Assessment Run 62 2021

CK7

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CK7, typically used in the diagnostic work-up of cancer of unknown primary (CUP) origin. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for CK7 (see below).

Material
The slide to be stained for CK7 comprised:

1. Appendix, 2. Liver, 3. Pancreas, 4. Lung adenocarcinoma, 5. Breast adenocarcinoma, 6. Colon adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CK7 staining as optimal included:

- An at least weak, distinct cytoplasmic staining reaction of the majority of epithelial cells of the intercalating ducts in the pancreas, while epithelial cells of the large pancreatic ducts showed a strong cytoplasmic staining reaction.
- A strong, distinct cytoplasmic staining reaction of bile duct epithelial cells in the liver.
- An at least weak staining reaction in dispersed neuroendocrine cells and vascular endothelial cells of the appendix.
- A strong, predominantly cytoplasmic staining reaction of virtually all neoplastic cells of the lung and breast adenocarcinomas.
- No staining reaction of normal epithelial cells in the appendix, hepatocytes in the liver and neoplastic cells in the colon adenocarcinoma.

Participation

Number of laboratories registered for CK7, run 62	379
Number of laboratories returning slides	359 (95%)

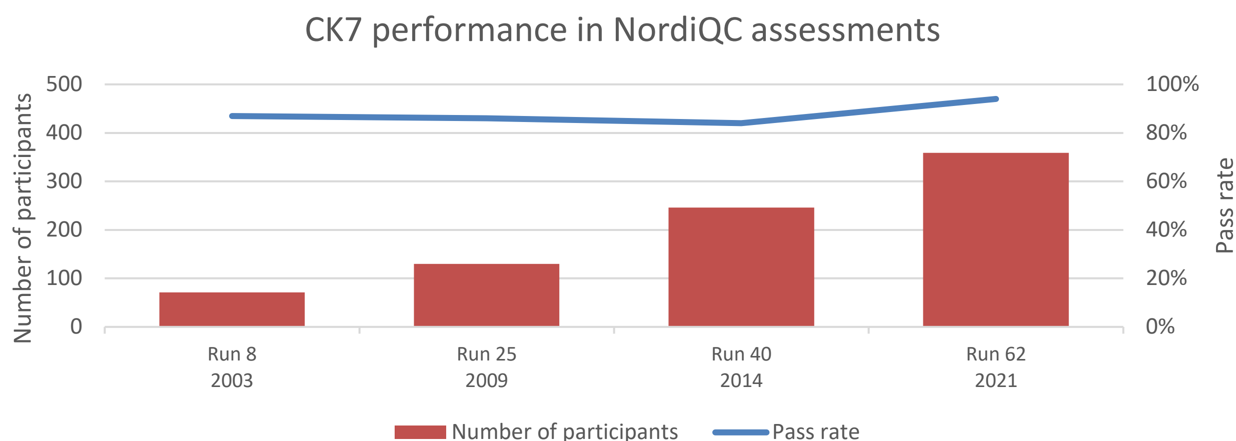
Results
At the date of assessment, 95% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

359 laboratories participated in this assessment and 94% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3).

The most frequent causes of insufficient staining were:
- Inefficient Heat Induced Epitope Retrieval (HIER) – too short time or use or acidic buffer.
- Omission of HIER, applying proteolytic pre-treatment or no pre-treatment at all.
- Too low concentration of the primary antibody or too short incubation time.
- Less sensitive detection systems used in combination with other low sensitivity protocol parameters.

Performance history
This was the fourth NordiQC assessment of CK7. A significant increase in pass rate was observed compared to previous runs (see Graph 1), which primarily is due to the use of robust primary antibodies and well calibrated Ready-To-Use (RTU) systems (see Table 1).

Graph 1. **Proportion of sufficient results for CK7 in the four NordiQC runs performed**



Conclusion
The mAbs clones **OV-TL 12/30**, **RN7, MX053, C1C10** and the rmAb clones **SP52** and **EP16** could all be used for demonstration of CK7. The mAb clone OV-TL 12/30 was the most frequently used antibody within a Laboratory Developed assay and efficient HIER (preferable in an alkaline buffer) and carefully calibration of the antibody titer adjusted to the sensitivity of the detection system employed, provided the highest proportion of optimal results. The RTU systems IR/GA619 (Dako/Agilent), PA0942/PA0138 (Leica Biosystems) and 790-4462 (Ventana/Roche) based on the clones OV-TL 12/30, RN7 and SP52, respectively, provided superior performance and applying vendor recommended protocol settings, all participants obtained a sufficient result.
Pancreas is an appropriate positive tissue control for CK7: Virtually all epithelial cells of the intercalating ducts must show an at least weak to moderate cytoplasmic staining reaction, whereas the epithelial cells of large ducts must display a strong cytoplasmic staining reaction. Appendix is recommended as negative tissue control and virtually all epithelial cells should be negative (dispersed columnar cells and endothelial cells may be positive).

Table 1. **Antibodies and assessment marks for CK7, Run 62**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **OV-TL 12/30**	87 14 7 3 2 2 2 1 1 1 1	Dako/Agilent Leica Biosystems Cell Marque Zytomed Systems BioGenex Thermo F. Scientific Immunologic Zeta Corporation Biocare Medical Diagnostic Biosystems GeneMed	74	33	13	1	88%	61%
mAb clone **BS28**	1	Nordic Biosite	0	1	0	0	-	-
mAb clone **RN7**	1	Leica Biosystems	0	1	0	0	-	-
mAb clone **IHC007**	1	GenomeMe Inc.	0	0	1	0	-	-
rmAb clone **EP16**	2	Epitomics	1	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **OV-TL 12/30, IR619**[3]	12	Dako/Agilent	11	1	0	0	100%	92%
mAb clone **OV-TL 12/30, IR619**[4]	12	Dako/Agilent	11	1	0	0	100%	92%
mAb clone **OV-TL 12/30, GA619**[3]	32	Dako/Agilent	31	1	0	0	100%	97%
mAb clone **OV-TL 12/30, GA619**[4]	30	Dako/Agilent	27	2	1	0	97%	90%
mAb clone **OV-TL 12/30, MAD-001004QD**	3	Master Diagnostica	2	0	1	0	-	-
mAb clone **OV-TL 12/30, 8296-0C10**	2	Sakura FineTek	1	1	0	0	-	-
mAb clone **OV-TL 12/30, PM061**	2	Biocare Medical	0	1	1	0	-	-
mAb clone **OV-TL 12/30, E061**	1	Linaris	1	0	0	0	-	-
mAb clone **OV-TL 12/30, 307M-97/88**	1	Cell Marque	0	1	0	0	-	-
mAb clone **RN7, PA0942**[3]	6	Leica Biosystems	1	5	0	0	100%	17%
mAb clone **RN7, PA0942/PA0138**[4]	11	Leica Biosystems	5	6	0	0	100%	45%
mAb clone **MX053 MAB-0828**	2	Fuzhou Maixin Biotech	2	0	0	0	-	-
mAb clone **C1C10, CCM-0992**	1	Celnovte Biotechnology	1	0	0	0	-	-
rmAb clone **BC1, PRM339**	1	Biocare Medical	0	0	0	1	-	-
rmAb clone **SP52, 790-4462**[3]	16	Ventana/Roche	11	5	0	0	100%	69%
rmAb clone **SP52, 790-4462**[4]	101	Ventana/Roche	86	12	2	1	97%	85%
Total	359		265	71	20	3	-	
Proportion			74%	20%	5%	1%	94%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).

Detailed analysis of CK7, Run 62
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **OV-TL 12/30**: Protocols with optimal results were typically based on HIER using Target Retrieval Solution (TRS, Dako/Agilent) pH 9 (3-in-1) (8/16)*, Cell Conditioning 1 (CC1, Ventana/Roche) (39/52), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (15/17), DBS Montage EDTA Antigen Retrieval Solution (1/1), TRIS-EDTA/EGTA pH 9 (2/3), Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (2/9) or Cell Conditioning 2 (CC2, Ventana/Roche) (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:30-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings, 63 of 67 (94%) laboratories produced a sufficient staining result (optimal or good).
One protocol based on combined pre-treatment (HIER in CC1+Protease 3), four protocols based on proteolytic pre-treatment and one protocol based on no pre-treatment at all, obtained an optimal mark.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP16**: One protocol with an optimal result was based on HIER using BERS2. The mAb was diluted 1:100, Bond Refine (DS9800, Leica Biosystems) was used as the detection system and staining was performed on BOND III.

Table 2. **Proportion of optimal results for CK7 for the most commonly used antibody as concentrate on the four main IHC systems***

Concentrated antibody	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica Biosystems Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **OV-TL 12/30**	4/7** (57%)	0/1	3/4	-	29/35 (83%)	1/1	8/9 (89%)	2/6 (33%)

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

Ready-To-Use antibodies and corresponding systems
mAb clone **OV-TL 12/30**, product no. **IR619**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 96-99°C), 20-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Using these protocol settings, 14 of 14 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **OV-TL 12/30**, product no. **GA619**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (efficient heating time 30 min. at 97°C), 12,5 min. incubation of the primary Ab and Envision FLEX (GV800) as detection system. Using these protocol settings, 37 of 37 (100%) laboratories produced a sufficient staining result.

mAb clone **OV-TL 12/30**, product no. **8296-C010**, Sakura FineTek, Tissue-Tek Genie Advanced:
One protocol with an optimal result was based on HIER using Tissue-Tek Genie High pH Antigen Retrieval Solution (efficient heating time 45 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek Genie Pro Detection Kit (8826-K250) as detection system.

mAb clone **RN7,** product.no. **PA0942/PA0138**, Leica Biosystems, BOND III/MAX:
Protocols with optimal results were typically based on HIER in BERS2 (efficient heating time 20 min. at 98-100°C), 15 min. incubation of the primary Ab and BOND Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 6 of 6 (100%) laboratories produced a sufficient staining result.

mAb clone **MX053**, product no. **MAB-0828**, Fuzhou Maixin Biotech, Titan S:
Protocols with optimal results were based on HIER using High pH buffer (Fuzhou Maixin, DNS-0811) (efficient heating time 20 min. at 99°C), 30 min. incubation of the primary Ab and Titan Super Detection Kit (TT-0805) as detection system.

rmAb clone **SP52**, product no. **790-4462**, Ventana/Roche, BenchMark GX/XT/ULTRA:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 16-32 min. incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings, 86 of 86 (100%) laboratories produced a sufficient staining result.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly according to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for CK7 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb OV-TL 12/30 **IR619**	100% (12/12)	92% (11/12)	100% (11/11)	91% (10/11)
Dako Omnis mAb OV-TL 12/30 **GA619**	100% (32/32)	97% (31/32)	100% (26/26)	92% (24/26)
Leica Bond III/MAX mAb RN7 **PA0942/PA0138**	100% (6/6)	17% (1/6)	100% (11/11)	45% (5/11)
VMS Ultra/XT rmAb SP52 **790-4462**	100% (16/16)	69% (11/16)	97% (98/101)	85% (86/101)

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

Comments

In concordance with the previous NordiQC assessments for CK7, the prevalent feature of an insufficient staining result was characterized by a general too weak or false negative staining reaction of cells and structures expected to be demonstrated. This was observed in 65% (15/23) of the insufficient results. In the remaining insufficient results, most displayed a false positive staining result often in combination with a too weak or false negative staining reaction. Almost all laboratories could detect CK7 in high expressing tissue structures as epithelial cells of large ducts in the pancreas or bile ducts of the liver, whereas demonstration of CK7 in intercalated ducts of the pancreas, dispersed endothelial of the appendix and the neoplastic cells of the lung adenocarcinoma was more challenging, requiring an optimally calibrated protocol.

The mAb clones OV-TL 12/30, RN7 and the rmAb clone SP52 were the most widely used antibodies for demonstration of CK7 and applied by 98% (351/359) of the laboratories (see Table 1). Assays based on these robust primary antibodies contributed to the overall high pass rate of 94% obtained in this assessment, emphasizing the importance of selecting high performance antibodies with focus on the analytical sensitivity and specificity. Only 6% (20/351) of the results based on one of these three clones were assessed as insufficient (borderline or poor). Used as concentrated format within Laboratory Developed (LD) assays, the mAb clone OV-TL 12/30 gave a pass rate of 88% (107/121) of which 61% (74/121) of the protocols gave an optimal result. The most frequent causes of an insufficient result were related to use of low sensitivity protocol settings (often in combination) as no or proteolytic pre-treatment, HIER in acidic buffer, too short HIER time, too diluted primary ab and/or use of a less sensitive 2-step multimer/polymer detection system (e.g., UltraView versus OptiView). Although proteolytic pre-treatment could provide optimal result, 21% (3/14) of the protocols based on proteolysis using otherwise optimal protocol settings gave an insufficient result. With offset in this observation, and in combination with the general variability of results based on proteolytic pre-treatment being dependent on multiple parameters as type of enzyme, concentration of the enzyme, incubation time, temperature, type of tissue and especially length of formalin fixation, it is highly advisable to perform HIER as pre-treatment. HIER in general provides consistent retrieval of antigens over a broader fixation range in formalin and is especially recommendable for antigens (e.g., CK7) that previously have shown superior performance applying this retrieval procedure.
As shown in Table 2, the mAb clone OV-TL 12/30 used within a LD-assay could provide optimal results on the four main automated platforms. For protocols based on HIER, the performance of the assays was influenced by the type of HIER buffer applied. Overall, and applying optimal protocol settings on the main four platforms as described above, the proportion of optimal results was only 33% (3/9) using HIER in an acidic (low pH) buffer compared to 80% (45/56) if protocols were based on HIER in an alkaline (high pH) buffer. In addition, to the importance of using HIER in an alkaline buffer, the primary Ab should be carefully calibrated as low concentration (titer > 1:300) provided significantly lower proportion of optimal results (62%, 18/29) compared 80% (44/55) if primary Ab was diluted in the optimal dilution range of 1:30-300. All these key elements should be adjusted to the choice of detection system employed to provide accurate analytical sensitivity and specificity of the IHC test.

In total, 65% (233/359) of the laboratories used a RTU format. As shown in Table 3, the RTU systems from all the major vendors provided superior performance. Grouped together, and following Vendor Recommended Protocol Settings (VRPS), all protocols (66/66) were assessed as sufficient. However, proportion of optimal results was significantly higher using e.g., the RTU systems IR/GA619 (Dako/Agilent) based on the mAb clone OV-TL 12/30 compared to the RTU systems PA0942/PA0138 (Leica Biosystems) and 790-4462 (Ventana/Roche) based on the mAb clone RN7 and the rmAB clone SP52, respectively.
For the Leica Biosystems RTU system PA0942/PA0138, the VRPS is based on use of HIER in acidic buffer (BERS1) and this might explain for the lower proportion (17%, 1/6) of optimal results. In support of this observation, and for participants applying Laboratory Modified Protocol Settings (LMPS) based on HIER in alkaline buffer (BERS2), the proportion of optimal results increased to 83% (5/6) optimal results.
For the Ventana/Roche RTU system 790-4462, the proportion of optimal results was 69% (11/16) using VRPS but 85% (86/101) applying LMPS. The increase in proportion of optimal results using LMPS can be related to the use of UltraView with amplification or OptiView as the detection system, whereas the vendor recommended protocol is based on use of the less sensitive UltraView as the detection system. All protocols (38/38) applying a 3-step multimer detection system (e.g., OptiView) were assessed as optimal, typically using HIER in CC1 for 24-64 min. and incubation time in primary Ab for 16-32 min.

This was the fourth NordiQC assessment of CK7. A significant increase in the pass rate was observed from 84% in Run 40 (2011) to 94% in Run 62 (2021). The most important factors influencing the final result in positive direction were the use of very robust Ab clones for demonstration of CK7 and well calibrated RTU systems from the three major vendors. In this assessment, and using VRPS, the RTU system GA619 (Agilent/Dako) based on the mAb clone OV-TL 12/30 gave the highest proportion of optimal results (97%). Importantly, protocols must stain accordingly to the expected antigen level in which pancreas and appendix are central immunohistochemical critical assay performance controls (ICAPCs) to guide the level of analytical sensitivity and specificity (see below).

Controls
Pancreas is recommended as positive tissue control for CK7. Virtually all ductal and intercalated duct epithelial cells must show an at least weak to moderate, distinct cytoplasmic staining reaction, whereas epithelial cells of large ducts should display a strong staining intensity. Acinar epithelial cells should be negative.
Appendix can be used as negative tissue control for CK7. In general, no staining reaction should be seen in the epithelial cells. Scattered columnar epithelial cells and endothelial cells may show a weak to moderate cytoplasmic staining reaction. The recommendations of the mentioned tissue controls for IHC are concordant with the guidelines published by the International Ad Hoc Expert Committee[1].

[1]Torlakovic EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18. doi: 10.1097/PAI.0000000000000163. Review. PubMed PMID: 25474126.

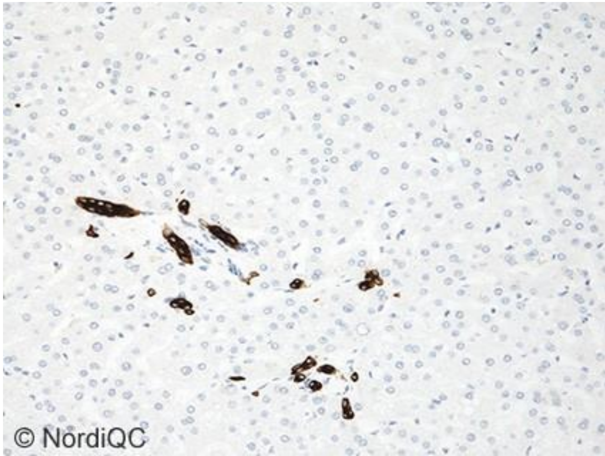


Fig. 1a (x100)
Optimal staining for CK7 of the liver using the mAb clone OV-TL 12/30 as a concentrate (1:100), efficient HIER in an alkaline buffer (BERS2, Leica) and a 3-step polymer based detection system (Bond Refine, Leica) - same protocol used in Figs. 2a - 6a. The bile ducts display a strong staining reaction. All hepatocytes are negative as expected.

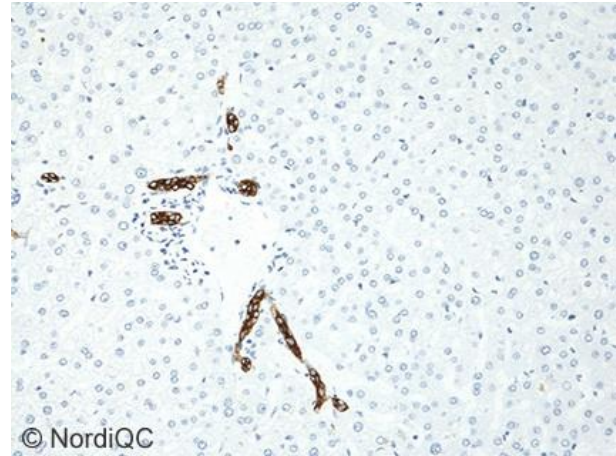


Fig. 1b (x100)
CK7 staining of the liver based on the mAb clone OV-TL 12/30 within a LD-assay, too diluted (1:2000), less efficient HIER in an acidic buffer (BERS1, Leica) and a 3-step polymer-based detection system (Bond Refine, Leica) - same protocol used in Figs. 2b – 6b. Although the staining showed the expected reaction pattern in the liver, the protocol overall provided too low analytical sensitivity – see Figs. 2a - 6b.

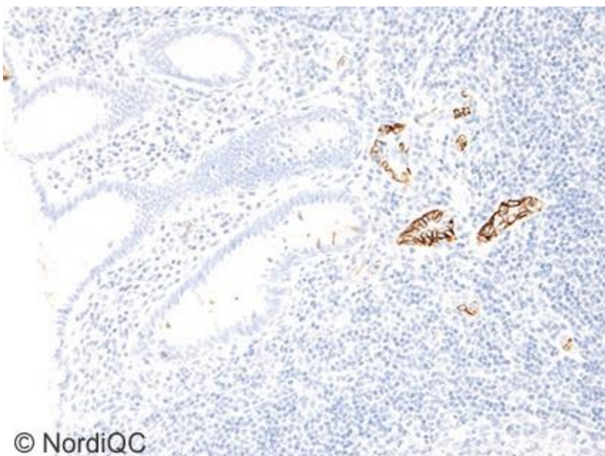


Fig. 2a (x200)
Optimal CK7 staining of the appendix using same protocol as in Fig. 1a. Scattered neuroendocrine and endothelial cells display a weak to moderate staining reaction and the epithelial cells are negative.

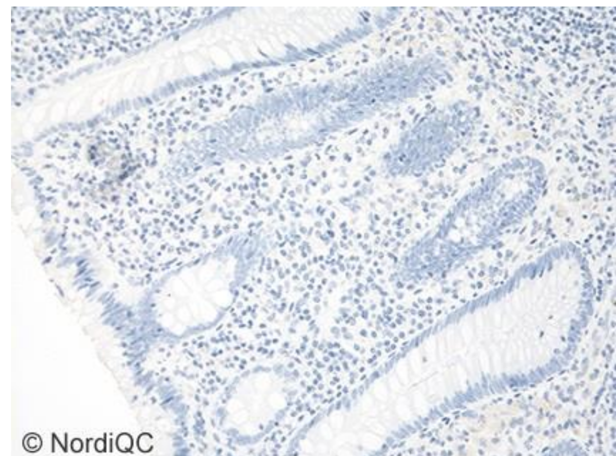


Fig. 2b (x200)
Insufficient CK7 staining of the appendix using same protocol as in Fig. 1b. Although the epithelial cells show the expected negative reaction, no staining is seen in neuroendocrine cells or in dispersed endothelial cells – compare with Fig. 2a.

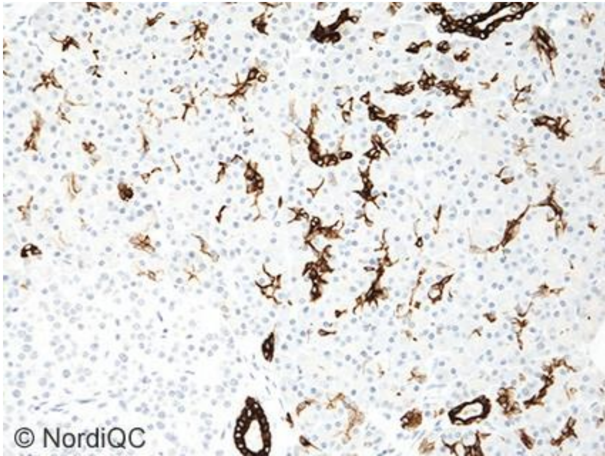


Fig. 3a (x200)
Optimal CK7 staining of the pancreas using same protocol as in Figs. 1a and 2a. Virtually all intercalating ducts show a weak to moderate staining reaction, whereas the epithelium of the large ducts display strong staining intensity. Epithelial cells of acinar structures are negative.

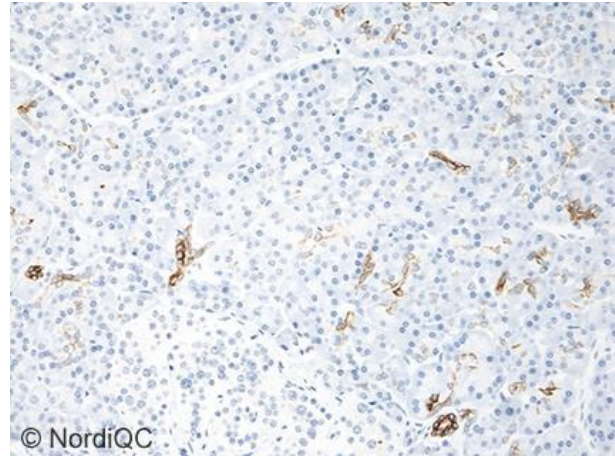


Fig. 3b (x200)
Insufficient Ck7 staining of the pancreas, using the same protocol as in Figs. 1b and 2b. The proportion and staining intensity of the intercalating ducts is significantly reduced. In addition, the epithelial cells of the large ducts only show a weak to moderate staining reaction. Compare with Fig. 3a.

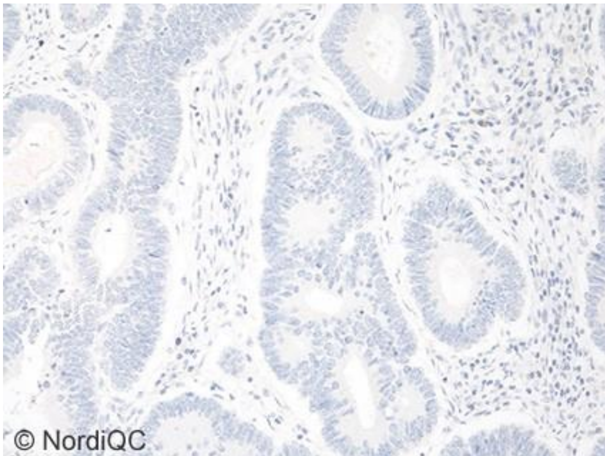


Fig. 4a (x200)
Optimal CK7 staining of the colon adenocarcinoma using same protocol as in Figs. 1a - 3a. All neoplastic cells are negative.

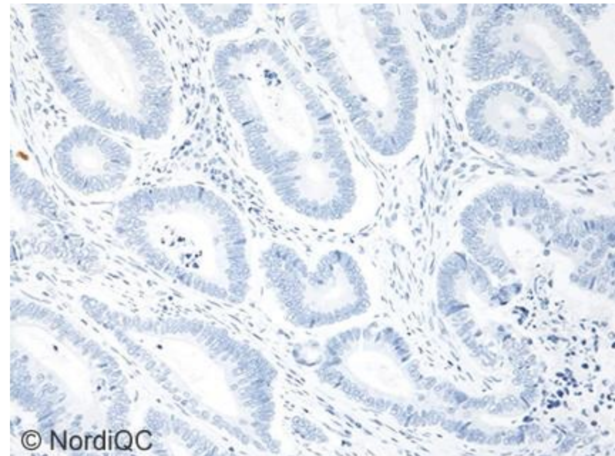


Fig. 4b (x200)
CK7 staining of the colon adenocarcinoma using the same protocol as in Figs. 1b - 3b. The staining displayed the expected reaction pattern, but as mentioned above, the protocol provided an overall too low analytical sensitivity - compare Fig. 2a-3b and Fig. 5a-6b.

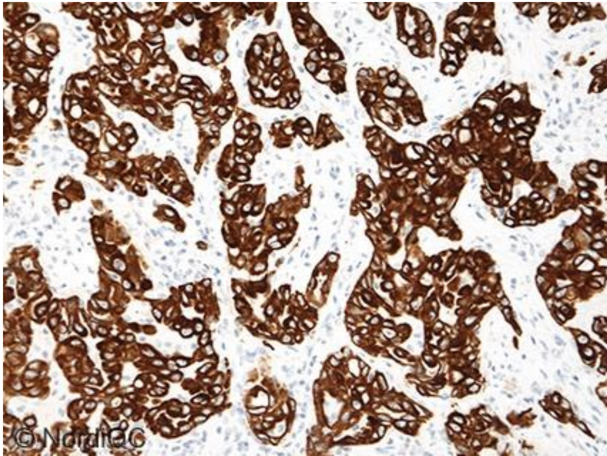


Fig. 5a (x200)
Optimal CK7 staining of the lung adenocarcinoma using same protocol as in Fig. 1a - 4a. All neoplastic cells show a strong, distinct cytoplasmic staining reaction.

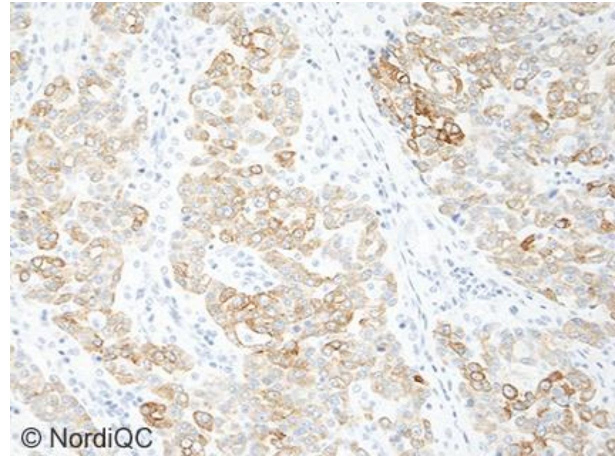


Fig. 5b (x200)
Insufficient CK7 staining of the lung adenocarcinoma using same protocol as in Fig. 1b - 4b. Virtually all neoplastic cells show a significantly reduced staining reaction or are only faintly demonstrated, risking misdiagnosis of tumours with low level of the antigen (CK7). Compare with Fig. 5a.

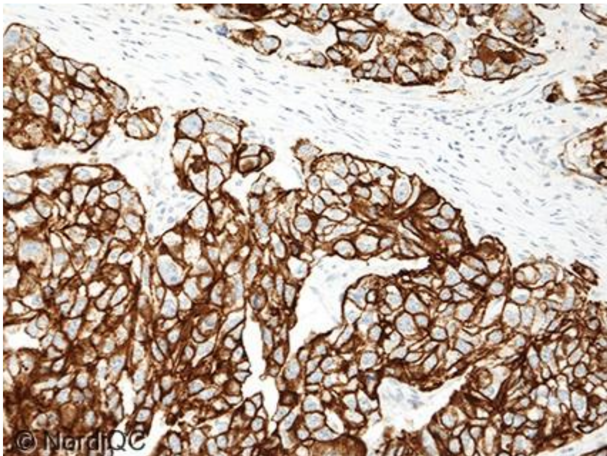


Fig. 6a (x200)
Optimal CK7 staining of the breast adenocarcinoma using same protocol as in Fig. 1a - 5a. All neoplastic cells show a strong, predominantly cytoplasmic staining reaction (membranous accentuation).

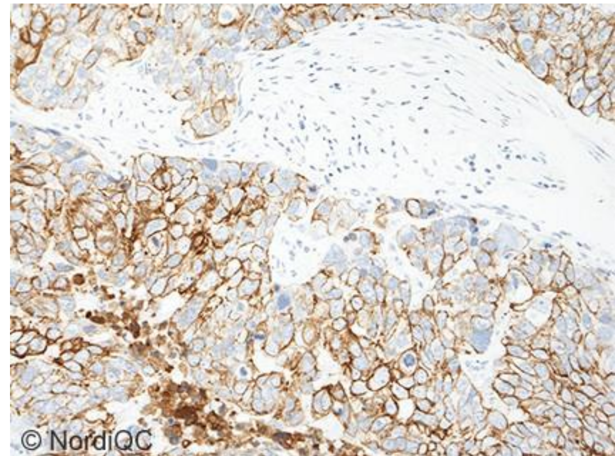


Fig. 6b (x200)
Insufficient CK7 staining of the breast adenocarcinoma using same protocol as in Fig. 1b - 5b. Virtually all neoplastic cells show a reduced staining intensity compared to the expected level in Fig. 6a.

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