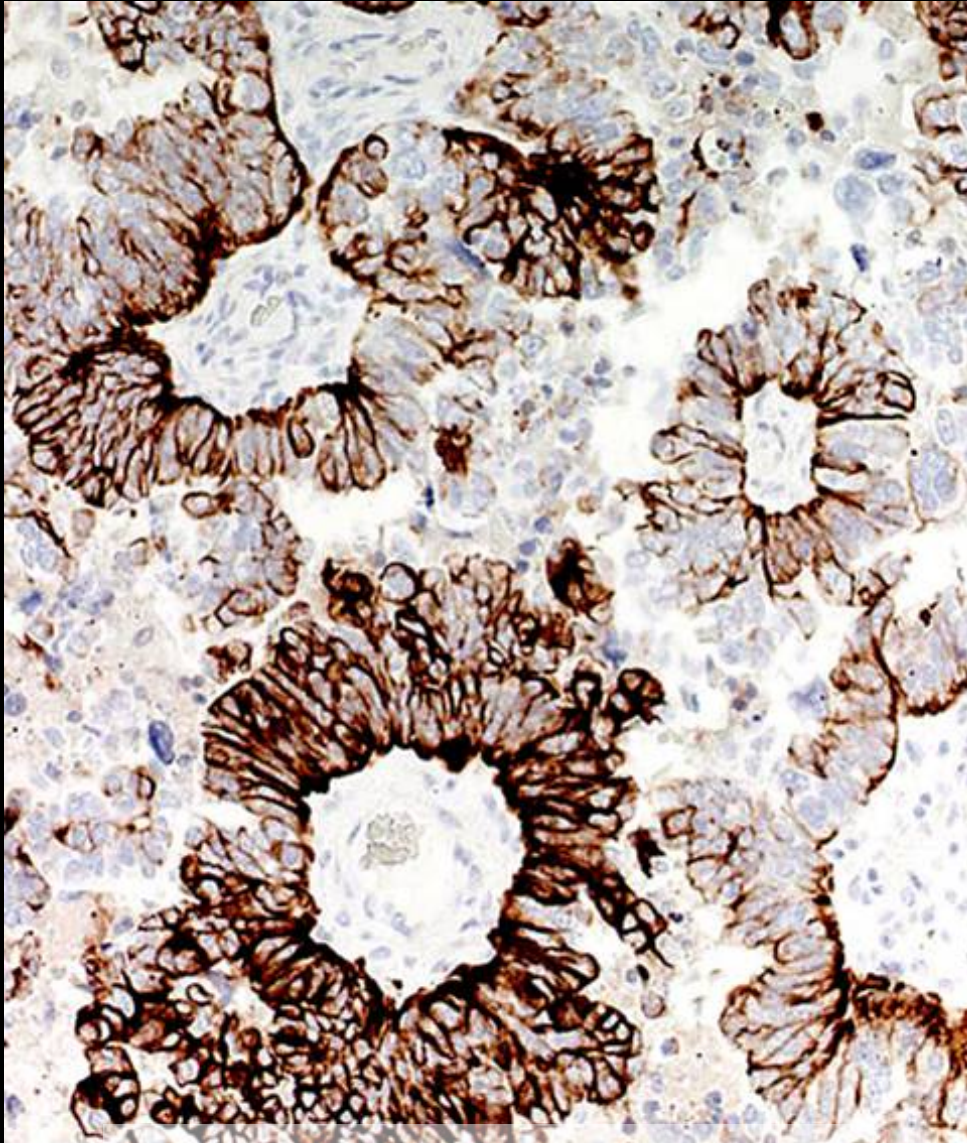


CK5: prostate adenocarc.

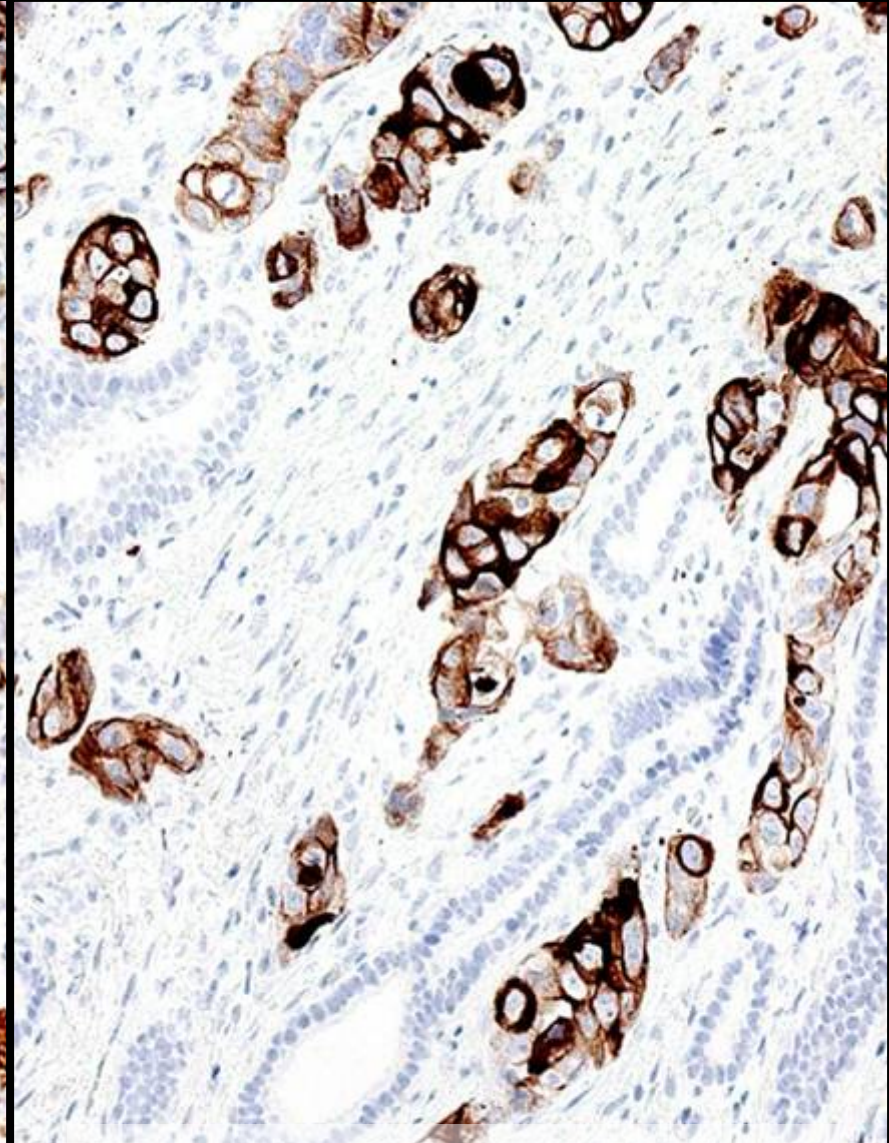
Cytokeratins in adenocarcinomas

Neutral/Basic (B, class II) Acidic (A, class I)	1 10	4	13	5	14	17	19	7	20	8 18
Squamous cell carcinoma	(+)	+	+	++	++	(+)	(+)	(+)	-	(+)
Transitional cell tumour	-	+	+	+	+	+	++	++	++	++
Malignant mesothelioma	-	-	-	++	++	+	++	+	-	++
Adenocarcinoma: complex epith. (lung, breast)	-	-	-	(+)	(+)	(+)	++	++	-	++
Adenocarc.: biliary tract, pancr., endom., ovary	-	-	-	(+) !	(+) !	(+) !	++	++	(+) !	++
Adenocarc.: stomach	-	-	-	-	-	-	++	+	+	++
Adenocarc.: intestine	-	-	-	-	-	-	++	+	++	++
Hepatocellular carcinoma	-	-	-	-	-	-	-	-	-	-
Renal cell carcinoma	-	-	-	-	-	-	-	+	-	++
Endocrine tumours: carcinoids	-	-	-	-	-	-	+	+	-	++
- Merkel cell carcinoma	-	-	-	-	-	-	+	-	++	++
- Thyroid carcinoma	-	-	-	-	-	-	+	++	-	++

Cytokeratins in adenocarcinomas



CK5: Serous ovarian carcinoma

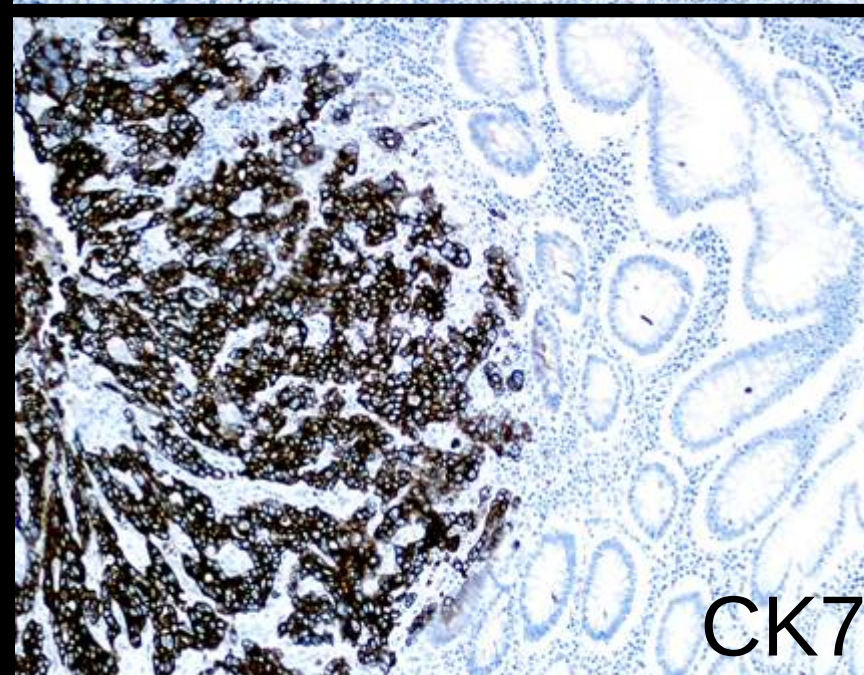
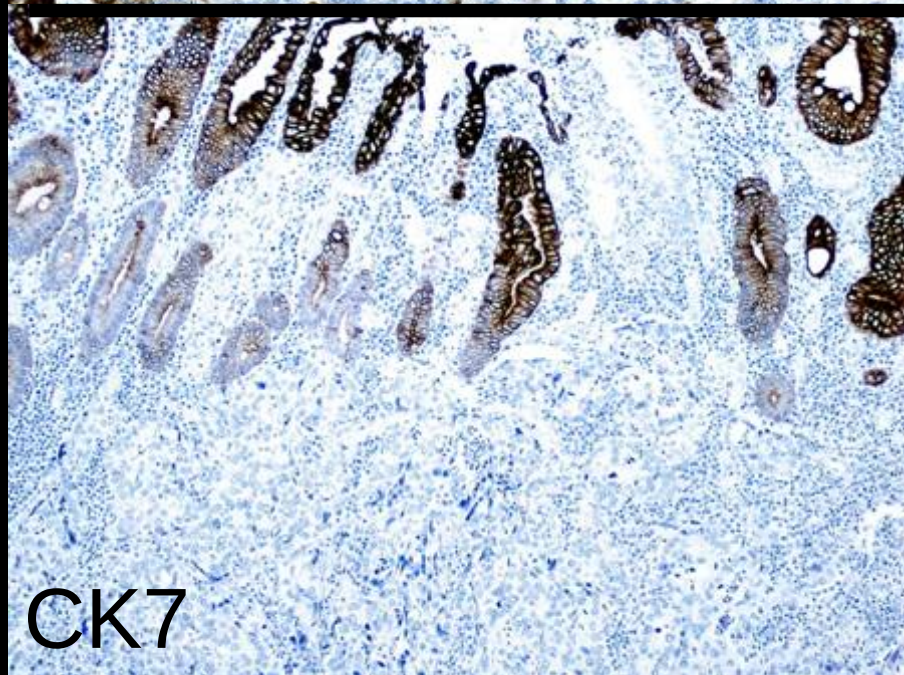
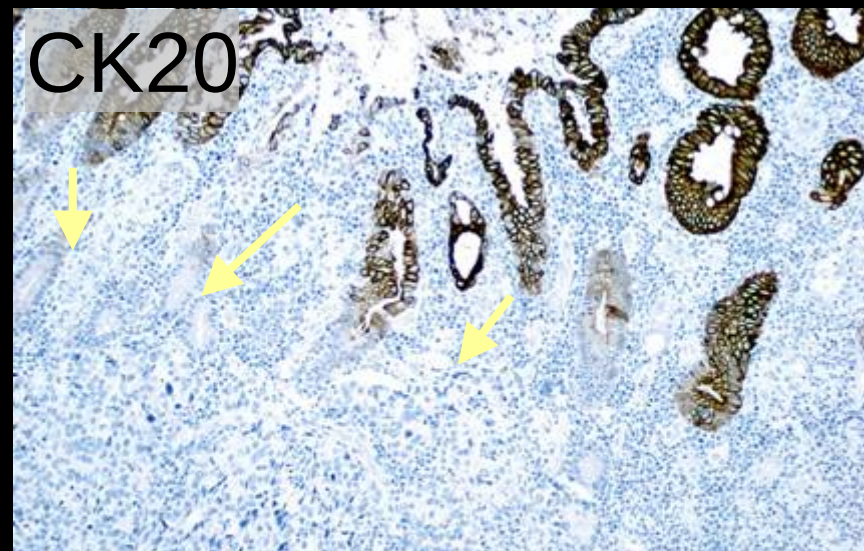
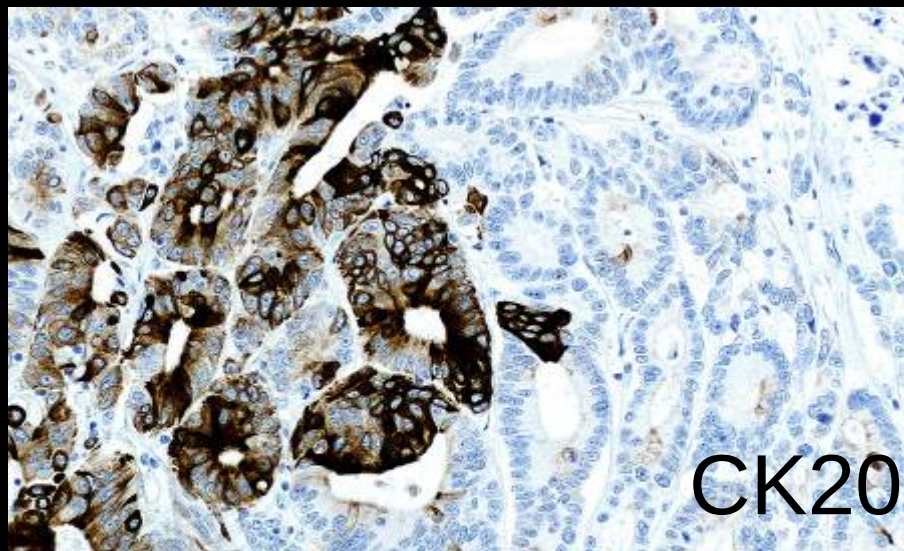


CK20: Gall bladder adenocarcinoma

Cytokeratins in adenocarcinomas

Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous cell carcinoma	(+)	+	+	++	++	(+)	(+)	(+)	-	(+)
Transitional cell tumour	-	+	+	+	+	+	++	++	++	++
Malignant mesothelioma	-	-	-	++	++	+	++	+	-	++
Adenocarcinoma: complex epith. (lung, breast)	-	-	-	(+)	(+)	(+)	++	++	-	++
Adenocarc.: biliary tract, pancr., endom., ovary	-	-	-	(+)	(+)	(+)	++	++	(+)	++
Adenocarc.: stomach	-	-	-	-	-	-	++	+	+	++
Adenocarc.: intestine	-	-	-	-	-	-	++	+ !	++	++
Hepatocellular carcinoma	-	-	-	-	-	-	-	+	-	++
Renal cell carcinoma	-	-	-	-	-	-	-	+	-	++
Endocrine tumours: carcinoids	-	-	-	-	-	-	+	+	-	++
- Merkel cell carcinoma	-	-	-	-	-	-	+	-	++	++
- Thyroid carcinoma	-	-	-	-	-	-	+	++	-	++

Cytokeratins in adenocarcinomas



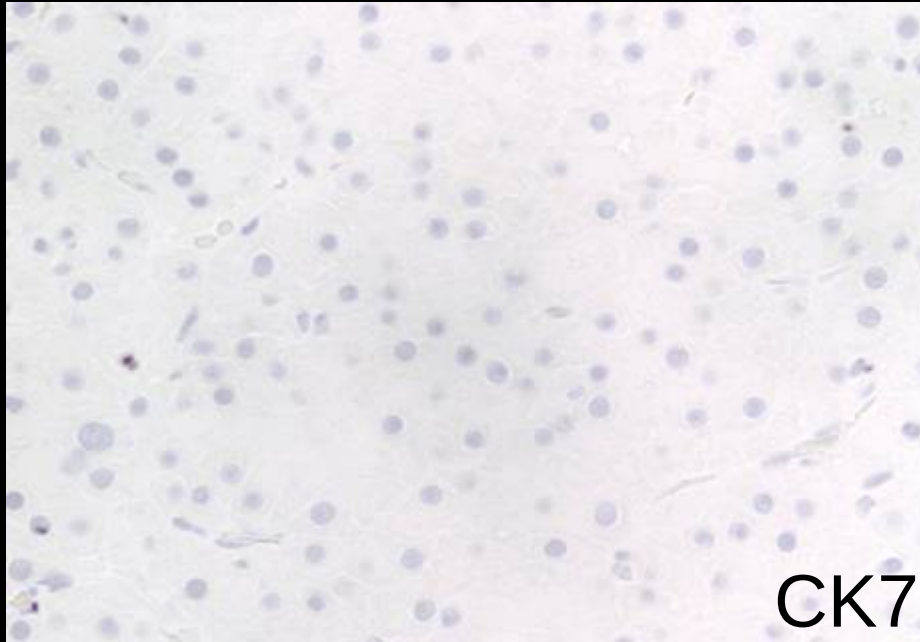
Colon: Typical

Colon: Deviant

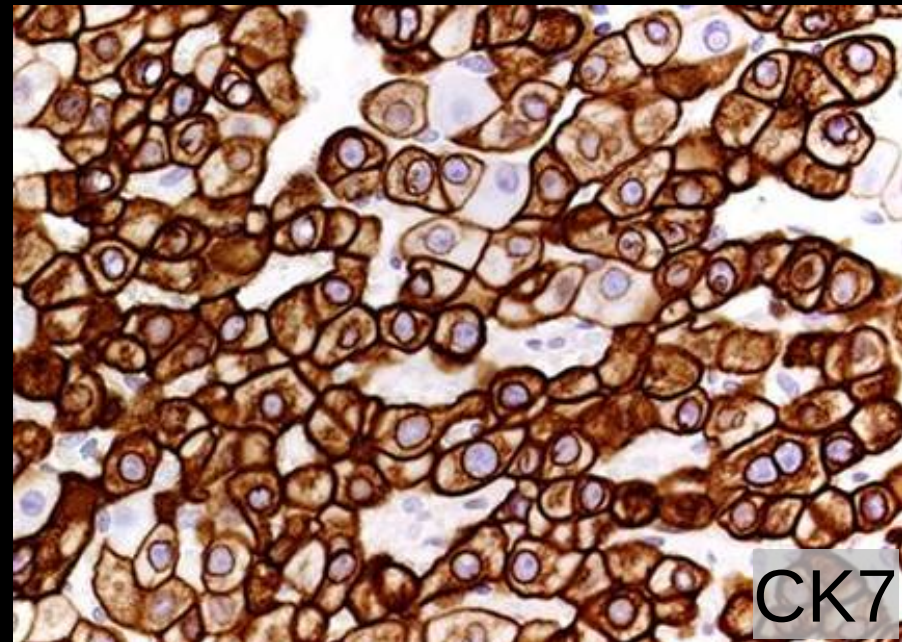
Cytokeratins in adenocarcinomas

Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous cell carcinoma	(+)	+	+	++	++	(+)	(+)	(+)	-	(+)
Transitional cell tumour	-	+	+	+	+	+	++	++	++	++
Malignant mesothelioma	-	-	-	++	++	+	++	+	-	++
Adenocarcinoma: complex epith. (lung, breast)	-	-	-	(+)	(+)	(+)	++	++	-	++
Adenocarc.: biliary tract, pancr., endom., ovary	-	-	-	(+)	(+)	(+)	++	++	(+)	++
Adenocarc.: stomach	-	-	-	-	-	-	++	+	+	++
Adenocarc.: intestine	-	-	-	-	-	-	++	+	++	++
Hepatocellular carcinoma										
Renal cell carcinoma	-	-	-	-	-	-	-	+	-	++
Endocrine tumours: carcinoids	-	-	-	-	-	-	+	+	-	++
- Merkel cell carcinoma	-	-	-	-	-	-	+	-	++	++
- Thyroid carcinoma	-	-	-	-	-	-	+	++	-	++

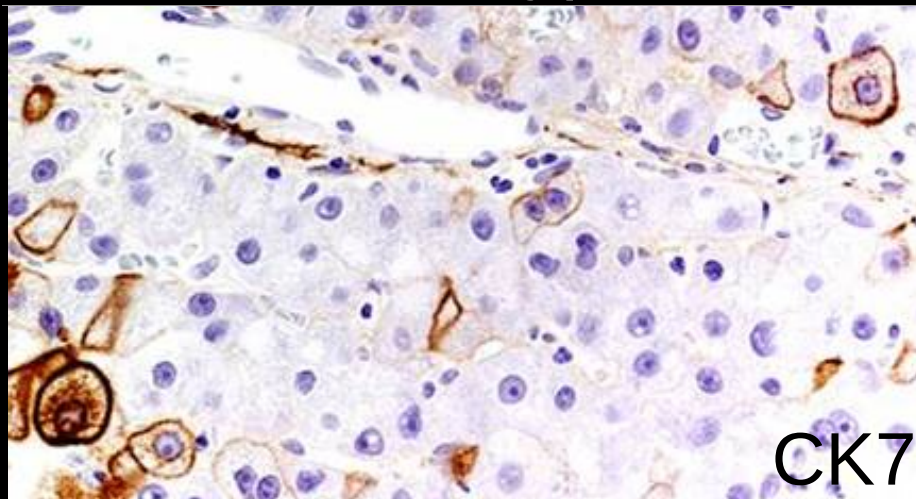
Cytokeratins in adenocarcinomas



HCC: typical



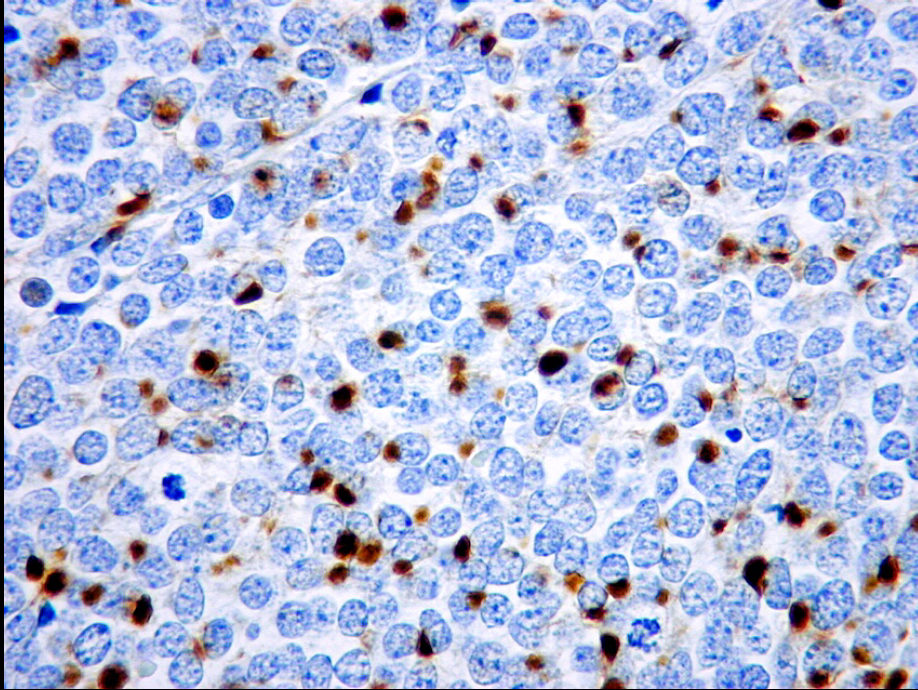
HCC: deviant



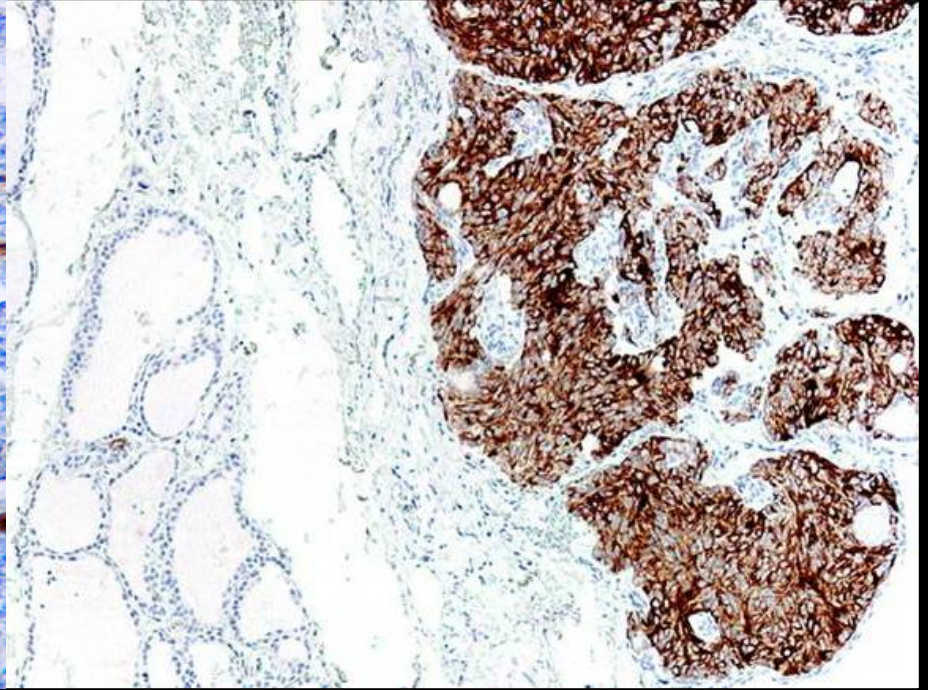
Cytokeratins in endocrine tumours

Neutral/Basic (B, class II)	1	4	13	5	14	17	19	7	20	8
Acidic (A, class I)	10									18
Squamous cell carcinoma	(+)	+	+	++	++	(+)	(+)	(+)	-	(+)
Transitional cell tumour	-	+	+	+	+	+	++	++	++	++
Malignant mesothelioma	-	-	-	++	++	+	++	+	-	++
Adenocarcinoma: complex epith. (lung, breast)	-	-	-	(+)	(+)	(+)	++	++	-	++
Adenocarc.: biliary tract, pancr., endom., ovary	-	-	-	(+)	(+)	(+)	++	++	(+)	++
Adenocarc.: stomach	-	-	-	-	-	-	++	+	+	++
Adenocarc.: intestine	-	-	-	-	-	-	++	+	++	++
Hepatocellular carcinoma	-	-	-	-	-	-	-	+	-	++
Renal cell carcinoma	-	-	-	-	-	-	-	+	-	++
Endocr. tumours: carcinoids	-	-	-	-	-	-	+	+	-	++
- Merkel cell carcinoma	-	-	-	-	-	-	+	-	++	++
- Thyroid carcinoma	-	-	-	-	-	-	+	++	-	++

Cytokeratins in endocrine tumours



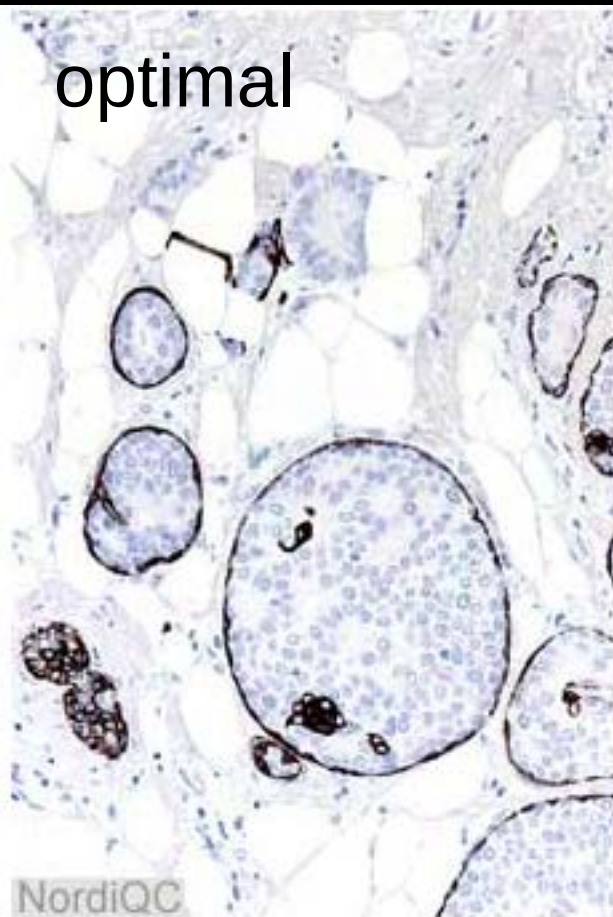
CK20: Merkel cell carc.



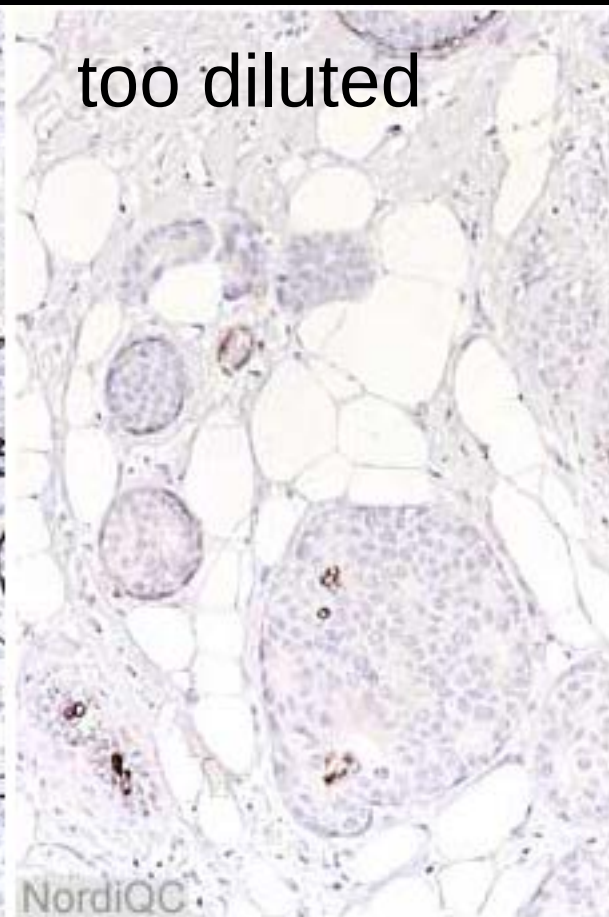
CK19: Thy. papill. carc.

Cytokeratins: Selection of antibodies

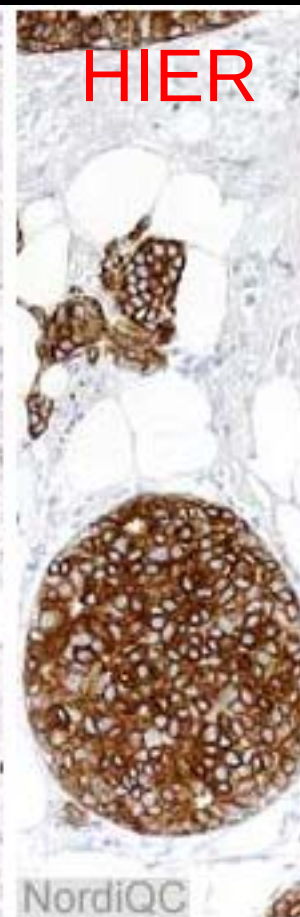
optimal



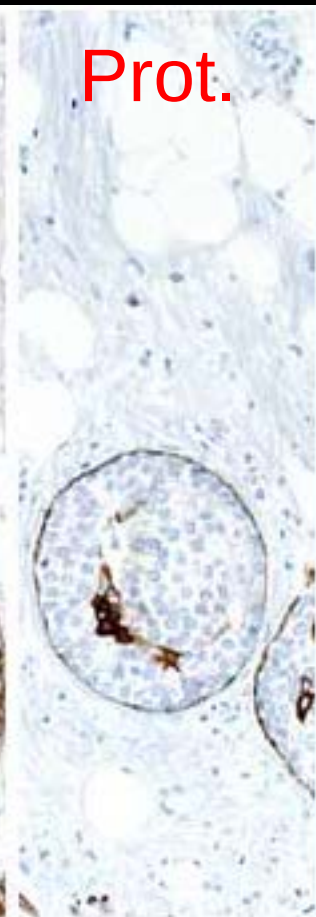
too diluted



HIER



Prot.



CK5: XM-26

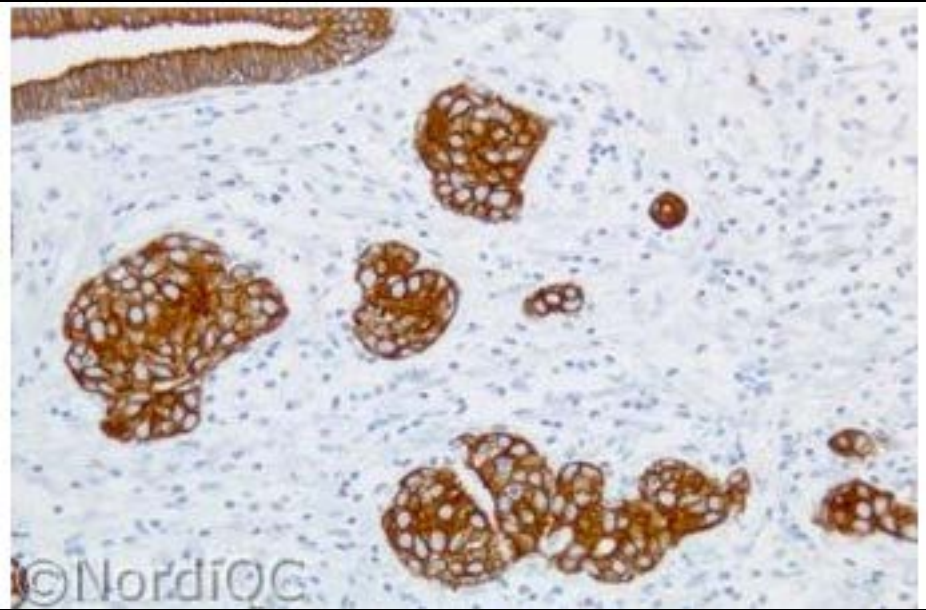
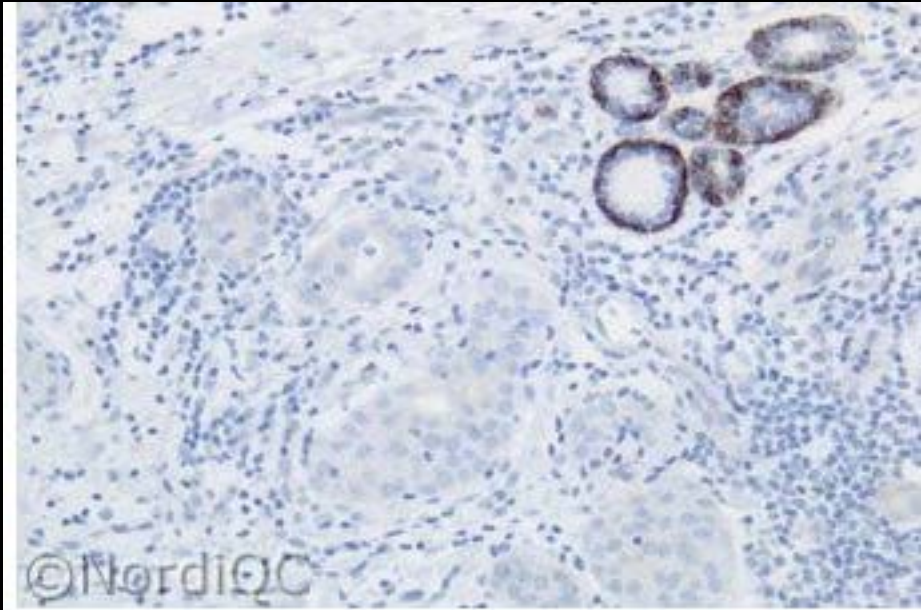
CK5/6: clone D5/16 B4



34BE12:

CK5/14/1/10/19?

Cytokeratins: Selection of antibodies



CK5: XM-26

CK5/6: clone D5/16 B4

34BE12:

CK5/14/1/10/19?





RESEARCH ARTICLE

(Appl Immunohistochem Mol Morphol 2017;25:673–678)

NordiQC Assessments of Low Molecular Weight Keratin 8/18 Immunoassays

Mogens Vyberg, MD,† Charlotte Diernæs, MD,* Rasmus Røge, MD,*† and Søren Nielsen, HT**

ISIMM-NordiQC Immunoassay Performance Assessment Series, No. 2

Clone Concentrates	Epitope	No.	% suff.
rmAb EP17 (/EP30)	K8(/K18)	17	100
mmAbs B22.1/B23.1	K8/18	12	84
mmAb DC10	K18	93	83
mmAb TS1	K8	16	75
mmAb 5D3	K8/18	88	55
mmAb CAM5.2	K8/7	59	44
Clone RTUs			
rmAbs EP17/EP30	K8/18	16	100
mmAbs B22-1/B23.1	K8/18	59	83
mmAb DC10	K18	36	83
mmAb 5D3	K8/18	16	70
mmAb CAM5.2	K8/7	35	31
mmAb 35betaH11	K8	15	5



Breast dc

Renal ccc



K8: EP17 (Epitomics)

K8/18: EP17/EP30 (Agilent)

K8/18: B22.1/B23.1 (Roche, CM)

A1

A2

A3

B1

B2

B3

C1

C2

C3

D1

D2

D3

K18: DC10 (several)

K8(7): CAM5.2 (several)

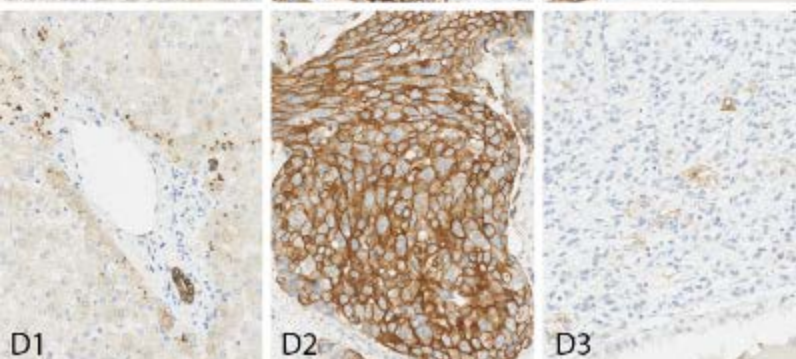
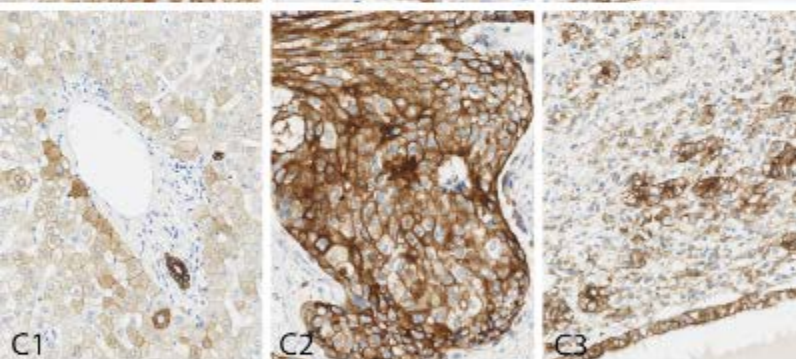
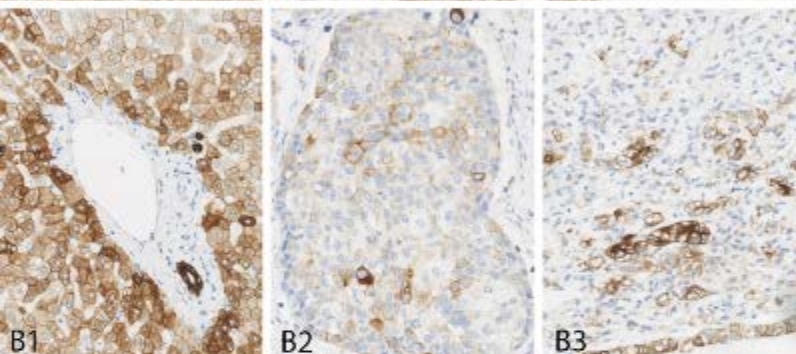
K8: 35 β H11 (Roche)





Breast dc

Renal ccc



K8: EP17 (Epitomics)

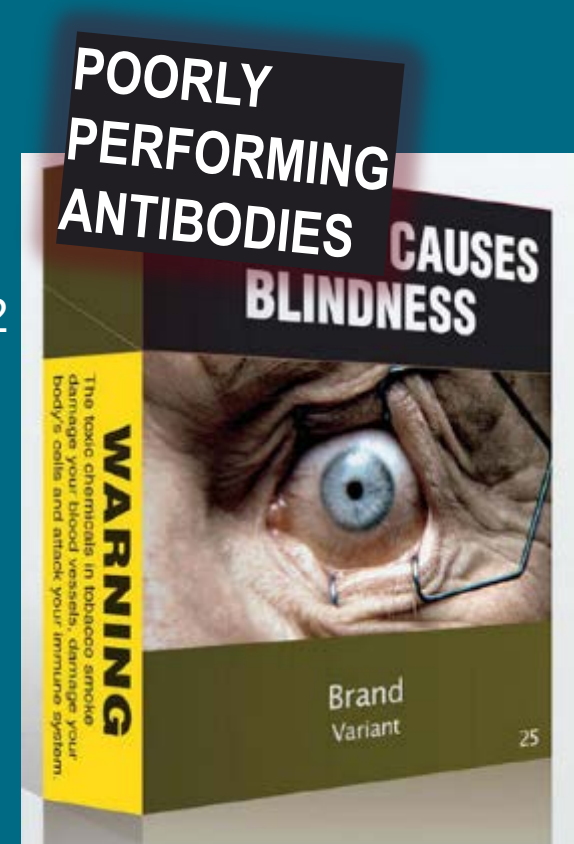
K8/18: EP17/EP30 (Agilent)

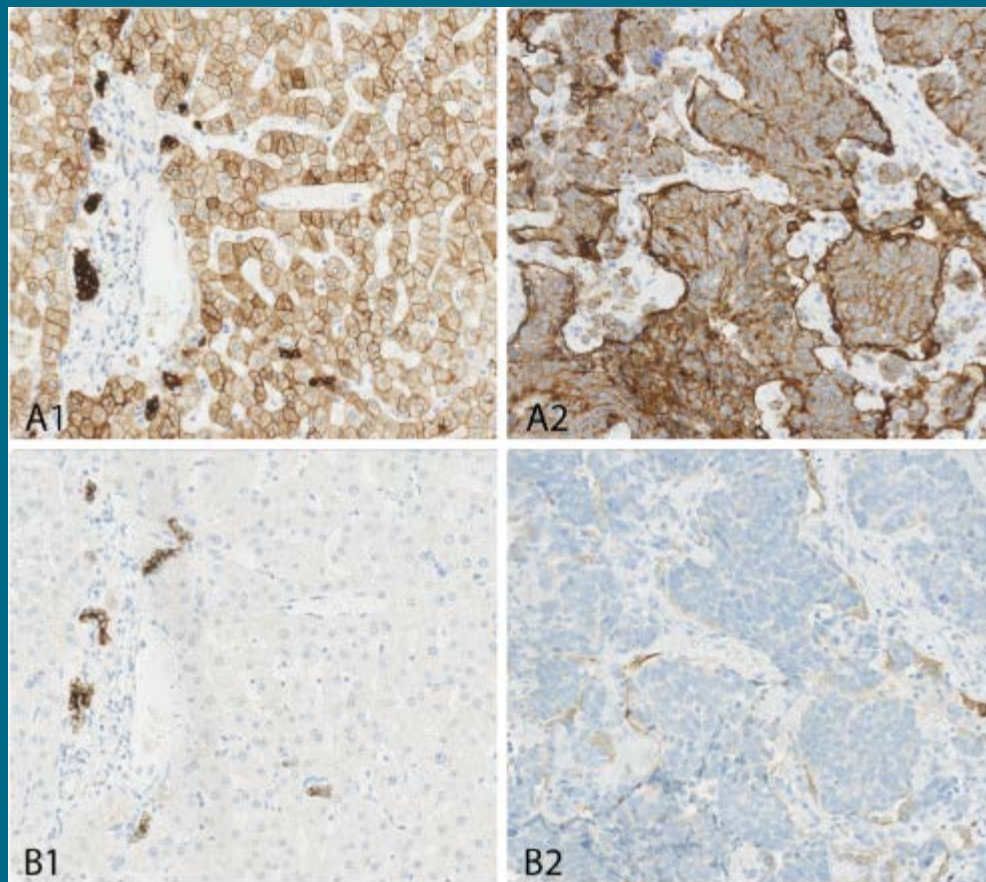
K8/18: B22.1/B23.1 (Roche, CM)

K18: DC10 (several)

K8(7): CAM5.2

K8: 35 β H11

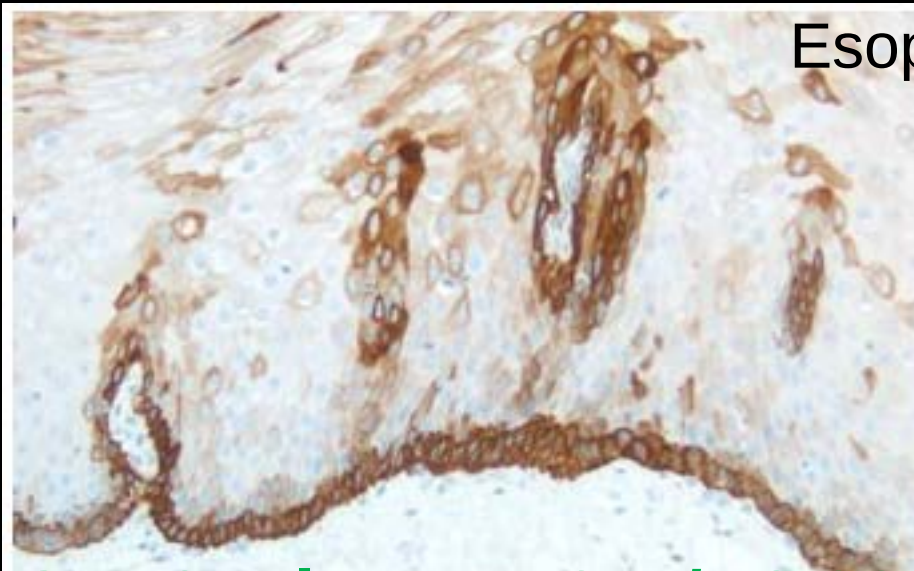




Comparison of **clone 5D3** used with optimized protocols on two platforms: **Leica Bond (A)** and **Ventana BenchMark (B)** to detect LMW Ks in serial sections of **liver (1)** and **breast ductal carcinoma (2)**.

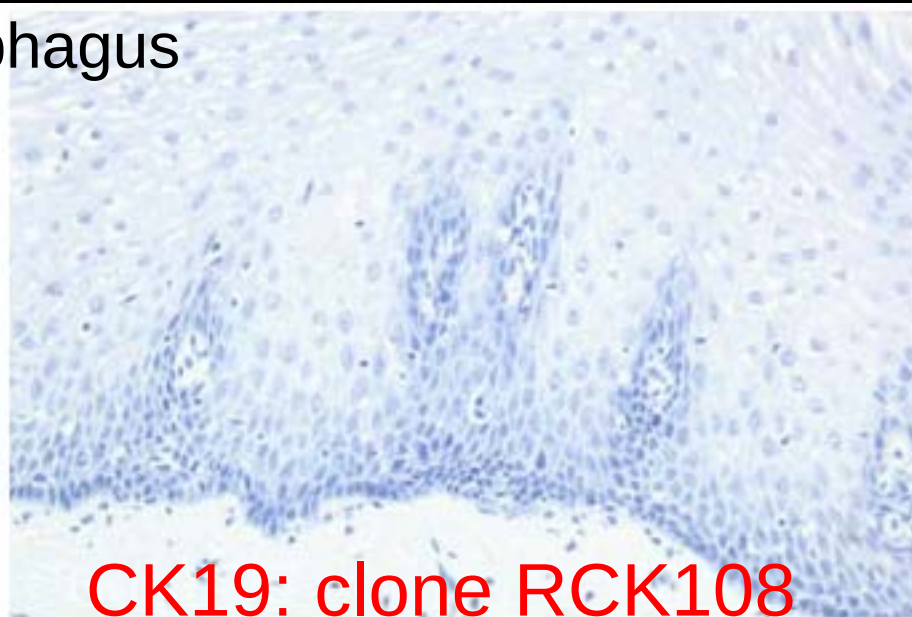
Clone 5D3 gives strong reactions on Leica Bond but consistently **poor** reactions when used on **BenchMark**.

Cytokeratins: Selection of antibodies

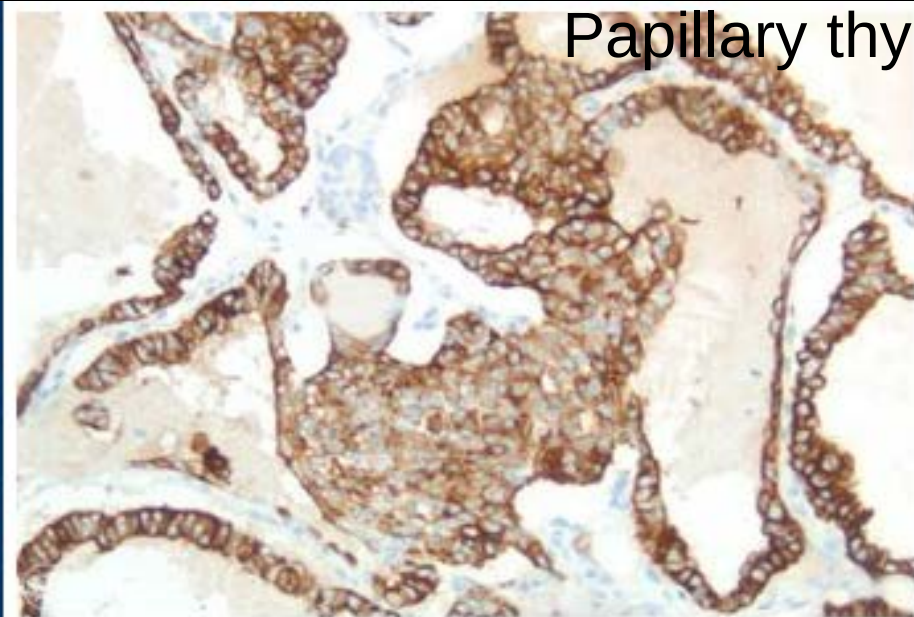


Esophagus

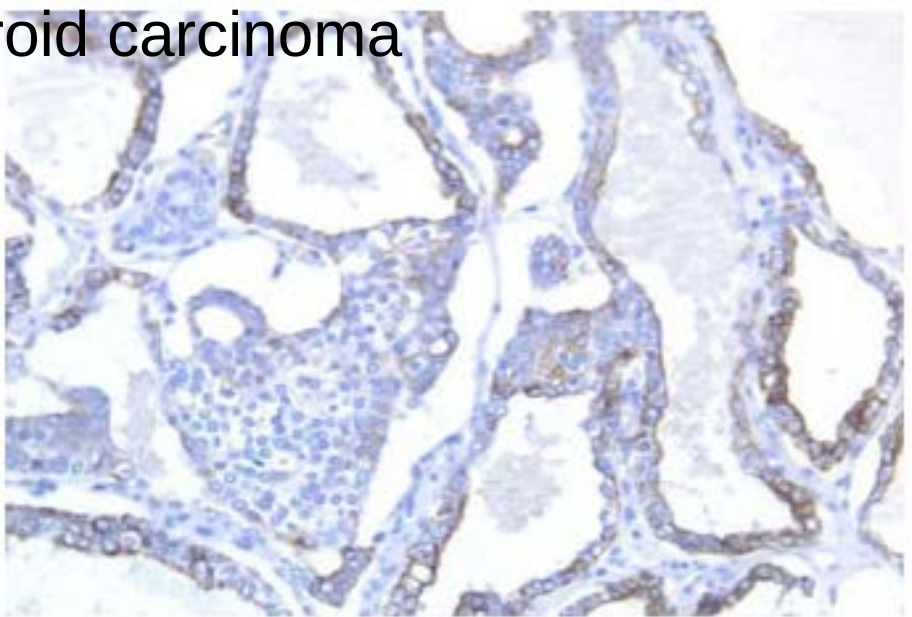
CK19: clone A53-B/A2.26



CK19: clone RCK108



Papillary thyroid carcinoma



Secondary panels for '**carcinoma**' identification/subclassification

- Apart from cytokeratins and
"general epithelial markers" (EpCAM, Cl4)

- "GI-markers" CDX2, CAD17, SATB2,
SMAD4, CEA
- "Fem.gen.tract markers" CA125, PAX8, WT1, ER, p53
- "Liver cell markers" Arginase, Glypican3, GlutSynt,
canCD66a, canCD10, AFP
- Neuroendocr. markers Synaptophysin, Chromogranin
- "Breast markers" GATA3, ER, GCDFP15, Mam.glob.

Secondary panels for **carcinoma** identification/subclassification

- "Lung markers" TTF-1, Napsin, p40
- "Mesothelioma makers" Calretinin, Popoplanin, WT1, CA125
- "Adrenal cortic.markers" Inhibin, Melan A, Synaptophysin
- Germinal cell markers SALL4, OCT3/4, PLAP, AFP, CD117, CD30
- Prostate markers NKX3.1, PSA, Prostein, AMACR, ERG
- Urinary tract markers Uroplakin II, GATA3, (CK5, CK20)

“GI markers”

CDX2

CEA

CAD17

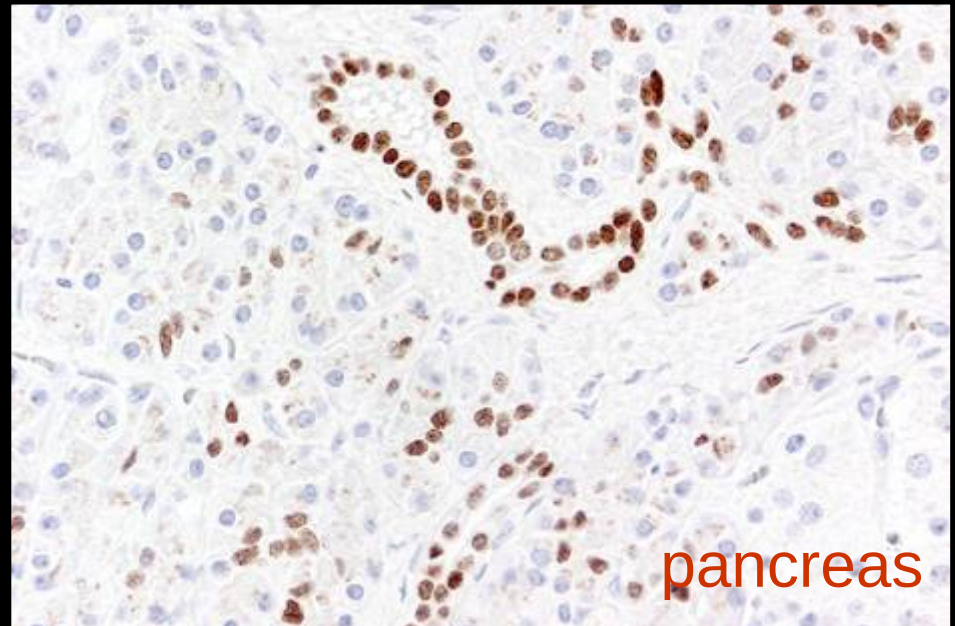
SATB2

SMAD4

CDX-2 protein

Drosophila caudal related
homeobox gene 2 product:
Nuclear transcription factor
for intestinal differentiation

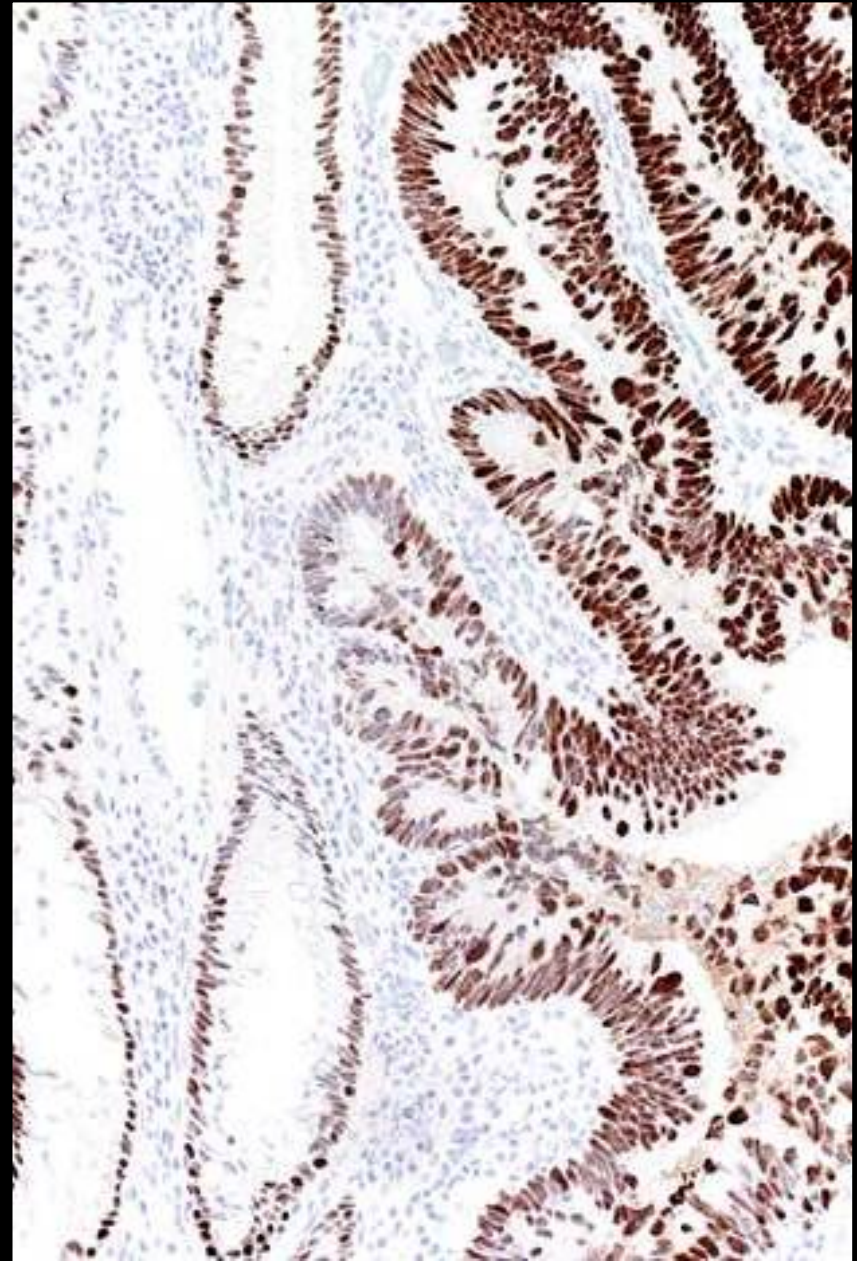
- Intestine
 - all cell types incl. endocrine
- Intestinal metaplasia
 - chronic gastritis
 - Barrett's esophagus
- Pancreas/bil.tract
 - heterogenous



CDX-2 protein in adenocarcinoma

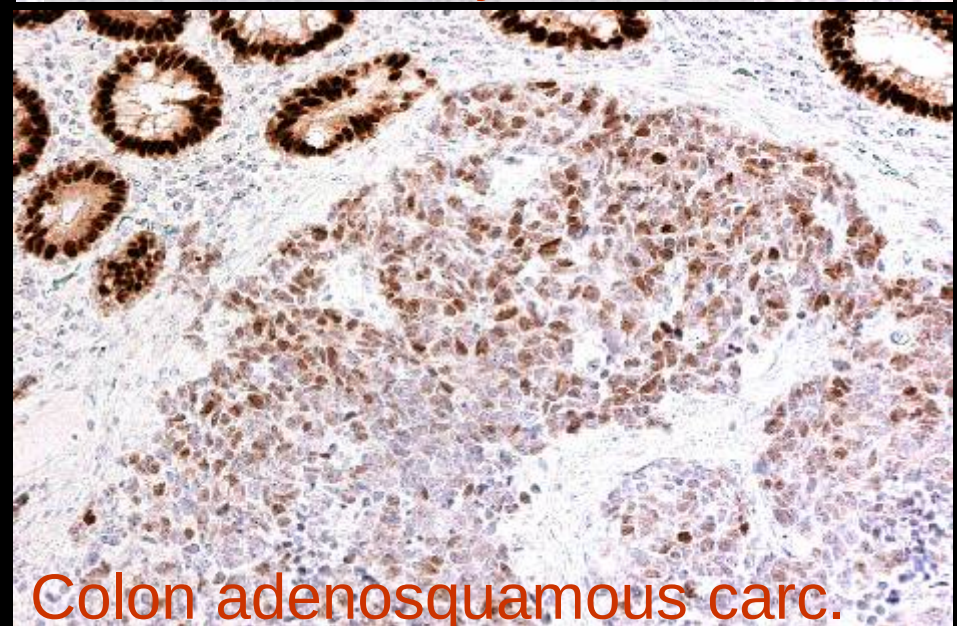
- Colorectum +(-)
- Mucinous ovar. +(-)
- Yolk sac tumour +
- Esoph./Stom. +/-
- Pancr./biliary -/+
- Lung -/+
- Prostate -(+)
- Urothelial -(+)
- Endometrioid -(+)

Colon adenocarcinoma



CDX-2 protein in adenocarcinoma

- Colorectum +(-)
- Mucinous ovar. +(-)
- Yolk sac tumour +
- Esoph./Stom. +/-
- Pancr./biliary -/+
- Lung -/+
- Prostate -(+)
- Urothelial -(+)
- Endometrioid -(+)



Endometrioid carcinoma: ER & CDX-2

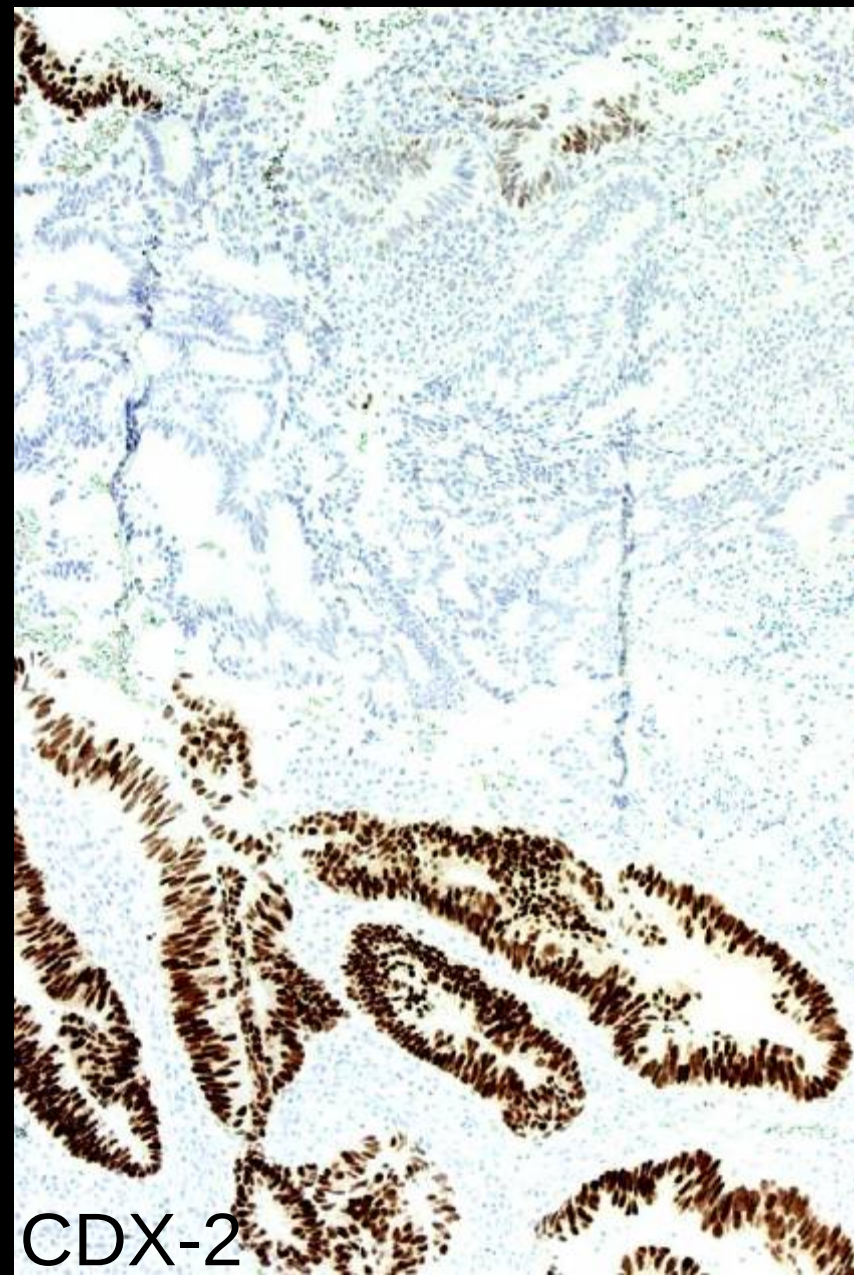
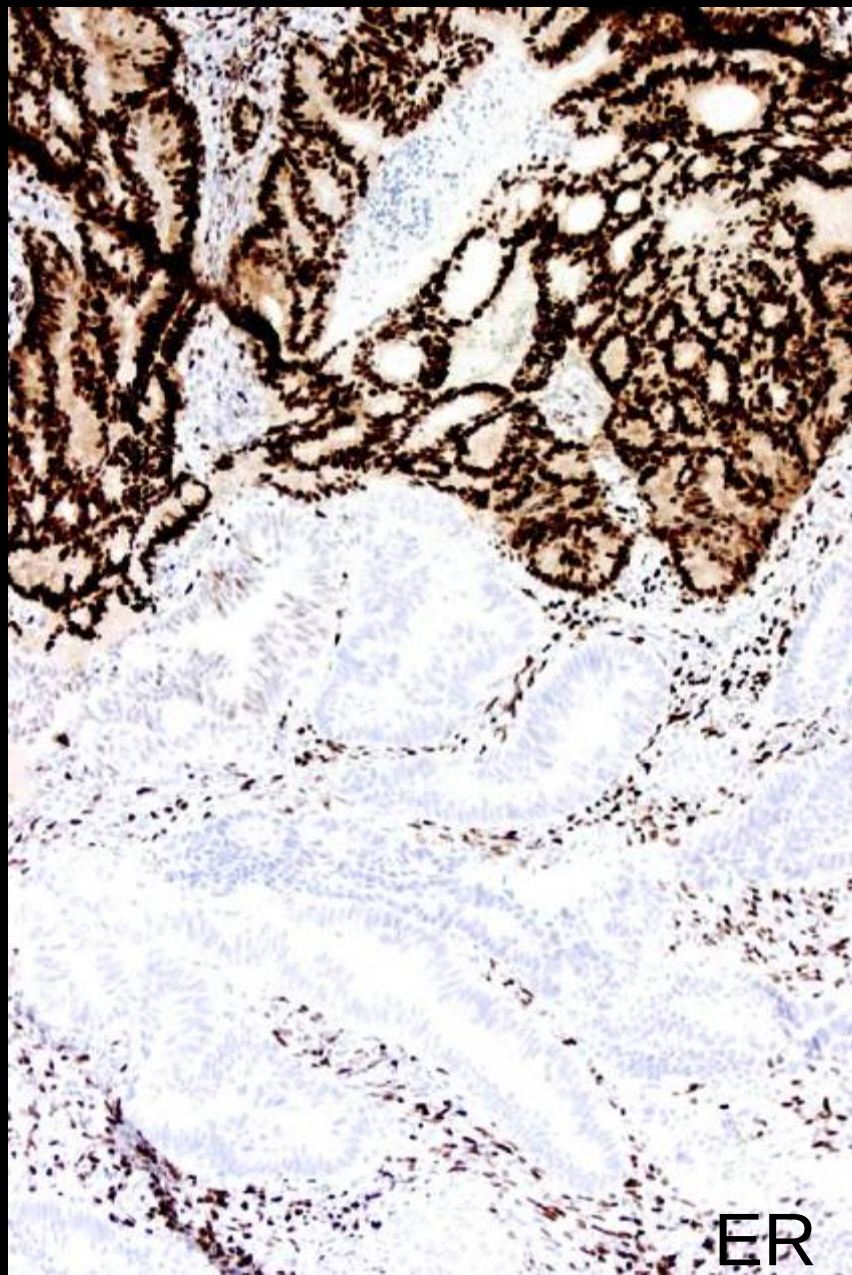


Table 1. Antibodies and assessment marks for CDX2, run 48

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone AMT28	2	Leica/Novocastra	0	0	0	2	-	-
mAb clone CDX2-88	2	Biocare	0	0	1	3	-	-
	2	Biogenex						
mAb clone DAK-CDX2	31	Agilent/Dako	6	9	7	9	48%	57%
rmAb clone EPR2764Y	31	Cell Marque	28	14	7	3	81%	81%
	5	Thermo/Neomarkers						
	4	Immunologic						
	4	Zytomed						
	2	Monosan						
	2	Zeta Corporation						
	1	A.Menarini						
	1	Abcam						
	1	Nordic Biosite						
	1	Thermo/Pierce						

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

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

Demonstration of CDX2 is Highly Antibody Dependant

Martine Borrisholt, MS, Søren Nielsen, HT, and Mogens Vyberg, MD

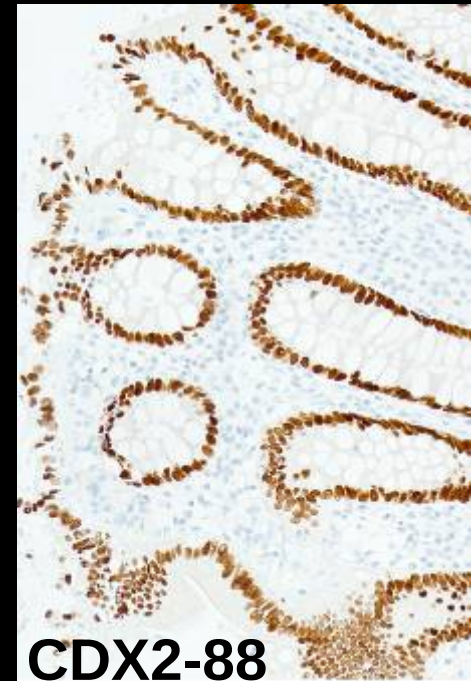
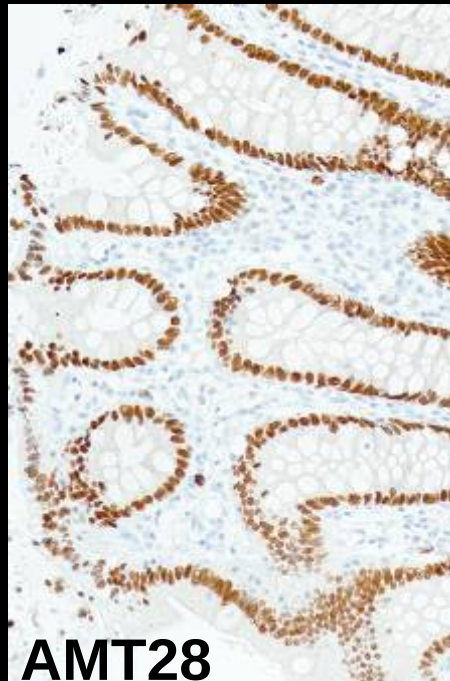
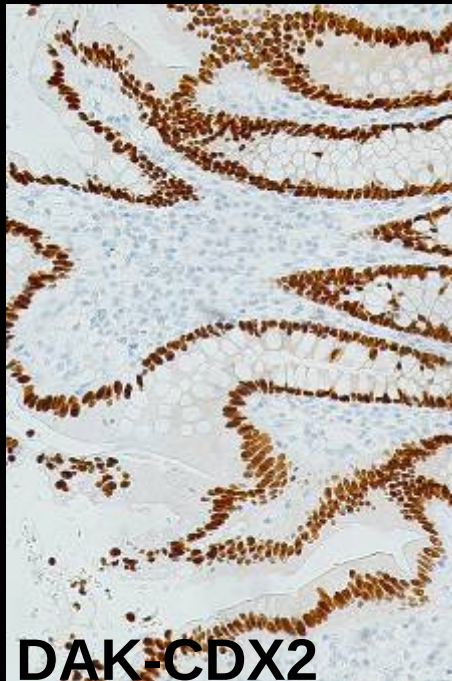
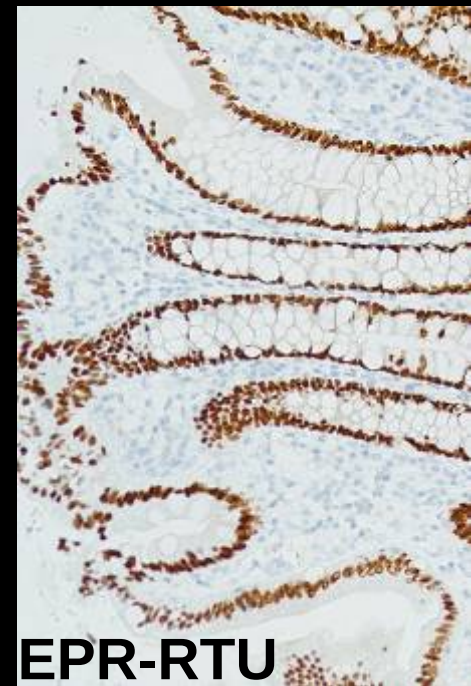
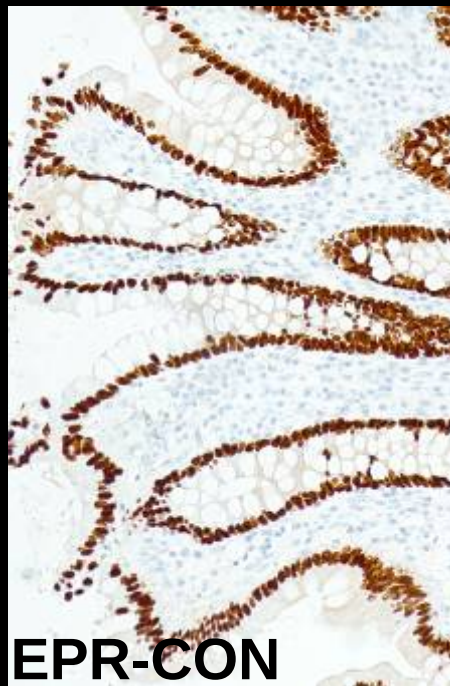
(Appl Immunohistochem Mol Morphol 2013;21:64–72)

CDX2

Normal
colon 1

Optimized
protocols

EPR =
EPR2764Y



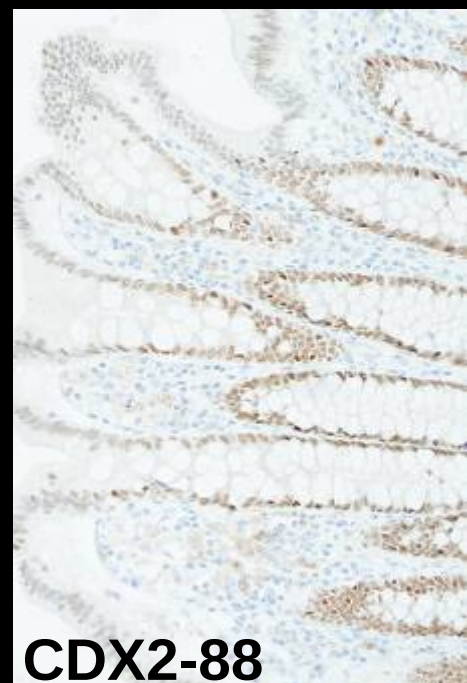
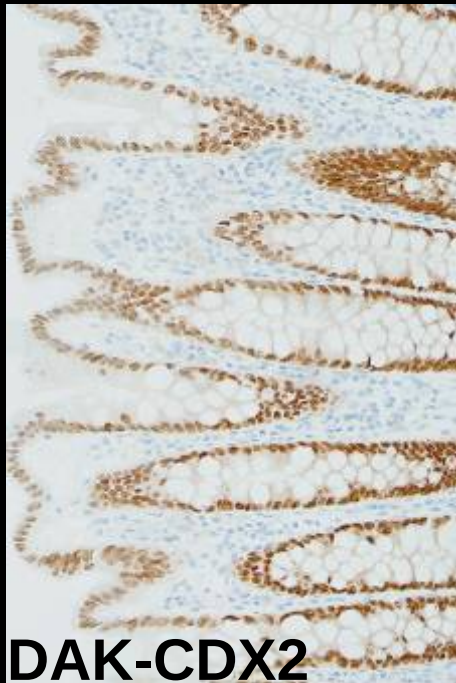
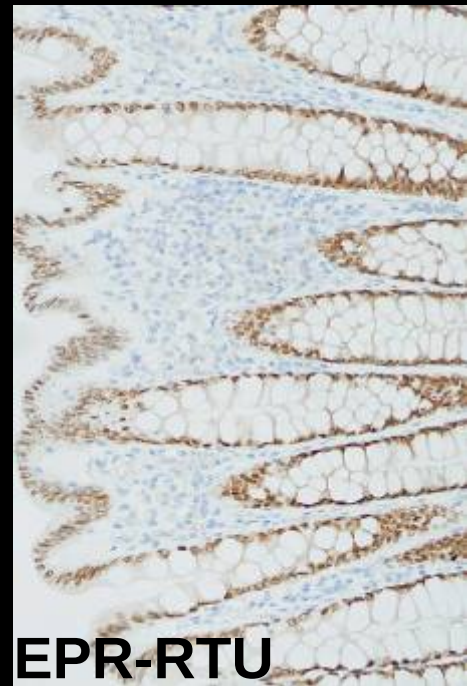
CDX2

Normal

colon 2

Optimized
protocols

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EPR2764Y

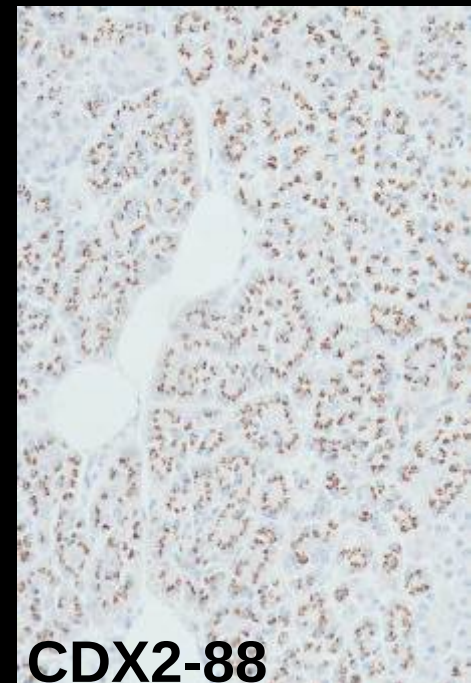
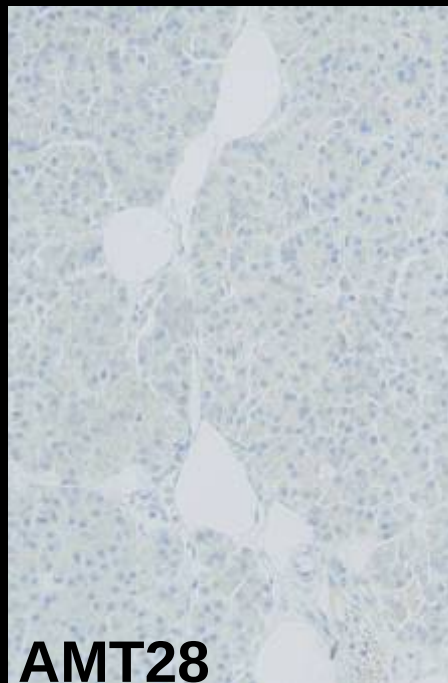
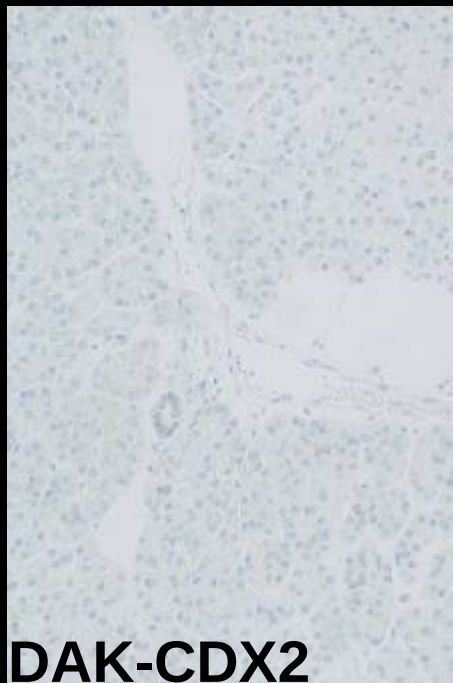
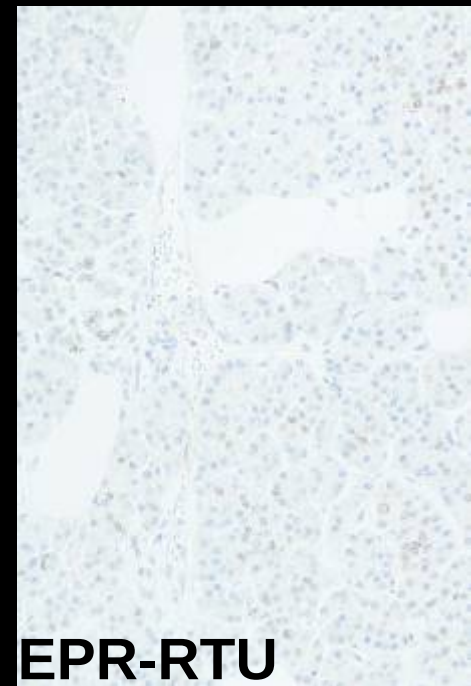
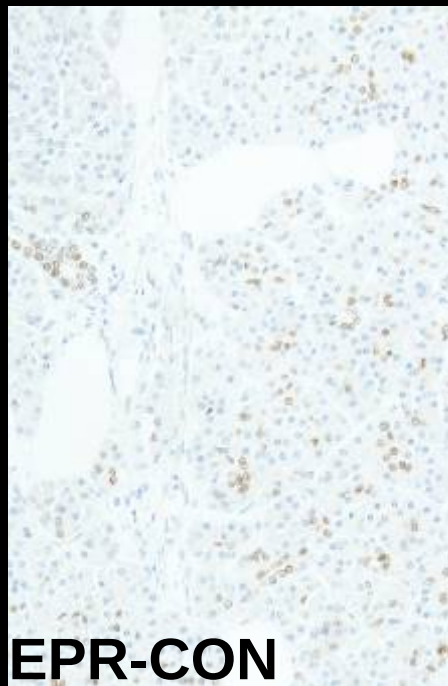


CDX2

Normal
pancreas

Optimized
protocols

EPR =
EPR2764Y



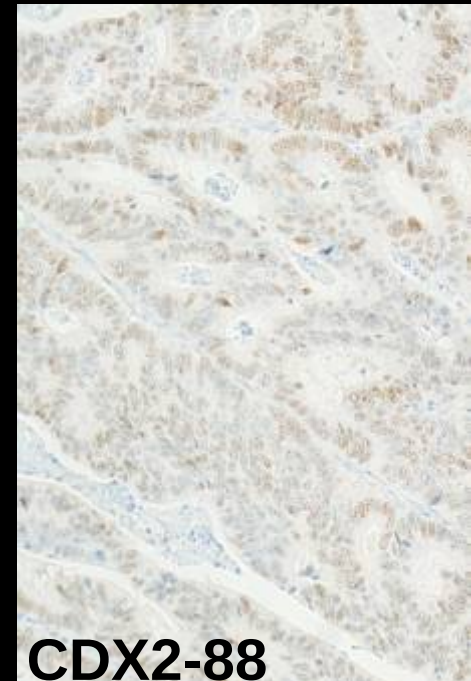
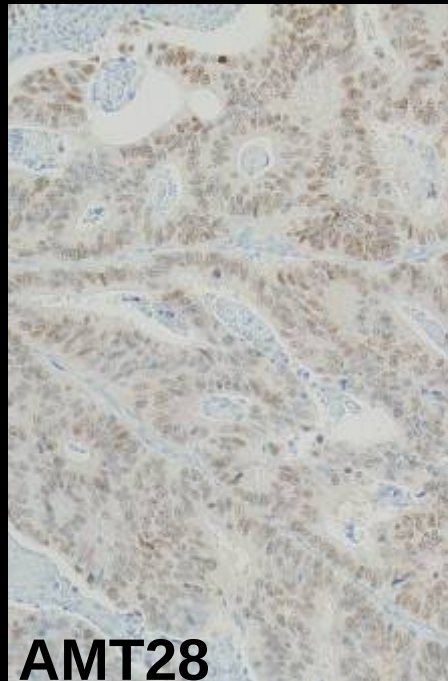
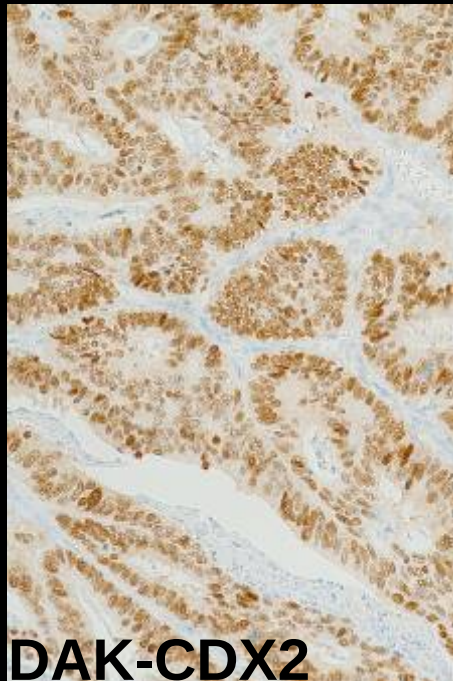
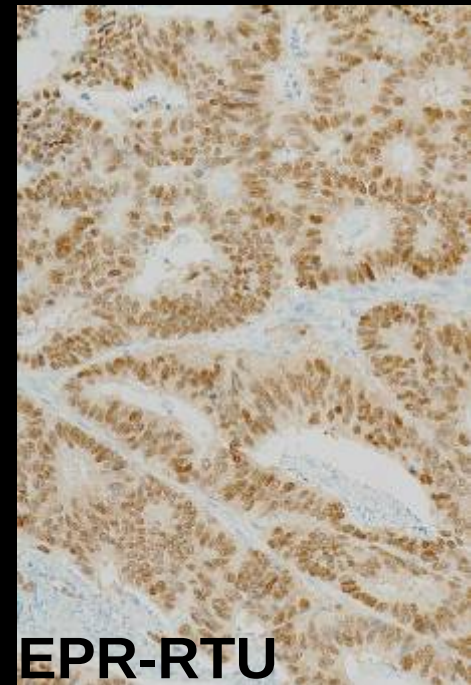
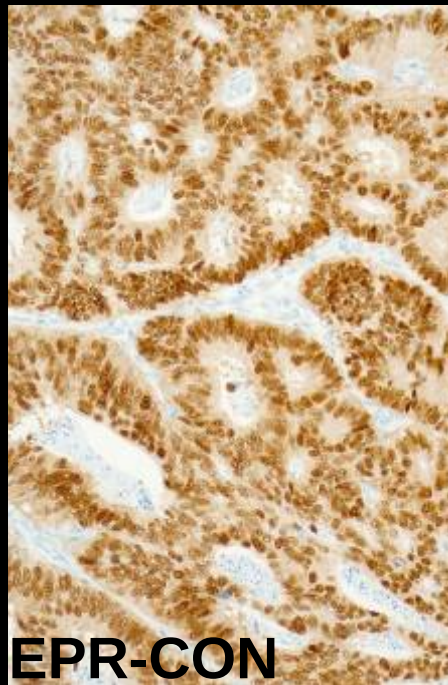
CDX2

Colon

adenocarc.1

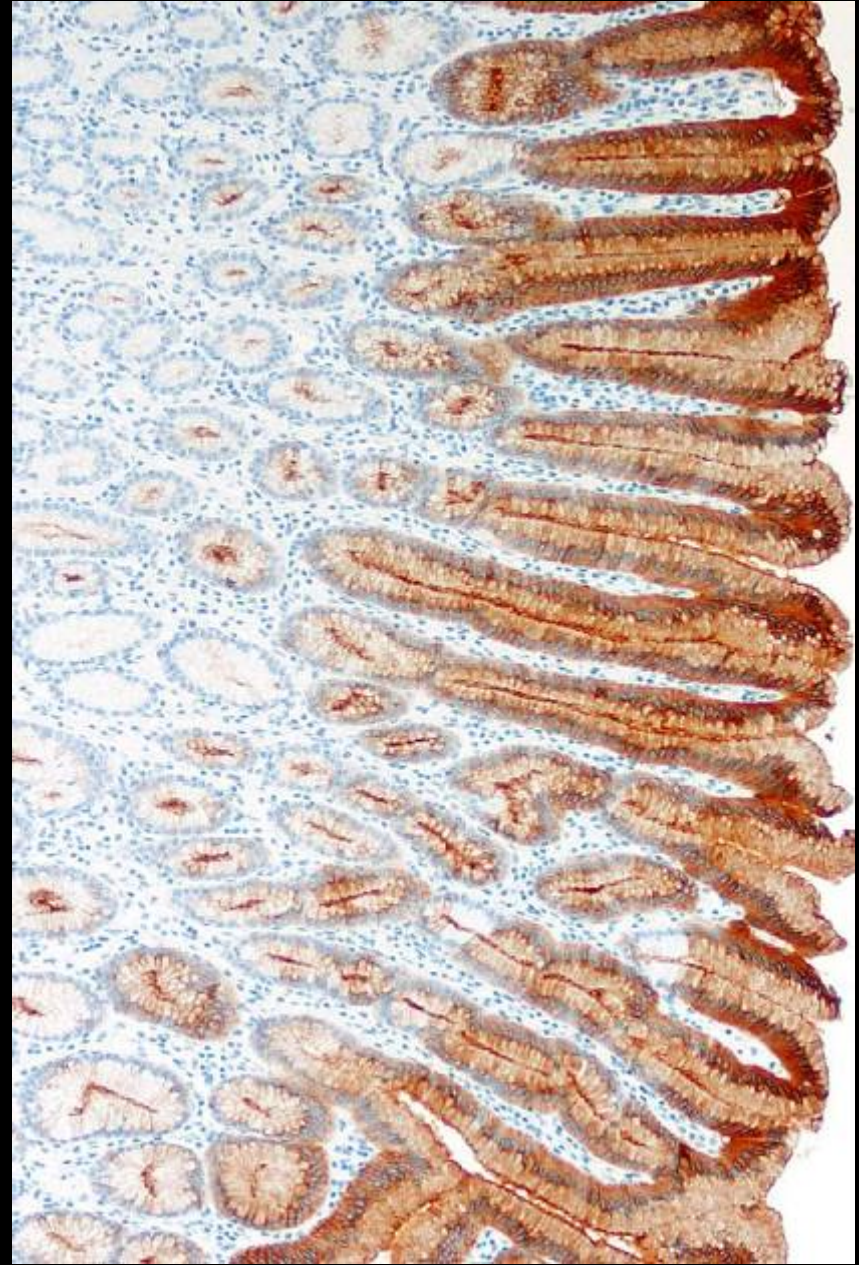
Optimized
protocols

EPR =
EPR2764Y



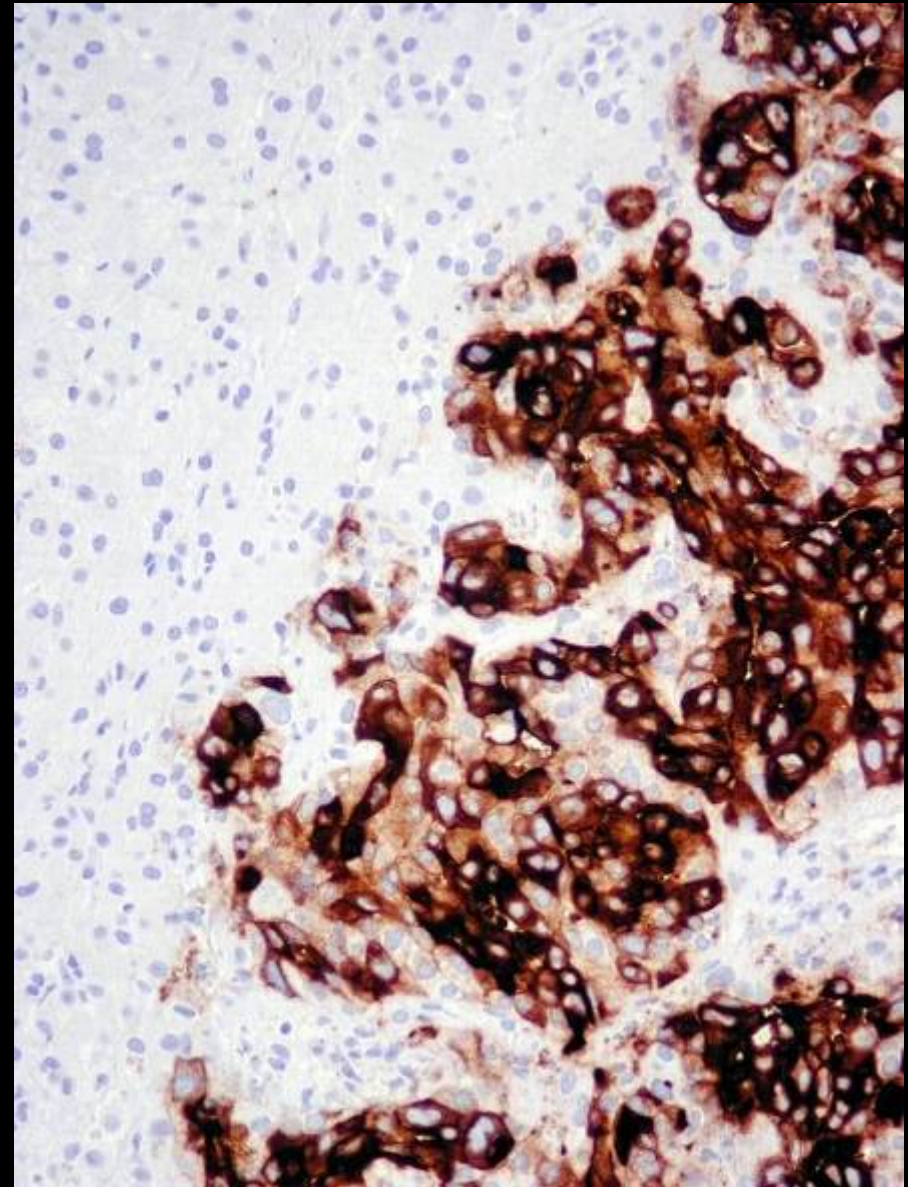
Carcinoembryonic antigen (CD66e)

- Adhesion molecule espc. associated with intestine



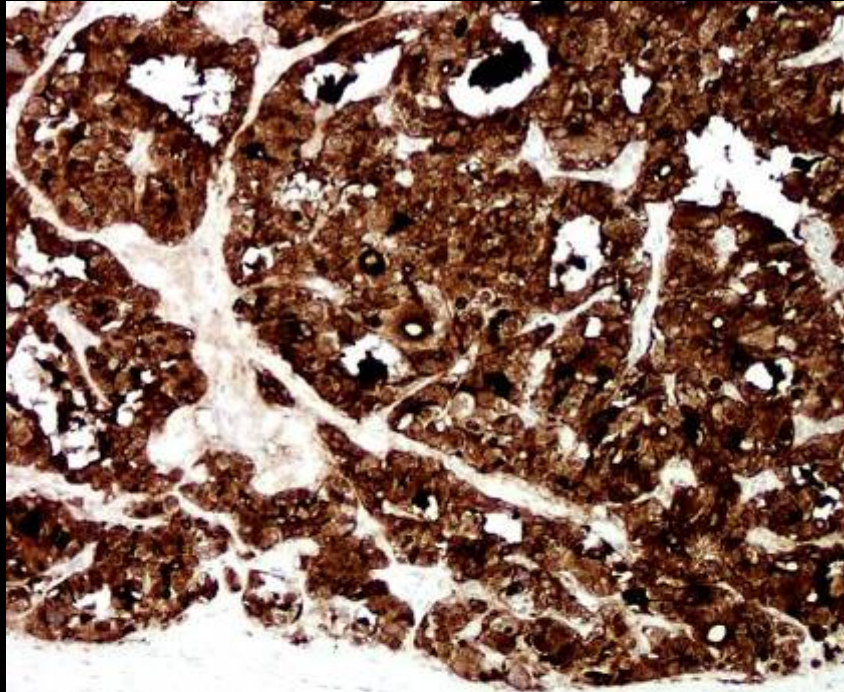
Carcinoembryonic antigen (CD66e) in adenocarcinomas

■ Colorectal	+
■ Medull. thyroid	+
■ Pancreas/biliary tract	+/-
■ Stomach	+/-
■ Lung	+/-
■ Ovary, mucinous	+/-
■ Ovary, non-muc.	-/+
■ Prostate	-
■ Kidney	-
■ Liver (!)	-

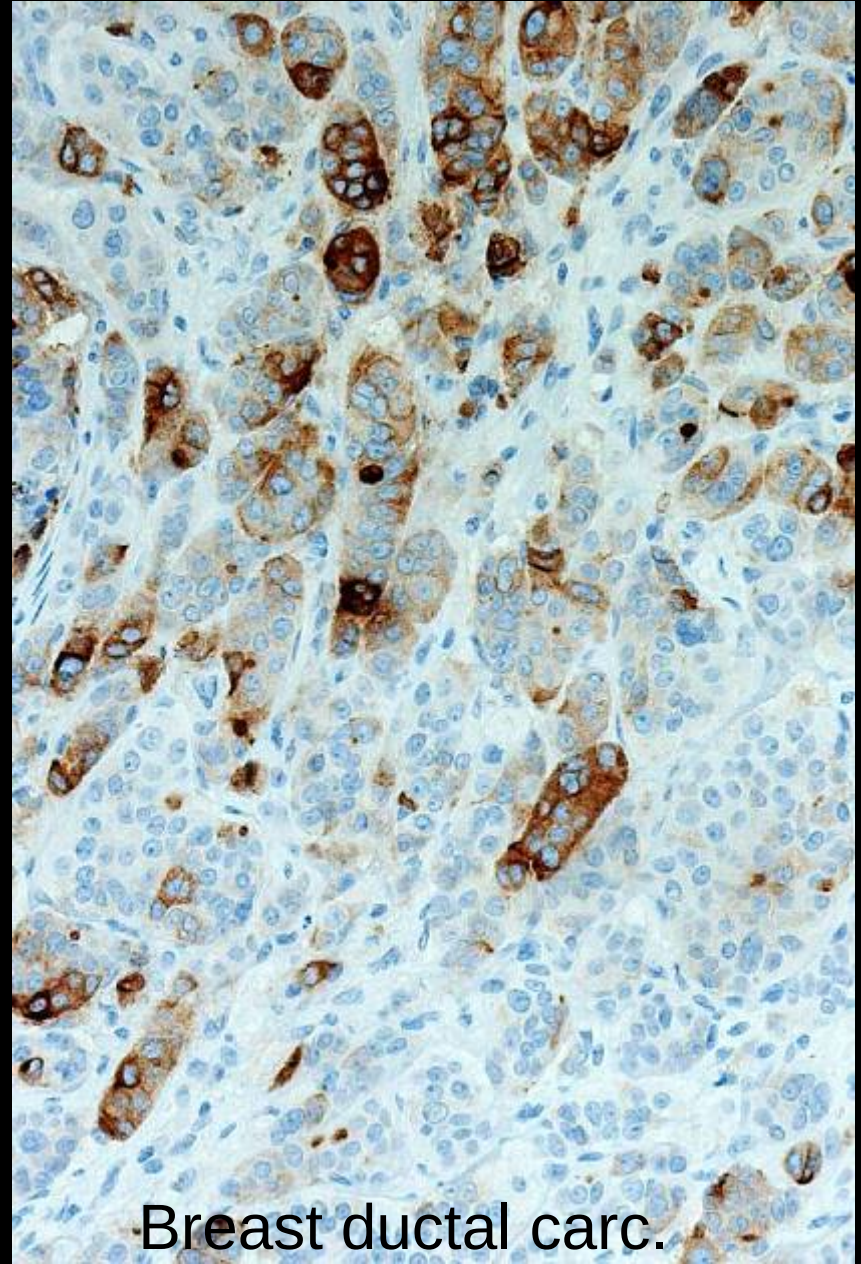


metast. colon adenoc

Carcinoembryonic antigen



Medul. thyroid carc.



Breast ductal carc.

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

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

Table 2. Proportion of sufficient results for CEA in the four NordiQC runs performed

	Run 12 2004	Run 27 2009	Run 37 2013	Run 47 2016
Participants, n=	60	123	190	255
Sufficient results	86%	75%	59%	42%

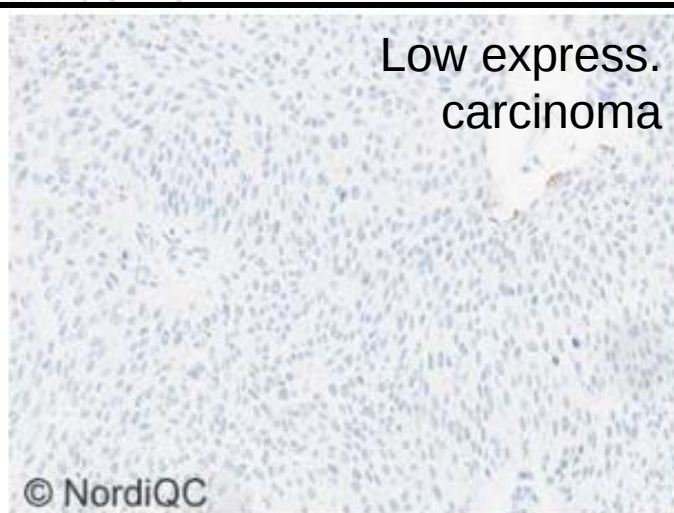
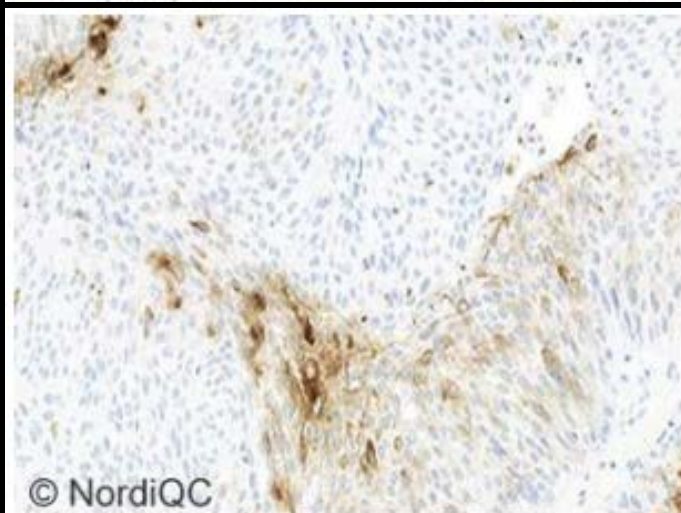
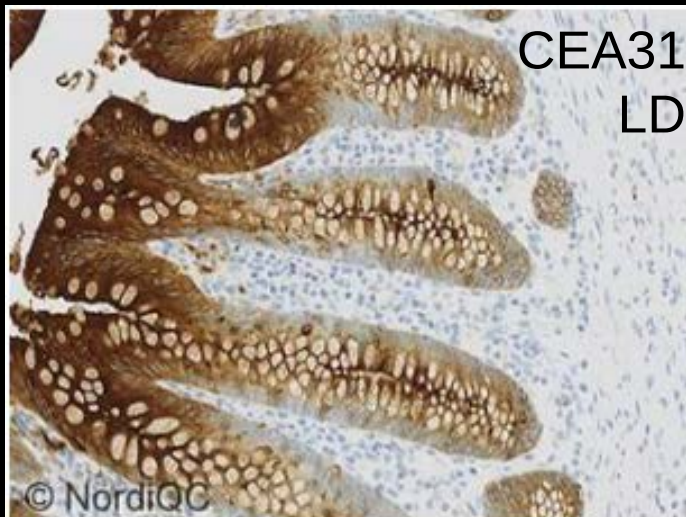
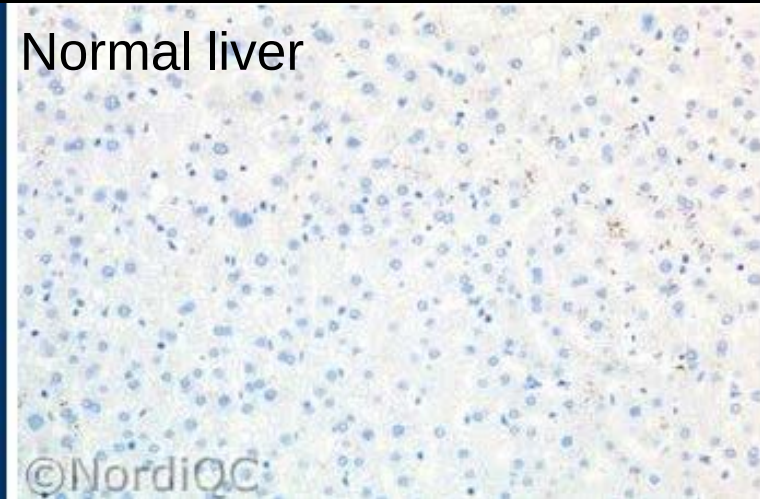


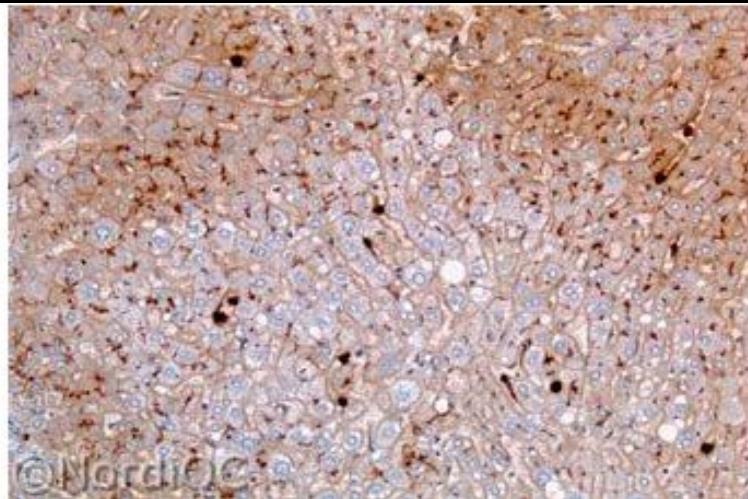
Table 2. Proportion of sufficient results for CEA in the four NordiQC runs performed

	Run 12 2004	Run 27 2009	Run 37 2013	Run 47 2016
Participants, n=	60	123	190	255
Sufficient results	86%	75%	59%	42%

Normal liver



II-7



TF3H8-1

“Female genital tract markers”

PAX8

CA125

WT1

p53

ER

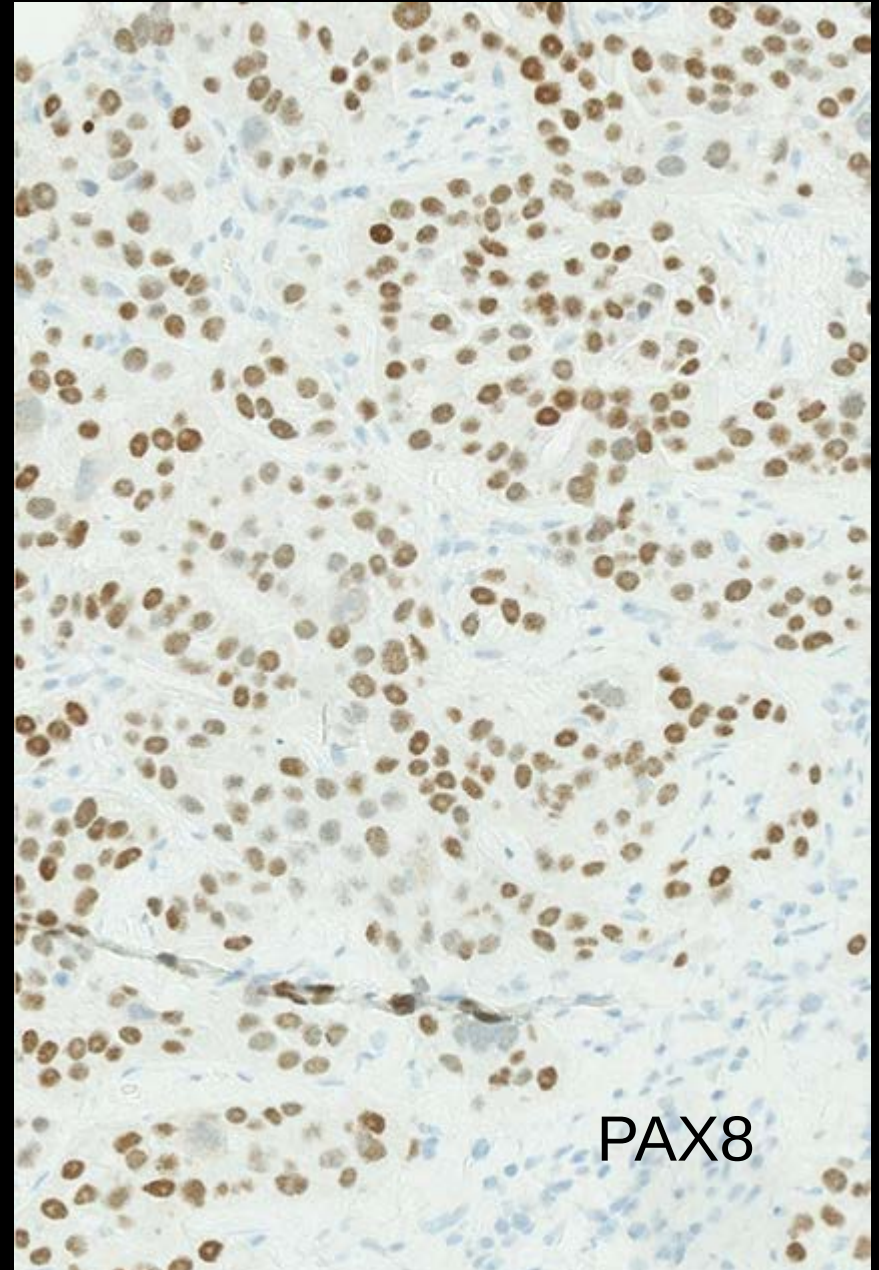
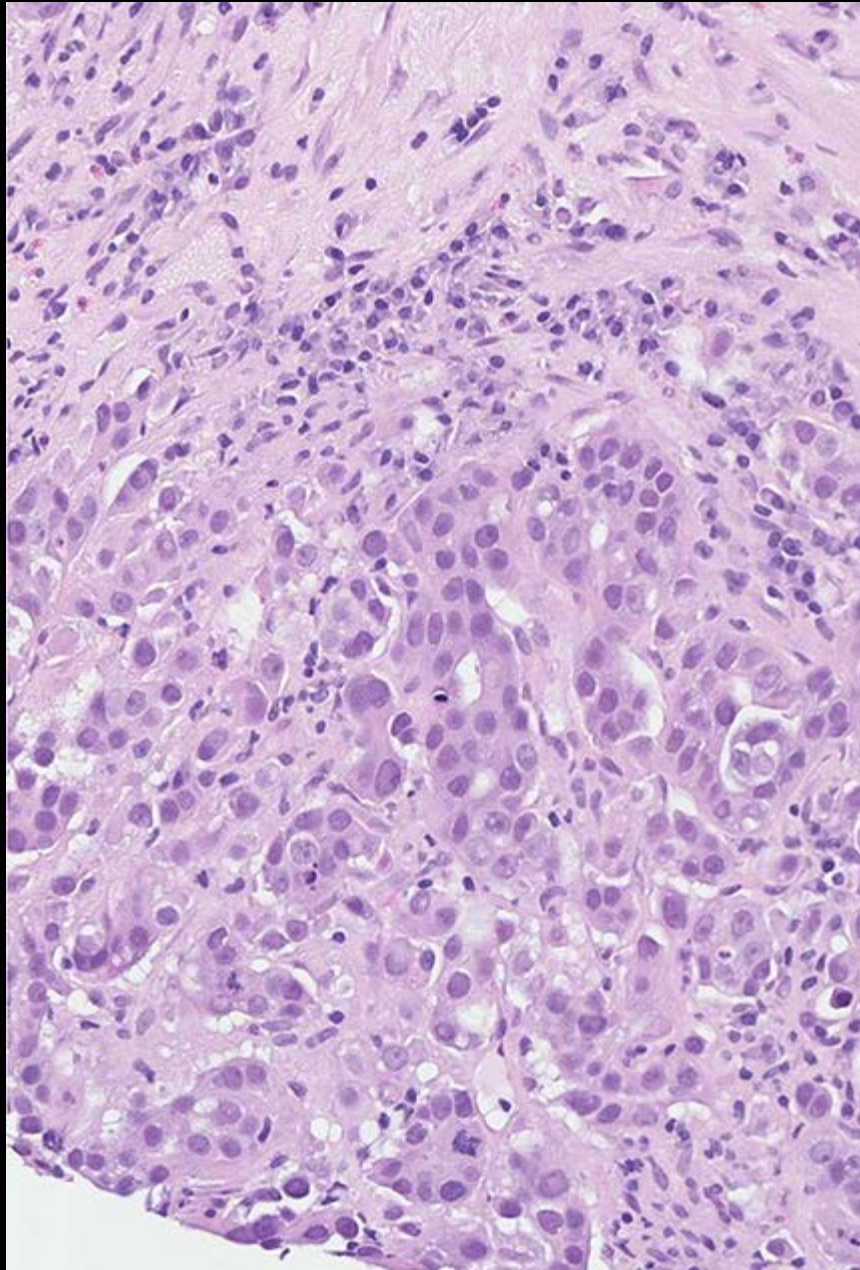
Crucial to the organogenesis and development of

- Urogenital tract
- Adult genital tract
- Thyroid
- Neuroendocrine system

PAX8

Ovary - serous carcinoma LG	+
- serous carcinoma HG / clear cell	+
- endometrioid carcinoma	+/-
- mucinous carcinoma	-(+)
Kidney - clear cell / papillary carcinoma	+
- chromophobe / collect. duct carc.	+
- RCC, sarcomatoid	+/-
NET - pancreas, duodenum, rectum	-(+)?
Thyroid carcinoma	+
Other carcinomas	-(+)
Malignant mesothelioma	-

Ovarian serous carcinoma



PAX8 in clear cell renal cell carcinoma

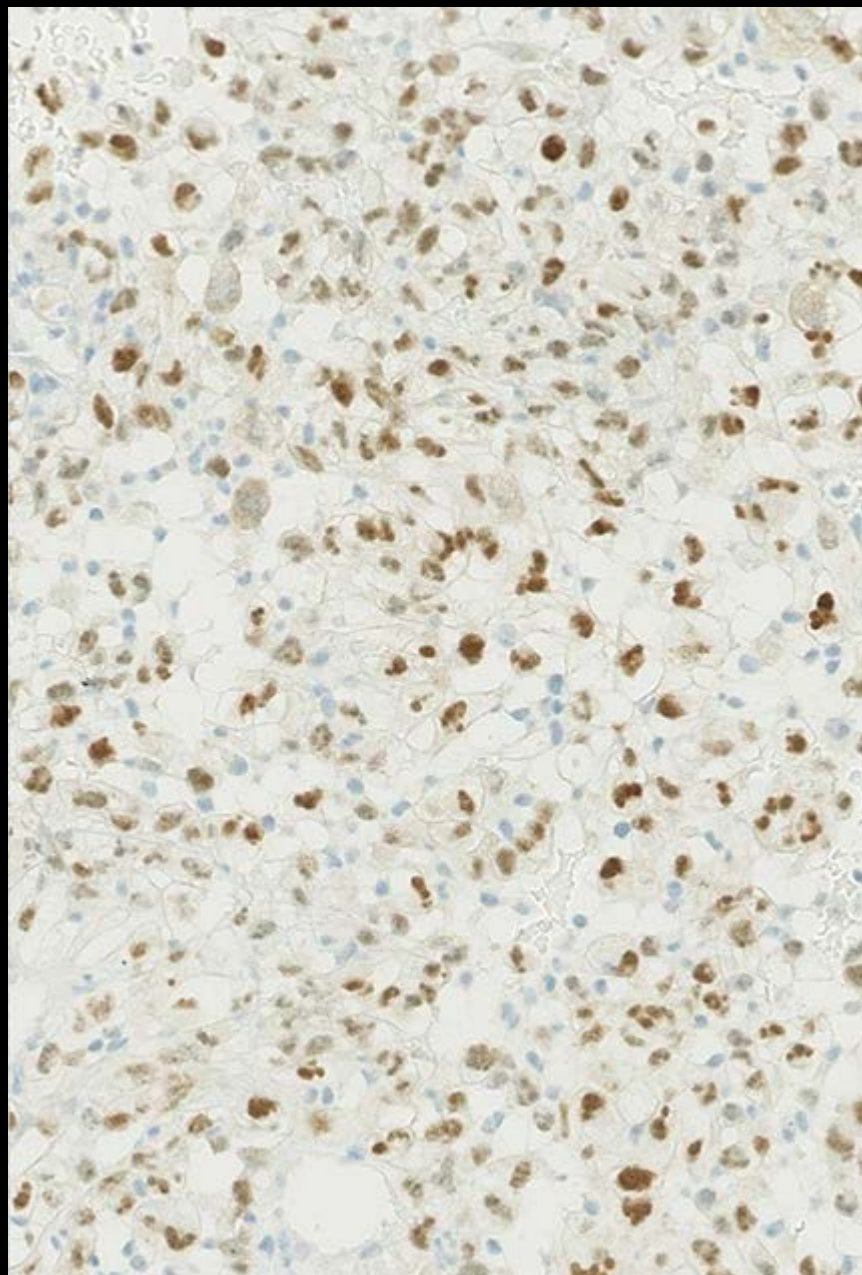
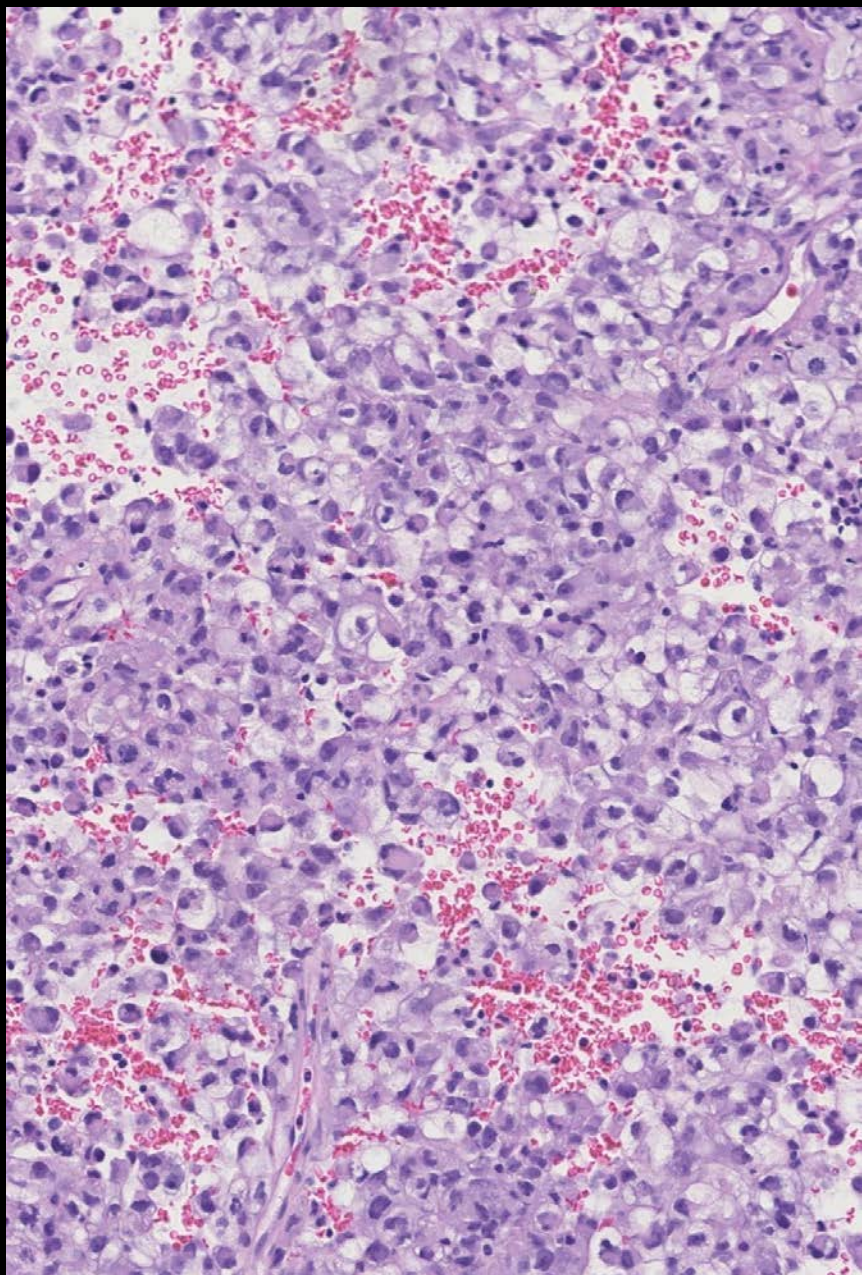


Table 1. Antibodies and assessment marks for PAX8, run 51

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone BC12	11	Biocare	1	4	0	7	42%	100%
	1	Zytomed Systems						
mAb clone DBM15.48	1	Diagnostic Bioystems	0	1	0	0	-	-
mAb clone MRQ-50	63	Cell Marque	17	24	13	10	64%	69%
	1	Menarini Diagnostics						
mAb clone PAX8R1	2	abcam	0	0	2	0		
rmAb clone EP298	4	Cell Marque	3	3	0	1	86%	100%
	2	Epitomics						
	1	BIO SB						
rmAb clone SP348	1	Spring Biosciences	1	0	0	0	-	-
rmAb clone ZR-1	3	Zeta Corporation	2	2	1	0	80%	100%
	1	Abcam						
	1	Nordic Biosite						
pAb, 10336-1-AP	29	Protein Tech	8	10	9	2	62%	83%
Ready-To-Use antibodies								
mAb clone MRQ-50 760-4618	11	Ventana/Cell Marque	0	4	5	2	36%	-
mAb clone MRQ-50 363M	68	Cell Marque	3	25	27	13	41%	55%
mAb clone PAX8/1492 MAD-000753QD/V	2	Master Diagnostica	2	0	0	0	-	-

(Appl Immunohistochem Mol Morphol 2018;26:221–224)

NordiQC Assessments of PAX8 Immunoassays

Rasmus Røge, MD,† Ole Nielsen, HT,‡ Michael Bzorek, HT,§ Søren Nielsen, HT,*
and Mogens Vyberg, MD*†*

KEY POINTS FOR PAX8 IMMUNOASSAY

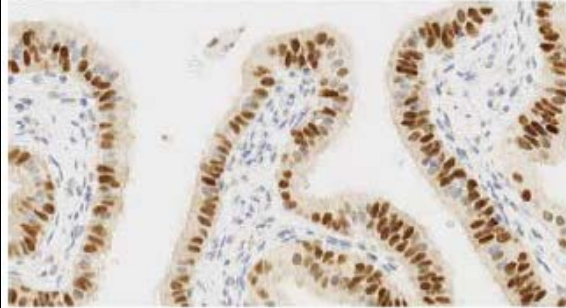
- Older clones, especially MRQ-50, are challenging especially on fully automated platforms (as Ventana Benchmark and Dako Omnis).
- Efficient HIER and use of sensitive 3-step detection systems are required.
- Fallopian tube, kidney, and tonsil are recommendable as on-slide controls.
- Caution when interpreting staining reaction, since some antibodies cross-react with other members of the PAX transcription factor family.

Optimal

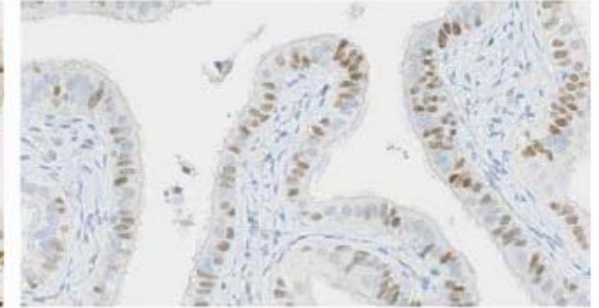
Too weak

Fallopian tube

A1

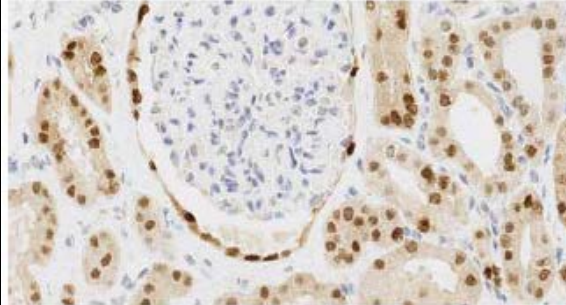


A2

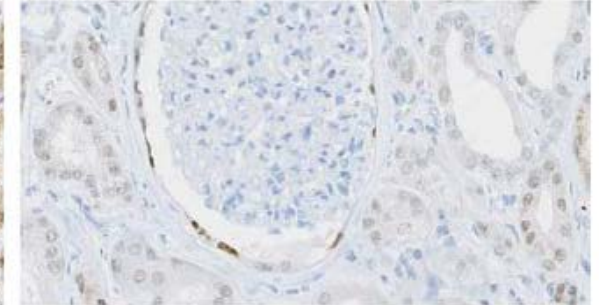


Kidney

B1

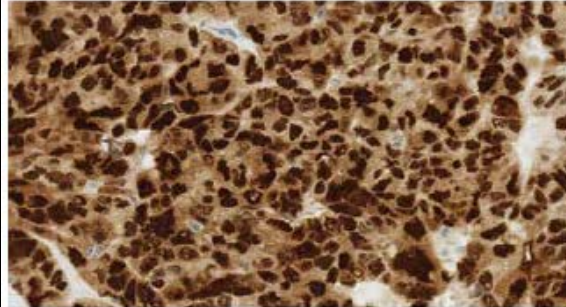


B2

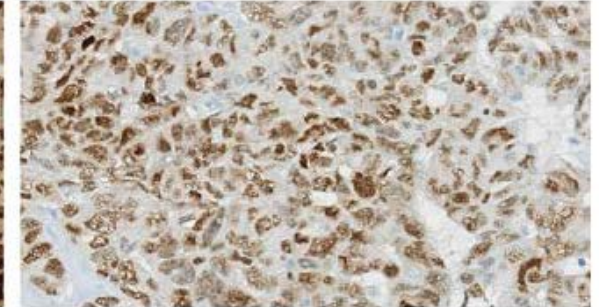


Ovarian serous carcinoma

C1

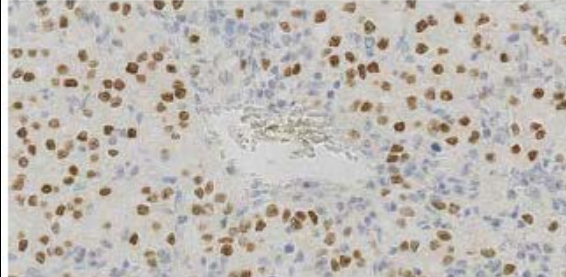


C2



Clear cell renal cell carcinoma

D1



D2



False negative

The unknown primary tumour: IHC Classification, antibody selection, protocol optimization, controls and EQA (part II)

Mogens Vyberg
Professor of Clinical Pathology
Director of NordiQC
Aalborg University Hospital,
Aalborg, Denmark