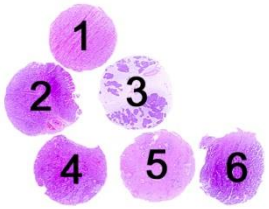
Assessment Run 25 2009

Cytokeratin 7 (CK7)

The slide to be stained for CK7 comprised:
1. Kidney, 2. Gastric corpus, 3. Pancreas, 4. Lung adenocarcinoma,
5. Pancreas adenocarcinoma, 6. Colon adenocarcinoma.
All tissues were fixed in 10 % neutral buffered formalin.



Criteria for assessing a CK7 staining as optimal included:

- A moderate to strong, distinct cytoplasmic reaction of virtually all the epithelial cells of the renal collecting ducts and the scattered epithelial cells in the Bowman capsule.
- A moderate, distinct cytoplasmic reaction of the majority of the foveolar epithelial cells of the stomach.
- A strong, distinct cytoplasmic reaction of virtually all the epithelial cells of the large pancreatic ducts while the epithelial cells of the intercalating ducts at least should show a weak to moderate cytoplasmic reaction.
- A moderate to strong, distinct cytoplasmic reaction in the majority of the neoplastic cells of the lung and the pancreas adenocarcinoma.
- No reaction in the proximal tubules of the kidney and the acinar cells of the pancreas.

130 laboratories participated in the assessment. 86 % achieved a sufficient mark. The results are summarized in Table 1.

Table 1. **Abs and scores for CK7, run 25**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **OV-TL 12/30**	93 5 3 3 2 2 1 1	Dako Novocastra NeoMarkers BioGenex Euro Diagnostica Monosan Bio-Optica Zymed	46	48	14	2	86 %	94 %
mAb clone **K72.7**	2 1	Biocare NeoMarkers	0	2	1	0	-	-
Ready-To-Use Abs								
mAb clone **OV-TL 12/30**	10	Ventana, 760-2224	7	3	0	0	100 %	100 %
mAb clone **OV-TL 12/30**	4	Dako, IR619	4	0	0	0	-	-
mAb clone **OV-TL 12/30**	2	Dako, N1626	0	1	0	1	-	-
mAb clone **OV-TL 12/30**	1	Linaris	0	1	0	0	-	-
Total	130		57	55	15	3	112	-
Proportion			44 %	42 %	12 %	2 %	86 %	95 %

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

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

Concentrated Ab
mAb clone **OV-TL 12/30**: the protocols giving an optimal result were based on either heat induced epitope retrieval (HIER) or enzymatic pre-treatment.

Protocols based on HIER, used Tris-EDTA/EGTA pH 9 (22/36)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (6/14)*, Cell Conditioning 1 (BenchMark, Ventana) (3/7), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/8), Target Retrieval Solution pH 6.1 (Dako) (1/2), or Citrate pH 6 (1/8) as retrieval buffer. The mAb was typically diluted in the range of 1:50 – 1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 64 out of 67 (96 %) laboratories produced a sufficient staining (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) (7/13), Proteinase K (Dako) (2/5), Bond Enzyme 1 (Bond, Leica) (1/2), Protease 3 (Benchmark, Ventana) (1/2), or Protease Type XIV Sigma (1/1). The mAb was typically diluted in the range of 1:50 – 1:1.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 19 out of 21 (90 %) laboratories produced a sufficient staining.

Ready-To-Use Abs
Using the mAb clone **OV-TL 12/30**, prod. no. 760-2224, Ventana, the protocols giving an optimal result were based either on HIER, enzymatic pre-treatment or combined enzymatic pre-treatment end HIER.

In the protocols using HIER, Cell Conditioning 1, mild, was used as retrieval buffer and the incubation time for the primary Ab was 32 min and UltraView or iView were used as detection system. Using these protocol settings 3 out of 3 (100 %) laboratories produced a sufficient staining.

Using enzyme as pre-treatment, the optimal protocols were based on Protease 1 for 4 or 8 min. The incubation time for the primary Ab was 20 - 32 min and UltraView or iView were used as detection system. Using these protocol settings 3 out of 3 (100 %) laboratories produced a sufficient staining.

One optimal protocol was based on combined enzyme pre-treatment and HIER. Protease 2 was used as enzymatic pre-treatment for 8 min, and Cell Conditioning 1, short as HIER, the incubation time for the primary Ab was 32 min and UltraView was used as the detection system.

Using the mAb clone **OV-TL 12/30**, IR619, Dako, the protocols giving an optimal result were all based on HIER using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH) or Tris/EDTA pH 9 and an incubation time in 20 min for the primary Ab and EnVision Flex as the detection system. Using these protocol settings 4 out of 4 (100 %) laboratories produced a sufficient staining.

The most frequent cause of insufficient staining were:
- Too low concentration of the primary antibody

In this assessment the prevalent feature of an insufficient staining was either a too weak general staining or a complete false negative reaction of the structures supposed to be demonstrated. Virtually all laboratories were able to demonstrate CK7 in high antigen expressing structures as the large ducts of the pancreas and the neoplastic cells of the pancreas adenocarcinoma, whereas the neoplastic cells of the lung adenocarcinoma, the foveolar epithelial cells of the stomach and the epithelial cells of the intercalating ducts in the pancreas expressed less CK7 and was thus more challenging and required an optimally calibrated protocol. In this run both HIER and enzymatic pre-treatment could be used to obtain an optimal staining, when the mAb clone OV-TL 12/30 was used. However from a general perspective HIER should be preferred to eliminate or reduce the influence of the fixation time in formalin, as enzymatic pre-treatment in generel shall be adjusted to the fixation time in formalin for an optimal result. In the optimal calibrated protocols a CK7 reaction frequently was seen in scattered endothelial cells and stromal cells. In this assessment pancreas was a recommended control for CK7 providing the epithelial cells of the intercalating ducts at least showed a weak to moderate reaction, while the acinar cells were negative.

This was the 2' assessment of CK7 in NordiQC, as CK7 also was assessed in run 8, 2003 and an identical proportion of sufficient results were seen in the two runs as shown in table 2. This shows that the immunohistochemical analysis for CK7 is relatively robust despite the number of participants almost has been doubled and new material has been circulated in this run.

Table 2. **Sufficient results with CK7 in two runs**

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

Conclusion
The mAb clone OV-TL 12/30 is a recommendable and robust marker for CK7.
Both HIER and enzymatic pre-treatment can be used to obtain an optimal staining for CK7. However HIER should be preferred to reduce the influence of the formalin fixation time. Pancreas is recommended as positive control in which the epithelial cells of the intercalating ducts shall show an as strong as possible reaction, while the acinar

cells shall be negative.

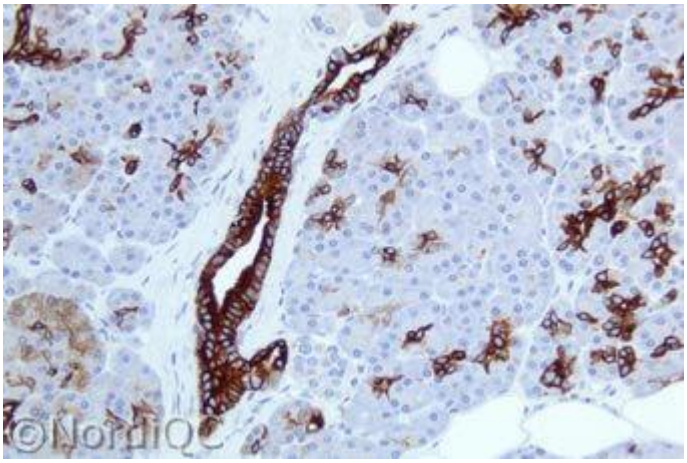


Fig. 1a
Optimal staining for CK7 of the pancreas using the mAb clone OV-TL 12/30, optimally calibrated and with HIER. The epithelial cells of the large interlobular ducts show a strong cytoplasmic reaction, while the epithelial cells of the intercalated ducts a weak to moderate reaction (same protocol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

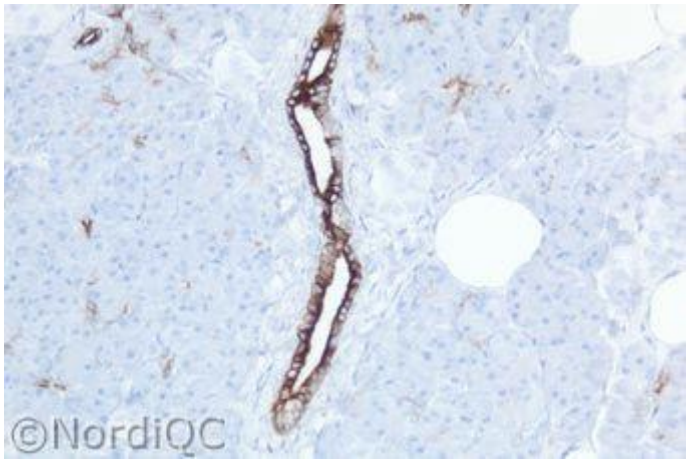


Fig. 1b
Insufficient staining for CK7 of the pancreas (using the mAb clone OV-TL 12/30 with HIER, but too diluted. Only the epithelial cells of the large interlobular ducts show a distinct staining, while the epithelial cells of the intercalated ducts are almost negative and only show a diffuse reaction (same protocol used in Fig. 2b).

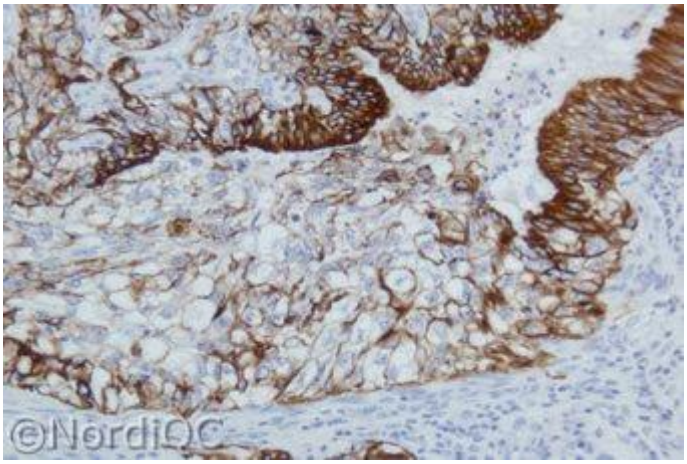


Fig. 2a
Optimal staining for CK7 of the lung adenocarcinoma. The majority of the neoplastic cells show a weak to moderate reaction, while the remnants of the normal lung epithelial cells show a moderate to strong reaction (same protocol used in Fig. 1a.)

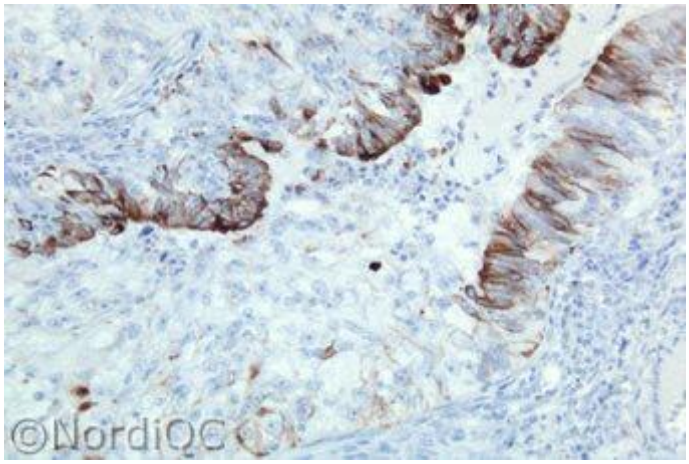


Fig. 2b
Insufficient staining for CK7 of the lung adenocarcinoma - same field as in Fig. 2a. Only the remnants of the normal lung epithelial cells are demonstrated, while the neoplastic cells are almost negative and only show a diffuse reaction (same protocol used in Fig. 1b).

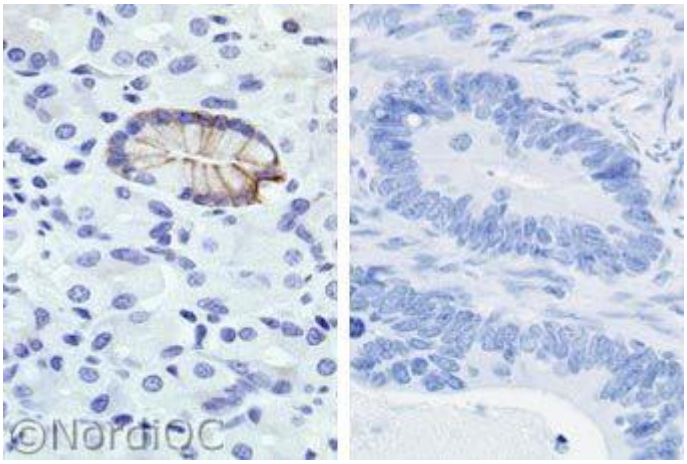


Fig. 3a
Optimal staining for CK7 using same optimal protocol as in Fig. 1a & 2a. based on a polymer based detection system.
Left: Gastric mucosa: The foveolar epithelial cells show a membranous reaction, while the chief cells are negative.
Right: Colon adenocarcinoma: The neoplastic cells are negative.

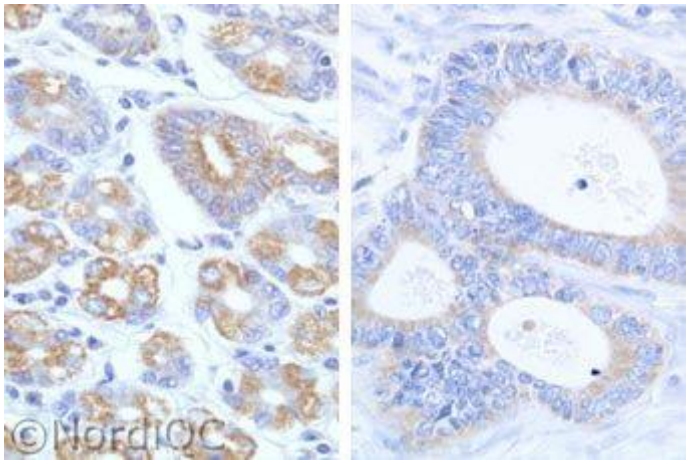


Fig. 3b
Insufficient staining for CK7 using the mAb clone OV-TL 12/30 with HIER and an avidin-biotin based detection system.
Left: Gastric mucosa: The foveolar epithelial cells (middle of the photo) show a membranous reaction, but also a distinct granular cytoplasmic reaction in the chief cells.
Right: Colon adenocarcinoma: The neoplastic cells show a positive cytoplasmic reaction.
The positive reaction in the two specimens is due to a false positive reaction of endogenous biotin. Also compare with Fig. 3a.

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