

Material

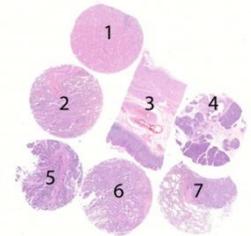
The slide to be stained for CK7 comprised:

1. Kidney, 2. Lung, 3. Gastric corpus, 4. Pancreas, 5. Colon adenocarcinoma, 6-7. Lung adenocarcinomas

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CK7 staining as optimal included:

- A moderate to strong, distinct cytoplasmic staining reaction of virtually all epithelial cells of the renal collecting ducts and the scattered epithelial cells in the Bowman capsule.
- A strong, distinct cytoplasmic staining reaction of all alveolar epithelial cells in the lung tissue.
- An at least weak to moderate predominantly cytoplasmic staining reaction of the majority of luminal foveolar epithelial cells of the gastric corpus mucosa.
- A strong, distinct cytoplasmic staining reaction of virtually all epithelial cells of the large pancreatic ducts, while the majority of the epithelial cells of the intercalating ducts at least should show a weak to moderate cytoplasmic staining reaction.
- A strong, distinct cytoplasmic staining reaction of all neoplastic cells in the lung adenocarcinoma no. 6.
- An at least moderate to strong cytoplasmic staining reaction of virtually all neoplastic cells in the lung adenocarcinoma no. 7.
- No staining reaction of neoplastic cells in the colon adenocarcinoma, epithelial cells of proximal tubules of the kidney or acinar cells of the pancreas.



Results

246 laboratories participated in this assessment. Of these 208 (84%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (See page 2).

The most frequent causes of insufficient stains were:

- Too low concentration of the primary antibody
- Inappropriate epitope retrieval – use of proteolysis
- Use of non-alkaline HIER buffer or insufficient HIER (too short efficient HIER time)

Performance history

This was the 3rd NordiQC assessment of CK7. The pass rate of this run was similar to the two former runs in 2003 (Run 8) and 2009 (Run 25) as shown in table 2.

Table 2. **Proportion of sufficient results for CK7 in three NordiQC runs performed**

	Run 8 2003	Run 25 2009	Run 40 2014
Participants, n=	71	130	246
Sufficient results	87%	86%	84%

Conclusion

The mAbs clones **OV-TL 12/30**, **RN7** and the rmAb clone **SP52** are all recommendable Abs for demonstration of CK7. Both HIER and enzymatic pre-treatment can be used to obtain an optimal result. However HIER should be preferred, as efficient HIER provides a consistent IHC result irrespective of the formalin fixation time, whereas proteolytic pre-treatment must be adjusted to the formalin fixation time for optimal performance.

The concentration of the primary Abs must be carefully calibrated to obtain optimal results.

The best performance was achieved with the Ready-To-Use system from Dako, Leica and Ventana. Pancreas is recommended as tissue control in which the epithelia cells of the intercalating ducts must show an at least moderate to strong staining reaction, while the acinar cells must be negative.

Table 1. **Antibodies and assessment marks for CK7, run 40**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone OV-TL 12/30	97	Dako	40	66	32	2	76%	94%
	14	Leica/Novocastra						
	14	BioGenex						
	4	Thermo S/ NeoMarkers						
	3	Monosan						
	2	Biocare						
	2	Cell Marque						
	2	Genemed						
	1	ZytoMed						
	1	Nordic Biosite						
mAb clone RN7	3	Leica/Novocastra	1	2	-	-	-	-
rmAb clone EPR1619Y	1	Abcam	-	-	1	-	-	-
mAb clone K72.7	1	Thermo S/ NeoMarkers	-	1	-	-	-	-
Ready-To-Use antibodies:								
mAb clone OV-TL 12/30, IR619	41	Dako	36	5	0	0	100%	100%
mAb clone OV-TL 12/30, MAD-001004QD	2	Master Diagnostica	1	1	0	0	-	-
mAb clone OV-TL 12/30, 307M-98	1	Cell Marque	1	0	0	0	-	-
mAb clone OV-TL 12/30, MON-RTU1074	1	Monosan	1	0	0	0	-	-
mAb clone OV-TL 12/30, PDM 097	1	Diagnostic Biosystem	0	1	0	0	-	-
mAb clone OV-TL 12/30, E061	1	Linaris	0	1	0	0	-	-
rmAb clone SP52, 790-4462	45	Ventana	26	18	1	0	98%	98%
mAb clone RN7, PA0942	7	Leica/Novocastra	2	4	1	0	86%	100%
rmAb clone BC1, PRM 339	1	Biocare	0	0	1	0	-	-
Clone unknown ZM-0071	1	Zhongshan	1	0	0	0	-	-
Total	246		109	99	36	2	-	
Proportion			44%	40%	15%	1%	84%	

1) Proportion of sufficient stains (optimal or good)

2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of CK7, Run 40

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Antibodies

mAb clone **OV-TL 12/30**: Protocols with optimal results were based on either heat induced epitope retrieval (HIER), enzymatic pre-treatment or a combination of proteolysis and HIER.

Protocols based on HIER, used either Tris-EDTA/EGTA pH 9 (4/13)*, Target Retrieval Solution pH 9 (3-in-1) (Dako) (6/17), Target Retrieval Solution pH 9 (Dako) (5/11), Target Retrieval Solution pH 6.1 (Dako) (1/1), Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/2), Bond Epitope Retrieval Solution 2 (Bond, Leica) (6/15), Citrate buffer pH 6.7 (1/1) or Cell Conditioning 1 (BenchMark, Ventana) (13/40). 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

reaction (optimal or good).

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

Protocols based on enzymatic pre-treatment used the following enzymes: Protease 1 (Benchmark, Ventana) (1/10) or Proteinase K either applied as Ready-To Use format (Dako, S3020) or an in-house solution (0.1%, Sigma, P6556) (2/3). The mAb was typically diluted in the range of 1:50 – 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 7 of 13 (54%) laboratories produced a sufficient staining reaction.

One protocol with an optimal result was based on a combined pre-treatment using HIER in CC1 (Benchmark, Ventana) followed by Protease 3 (Benchmark, Ventana). The mAb was diluted 1:4,000.

mAb clone **RN7**: The protocol with an optimal result was based on enzymatic pre-treatment using Proteinase K (RE7160-K, Leica). The mAb was diluted 1:200.

Table 3 summarizes the overall proportion of optimal staining results for the most frequently used concentrated Ab on the three most commonly used IHC stainer systems.

Table 3. **Proportion of optimal results for CK7 using mAb clone OV-TL 12/30 as concentrate on the 3 main IHC systems***

Concentrated antibody	Dako		Ventana		Leica	
	Autostainer Link / Classic		BenchMark XT / Ultra		Bond III / Max	
Buffer	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	11/28** (39%)	1/1	13/46 (28%)	-	6/15 (40%)	1/2

* 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 (RTU) Antibodies

mAb clone **OV-TL 12/30** (prod. no. IS/IR619, Dako): Protocols with optimal results were all based on HIER using Target Retrieval Solution pH 9 (3-in-1), Target Retrieval Solution pH 9 or Tris-EDTA/EGTA pH 9 (heating time 10-20 min at 95-100°C), 10-30 min incubation of the primary Ab and EnVision Flex/Flex+ (K8000/K8002) as detection systems. Using these protocol settings 41 of 41 (100%) laboratories produced a sufficient staining reaction (optimal or good).

rmAb clone **SP52** (prod. no. 790-4462, Ventana): Protocols with optimal results were based on HIER using mild or standard Cell Conditioning 1, 8-72 min incubation of the primary Ab and iView (790-091), UltraView (760-500), UltraView (760-500) with amplification (760-080) or OptiView (760-700) as detection systems. Using these protocol settings 43 of 44 (98%) laboratories produced a sufficient staining reaction.

mAb clone **RN7** (prod. no. PA0942, Leica/Novocastra): Protocols with optimal results were based on HIER using Bond Epitope Retrieval Solution 2 (Bond, Leica) (heating time 20-30 min at 95-100°C), 15-30 min incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 3 of 3 (100%) laboratories produced a sufficient staining reaction.

In concordance with the previous NordiQC assessment of CK7 (run 25, 2009), the prevalent feature of an insufficient result was a overall too weak or a false negative staining of the cells expected to be demonstrated. The majority of the laboratories were able to demonstrate CK7 in high level antigen expressing structures such as alveolar epithelial cells of normal lung tissue or the neoplastic cells of the lung adenocarcinoma tissue core no. 6, whereas the demonstration of CK7 in the intercalating ducts of the pancreas and the neoplastic cells of the lung adenocarcinoma tissue core no. 7 was more challenging and required an optimally calibrated protocol.

Optimal staining results could be obtained with both the well characterized mAb clone OV-TL 12/30 and the relatively recently introduced antibodies mAb clone RN7 and rmAb clone SP52. Comparing the stainings for CK7, the reaction pattern was slightly different in both the kidney and stomach depending on the clone used. In general, the mAb clone OV-TL 12/30 identified a higher proportion of epithelial cells in the renal collecting ducts – although a population of cells within the collecting ducts displayed a weaker intensity. In comparison, the mAb clone RN7 and rmAb clone SP52 gave a completely negative reaction of the cell population being weakly to moderately stained with the mAb clone clone OV-TL 12/30 (illustrated in fig.4 a-4b). A different staining pattern was also observed in the luminal epithelial cells of the gastric mucosa. Using the mAb clone OV-TL 12/30 with optimal calibrated protocols, weak to moderate

cytoplasmic staining reaction was seen in the majority of the epithelial cells, whereas the staining reaction was absent or only seen in scattered cells using the mAb clone RN7 and rmAb clone SP52. However, if the overall staining result and staining pattern in the other tissue cores tested were identical both reaction patterns were accepted in this assessment.

The mAb clone OV-TL 12/30 was the most widely used antibody for CK7. Optimal staining results could be obtained on all main system (Dako, Ventana and Leica, see table 3). Applied as a concentrate, protocols based on HIER (preferred in alkaline buffer) provided a significant higher pass rate of 89% (93 of 105) and 34% were assessed as optimal compared to the use of proteolysis as pre-treatment. If proteolysis was used as pre-treatment, the pass rate was only 32% (9 of 28) and 11% was optimal.

In this run, the proportion of sufficient results was unaffected by the choice of detection system, whereas the titre of the primary Ab had a high impact on the final results. Using protocol settings based on the concentrated format of the mAb clone OV-TL 12/30, HIER performed in either an alkaline or standard acidic (pH 6) buffer and applying a standard 2-step polymer/multimer based detection system, the Average Dilution Value (ADV) for optimal results was 1:157 (range 1: 50 – 1:400, n=15 protocols). Protocols giving a result assessed as good used an ADV of 1:285 (range 1:30 – 1:2,000, n=34 protocols) and finally an ADV of 1:650 (range 1:50 – 1:2,000, n=7 protocols) was seen for protocols with insufficient result (borderline).

The most frequently applied Ready-To-Use assays for CK7 in this assessment were based on the mAb clone OV-TL 12/30 (IS/IR619, Dako), the rmAb clone SP52 (790-4462, Ventana) and the mAb clone RN7 (PA0942, Leica/Novocastra). The RTU system from Dako (IS/IR619) provided the highest pass rate of 100% (41 of 41) and 89% were assessed as optimal. The RTU system from Ventana (760-4378) gave a high pass rate of 98% (44 of 45) and 58% were optimal and finally the RTU system from Leica/Novocastra (PA0492) provided a slightly lower pass rate of 86% (6 of 7) and 29% were assessed as optimal.

Although the number of participants has increased significantly from Run 25 to Run 40 (116 new laboratories), the pass rate for CK7 in this run is nearly unchanged. The high quality and extended use of the Ready-To-Use (RTU) systems for CK7 from the two main providers Dako and Ventana contributed in this assessment to a high pass-rate of 100% and 98% respectively, thus being superior to the pass-rates for the in-house validated protocols (overall pass rate 76%). The most important parameters that contributed to insufficient staining results in this assessment were: Inappropriate epitope retrieval (inadequately calibrated enzymatic pre-treatment) and/or too low titer of the primary antibody.

Controls

Normal pancreas is recommendable as positive tissue control for CK7: The vast majority of epithelial cells of the intercalating ducts must show an at least weak to moderate cytoplasmic staining reaction, whereas the epithelial cells of large pancreatic ducts must show an intense staining reaction. No staining reaction should be seen in the epithelial acinar cells.

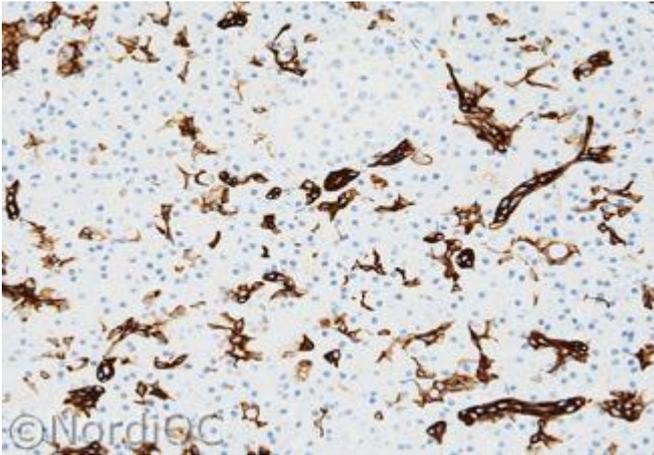


Fig. 1a
Optimal CK7 staining of the pancreas using the mAb clone OV-TL 12/30 optimally calibrated and with HIER. The epithelial cells of the large interlobular ducts show a strong cytoplasmic staining reaction, while the epithelial cells of the intercalated ducts show a weak to moderate staining reaction (same protocol used in Figs. 1a - 3a).

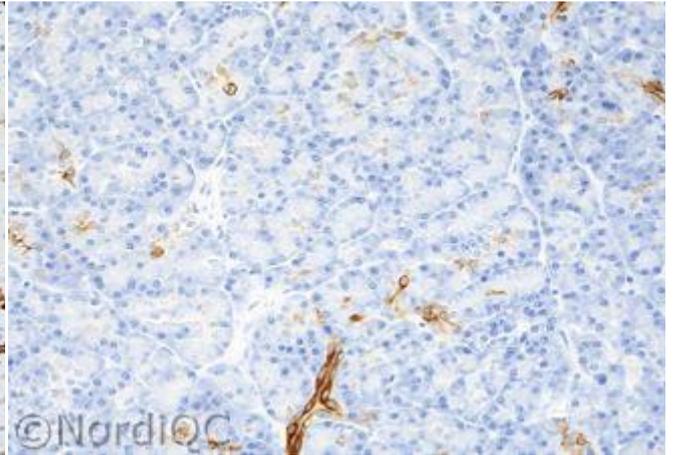


Fig. 1b
Insufficient CK7 staining of the pancreas using the mAb clone OV-TL 12/30 too diluted and with enzymatic pre-treatment - same field as Fig. 1a. Only the epithelial cells of the large interlobular ducts show a distinct staining reaction, while the epithelial cells of the intercalated ducts are almost negative and only show a diffuse staining reaction (same protocol used in Fig. 1b-3b).

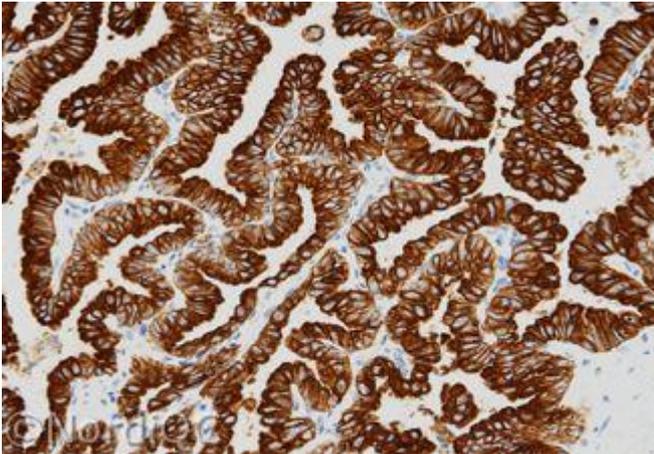


Fig. 2a
Optimal CK7 staining of the lung adenocarcinoma no. 6. All the neoplastic cells show a strong distinct cytoplasmic staining reaction.

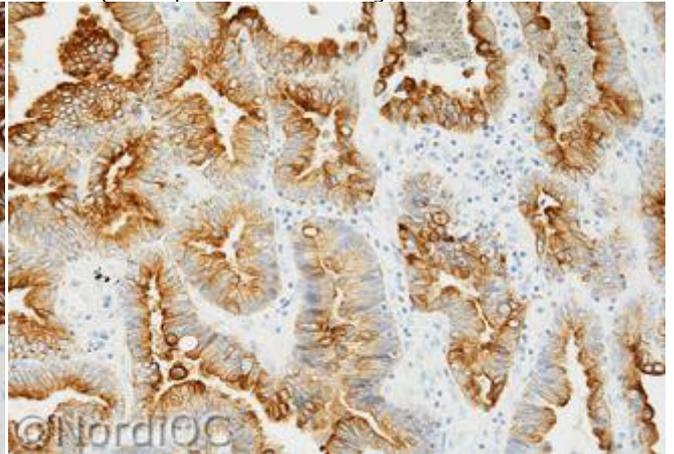


Fig. 2b
Insufficient CK7 staining of the lung adenocarcinoma no. 6 - same field as in Fig. 2a, using same protocol as in Fig. 1b. The neoplastic cells only show a weak to moderate cytoplasmic staining reaction. Compare with Fig. 2 a.

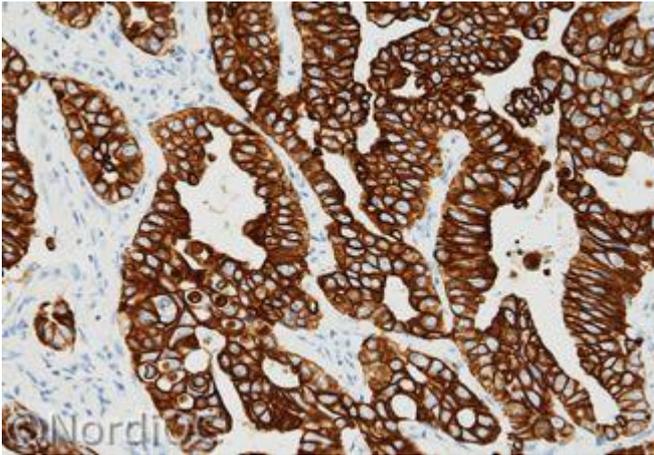


Fig. 3a
Optimal CK7 staining of the lung adenocarcinoma no. 7 using the same protocol as in Figs. 1a & 2a. Virtually all neoplastic cells show a moderate to strong cytoplasmic staining reaction.

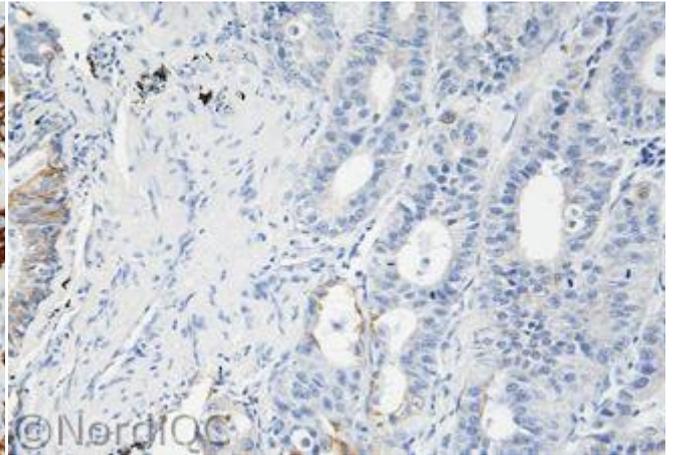


Fig. 3b
Insufficient CK7 staining of the lung adenocarcinoma no. 7 using the same protocol as in Figs. 1b & 2b - same field as Fig. 3a. The staining intensity is significantly reduced and only scattered neoplastic cells show a weak and diffuse cytoplasmic staining compared to the reaction seen in Fig. 3a.

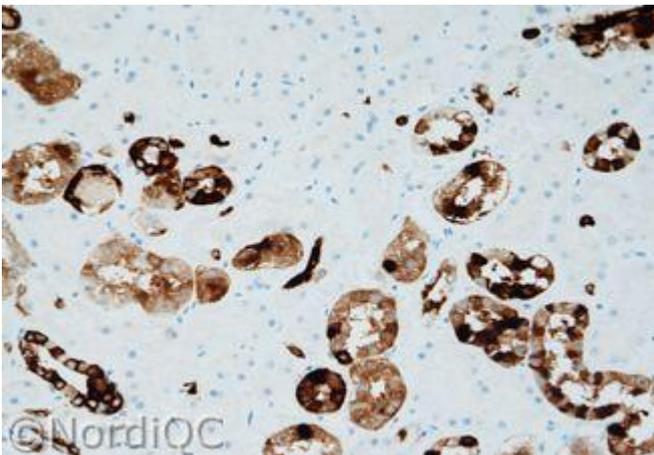


Fig. 4a
Optimal CK7 staining of the kidney using the mAb clone OV-TL 12/30 as concentrate, optimally calibrated, HIER in CC1 (Ventana) and OptiView (Ventana) as the detection system. Virtually all the epithelial cells of the collecting/distal ducts are stained, although a fraction of cells show a weaker intensity. Compare with Fig. 4b.

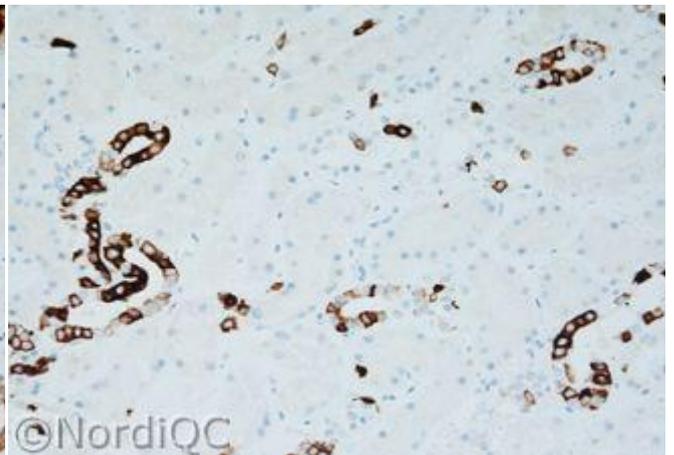


Fig. 4b
Optimal CK7 staining of the kidney using the rmAb clone SP52 as Ready-To-Use format (Ventana) and identical protocol settings as used in Fig. 4a - same field as Fig. 4a. Note, that the epithelial cells in the collecting/distal ducts, which display a weak to moderate staining with the mAb OV-TL 12/30, are completely negative. Otherwise the staining patterns in the other tissues as shown in Figs. 1a - 3a were identical. Thus, both results were assessed as optimal.

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