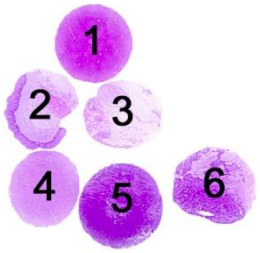
Assessment Run 25 2009

CK-LMW

The slide to be stained for CK-LMW comprised:
1. Liver, 2. Esophagus, 3. Breast, 4. Renal clear cell carcinoma, 5. Small cell lung carcinoma, 6. Colon adenocarcinoma.
All tissues were fixed in 10 % neutral buffered formalin.



Criteria for assessing a CK-LMW staining as optimal included:

- A strong, distinct cytoplasmic reaction of virtually all the bile ductal epithelial cells, and at least a moderate, predominantly membranous reaction of the large majority of hepatocytes.
- A strong, distinct cytoplasmic reaction of virtually all the breast ductal epithelial cells.
- A moderate to strong, distinct staining of the majority of the neoplastic cells of the renal cell carcinoma, the small cell lung carcinoma and the colon adenocarcinoma.
- No staining of the esophageal squamous epithelial cells, except for a staining of the basal cells, when using an Ab reacting with CK8.

108 laboratories submitted stains but 9 laboratories used an inappropriate antibody such as pan-CK or CK19. Among the 99 laboratories, 66 % achieved a sufficient mark. The results are summarized in Table 1.

Table 1. **Abs and scores for CK-LMW, run 25**

Concentrated Abs	CK types	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **DC10**	18	28 4 2 1	Dako Novocastra NeoMarkers ID Labs inc	26	7	1	1	94 %	97 %
mAb clone **CAM 5.2**	8/(7)	19	Becton Dickinson	1	7	4	7	42 %	100 %
mAb clone **5D3**	8/18	7 6 2 1 1 1	NeoMarkers Novocastra Santa Cruz BioCare BioGenex Diagnostic Bios.	6	7	4	1	72 %	82 %
mAb clone **35BH11**	8	8 1	Dako Biotrend	0	0	3	6	-	-
mAb clone **C51**	18*	7	Zymed	6	0	1	0	86 %	86 %
mAb clone **Ks-B17.2**	18	1	Sigma	0	0	1	0	-	-
mAb clone **TS1**	8	1	NeoMarkers	0	1	0	0	-	-
mAb clones **K8.8+DC10**	8/18	1	NeoMarkers	0	1	0	0	-	-
mAb clone **UCD/PR-10.11**	8/18	1	Zymed	0	0	0	1	-	-
Ready-To-Use Abs									
mAb clone **35βH11**	8	3	Ventana	0	0	0	3	-	-
mAb clones **B22.1 & B23.1**	8/18	2	Ventana	0	2	0	0	-	-
mAb clone **5D3**	8/18	1	Novocastra	0	1	0	0	-	-
mAb clone **DC10**	18	1	Dako	1	0	0	0	-	-
Total		99		40	26	14	19	-	-
Proportion				40 %	26 %	14 %	19 %	66 %	84 %

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
* Claimed by Zymed to be CK8.

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

Concentrated Abs
mAb clone **DC10**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (15/19)*, Cell Conditioning 1 (BenchMark, Ventana) (5/6), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (3/6), Bond Epitope Retrieval Solution 2 (Bond, Leica) (2/3) or Citrate pH 6 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:20 – 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 33 out of 34 (97 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **C51**: The protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (4/4) or Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako (2/3). The mAb was typically diluted in the range of 1:50 – 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 out of 7 (86%) laboratories produced a sufficient staining (optimal or good).

mAb clone **5D3**: The protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (3/7), Bond Epitope Retrieval Solution 2 (Bond, Leica) (2/2) or Citrate pH 6 (1/3) as retrieval buffer. The mAb was typically diluted in the range of 1:20 – 1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings 9 out of 11 (82%) laboratories produced a sufficient staining.

mAb clone **CAM 5.2**: The protocol giving an optimal result was based on enzyme pre-treatment with Protease 1 (BenchMark, Ventana) (1/5) and the Ab was used undiluted.

Ready-To-Use Abs
mAb clone **DC10**, IR618, Dako: The protocol giving an optimal result was based on HIER using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH) and an incubation time in 20 min for the primary Ab and EnVision Flex as the detection system.

The most frequent causes of insufficient staining were:
- Less successful antibodies (e.g., 12/12 protocols based on the mAb clone 35BH11 gave an insufficient result)
- Inappropriate epitope retrieval (e.g. enzymatic pre-treatment for the mAb clone 5D3)
- Too low concentration of the primary Ab.

In this assessment and in concordance with the previous CK-LMW assessments (run 16 and 20) the prevalent feature of an insufficient staining was a too weak or negative reaction of cells expected to stain. The majority of the laboratories were able to demonstrate CK-LMW in the bile duct epithelium and the colon adenocarcinoma. However, the demonstration of CK-LMW in the small cell lung carcinoma and especially the renal cell carcinoma was more difficult and only seen with appropriate protocol settings, e.g., a correct titre of the mAb clones DC10 and 5D3 combined with efficient HIER.
As observed in the previous assessments of CK-LMW, liver was a reliable positive control, as all laboratories that could demonstrate the membranous reaction in the hepatocytes also could demonstrate CK-LMW in the renal cell carcinoma, which in this run was the most challenging tumour.
The choice of Ab has a high impact on the pass rate, as e.g. the proportion of sufficient stains based on the mAb clone 5D3 was 72 % compared to 0 % ,when the mAb clone 35BH11 was used, despite the number of participants and otherwise applied protocol settings were similar for the two clones. In Table 2, the overall pass rates are summarized for the most widely used clones in the last three CK-LMW assessments.

Table 2. **Performance of five commonly used clones in three runs**

	Run 16 2006		**Run 20 2007**		**Run 25 2009**		**Total**	
	N	Suffic.	N	Suffic.	N	Suffic.	N	Suff. (%)
mAb clone DC10	16	14	21	19	36	33	73	66 (90 %)
mAb clone CAM5.2	27	10	20	11	19	8	66	29 (44 %)
mAb clone 35βH11	12	2	14	4	12	0	38	6 (16 %)
mAb 5D3	6	4	9	5	18	14	33	23 (70 %)
mAb C51	6	5	8	8	7	6	21	19 (91 %)

These data clearly indicates that the mAb clones CAM5.2 and 34BH11 have been less successful in three successive assessments for CK-LMW. The most robust markers for CK-LMW are the clones C51 and DC10, followed by the clone 5D3. The literature and vendors' information on clone C51 give conflicting information about the reactivity with CK types 8 and CK18. In an analysis in the NordiQC laboratory, the reaction pattern of clone C51 is

identical with CK18 antibodies like clone DC10 and different from Abs reacting with CK8.
This was the fifth assessment of CK-LMW in NordiQC. Identical pass rates has been achieved in the last two runs as shown in table 3.

Table 3. **Pass rates in five NordiQC tests for CK-LMW**

	Run 2 2000	Run 9 2003	Run 16 2006	Run 20 2007	Run 25 2009
Participants	9	54	66	74	99
Sufficient results	44 %	57 %	45 %	67 %	66 %

In the previous assessments of CK-LMW (run 16 and 20), a total of 61 laboratories obtaining an insufficient result have been given specific recommendations how to improve their protocol. 47 laboratories submitted a new stain in the subsequent run. 26 followed the recommendation, of which 20 improved to good or optimal (77 %). 19 laboratories did not follow the recommendation, and only 3 of these (16 %) obtained a sufficient staining in the subsequent run.

Conclusion
The mAb clones DC10, 5D3 and C51 seem to be robust and sensitive Abs for CK-LMW and should replace the old Abs clones CAM 5.2 and 35βH11. HIER, preferably in an alkaline buffer, seems mandatory for optimal performance for all three recommended clones.
Liver is an appropriate control for CK-LMW: The majority of hepatocytes shall show an at least moderate staining with an enhancement along the cell membranes.

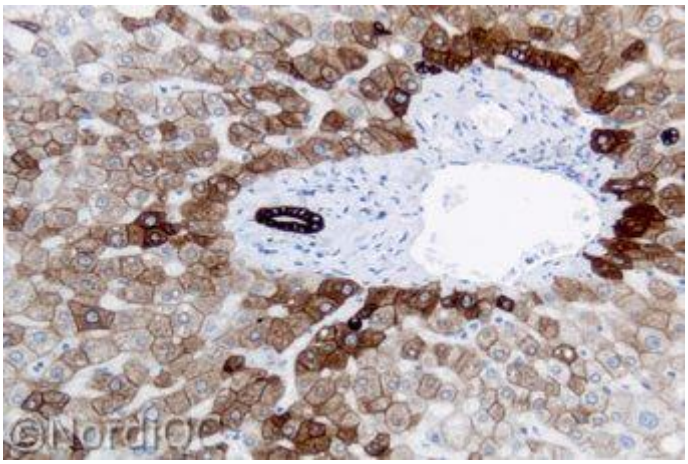


Fig. 1a
Optimal staining for CK-LMW of the liver using the mAb clone DC10 with HIER. The majority of the hepatocytes show a distinct, moderate to strong, predominantly membranous reaction, while the bile duct epithelial cells show an intense cytoplasmic reaction (same protocol used in Fig. 1a – 4a).

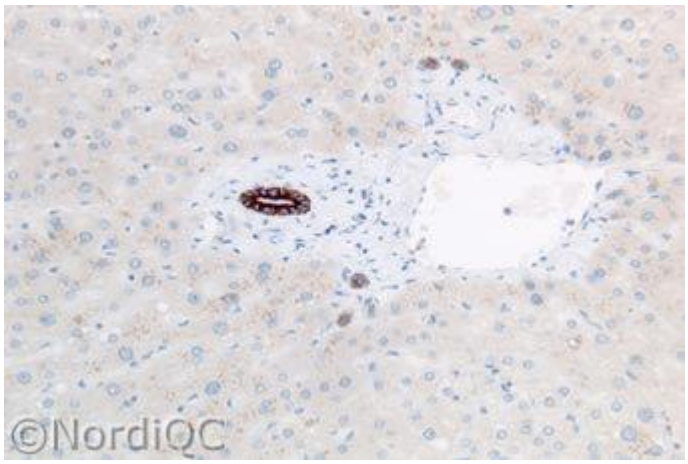


Fig. 1b
Insufficient staining for CK-LMW of the liver (same field as in Fig 2a) using the mAb clone 35BH11 with HIER. Only the bile duct epithelial cells are demonstrated, while the hepatocytes are almost negative and only show a diffuse reaction (same protocol used in Fig. 2b & 3b).

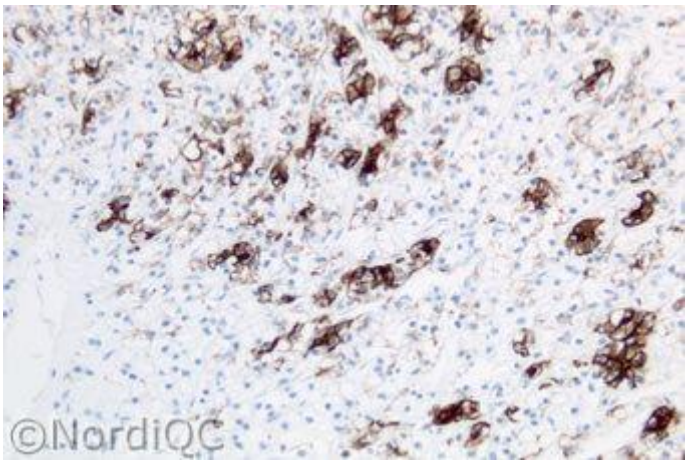


Fig. 2a
Optimal staining for CK-LMW of the renal clear cell carcinoma. The majority of the neoplastic cells show a moderate to strong and distinct reaction (same protocol used in Fig. 1a - 4a).

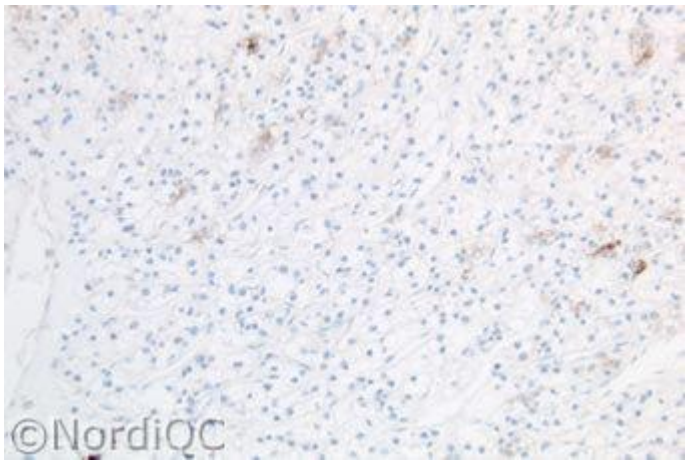


Fig. 2b
Insufficient staining for CK-LMW of the renal cell carcinoma - same field as in Fig. 2a. The proportion and intensity of the positive cells is highly reduced compared to the result in Fig. 2a (same protocol used in Fig. 1b & 3b).

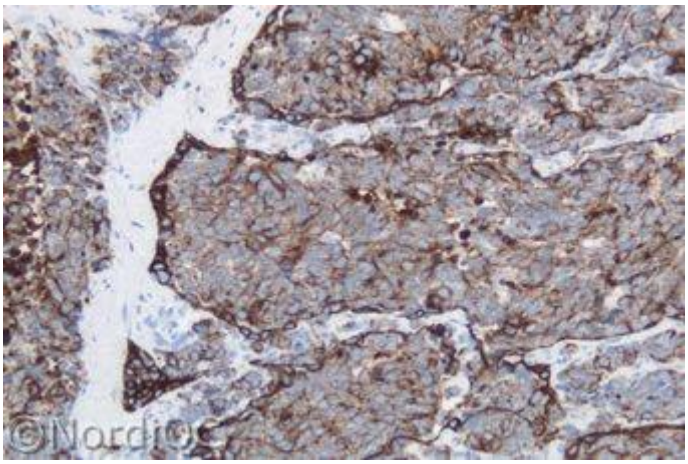


Fig. 3a
Optimal staining for CK-LMW of the small cell lung carcinoma. The majority of the neoplastic cells show a moderate cytoplasmic reaction, while the remnants of the normal lung epithelial cells (at the periphery of the tumor nests) show a strong cytoplasmic reaction (same protocol used in Fig. 1a - 4a).

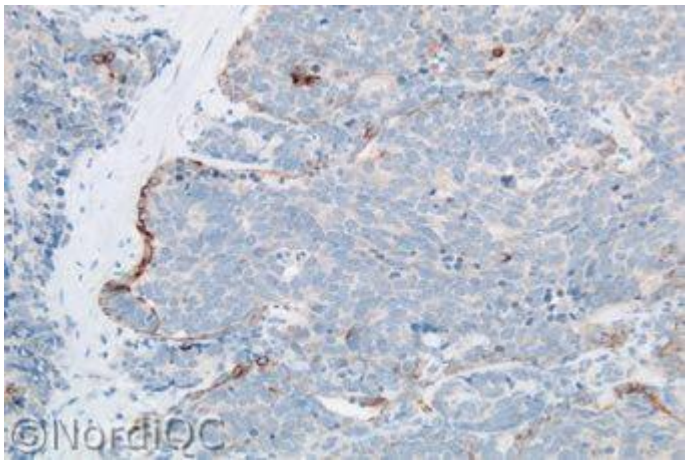


Fig. 3b
Insufficient staining for CK-LMW of the small cell lung carcinoma same - field as in Fig 3a. Only the remnants of the normal lung epithelial cells are demonstrated, while the neoplastic cells are almost negative and only show a diffuse reaction (same protocol used in Fig. 1b & 2b)

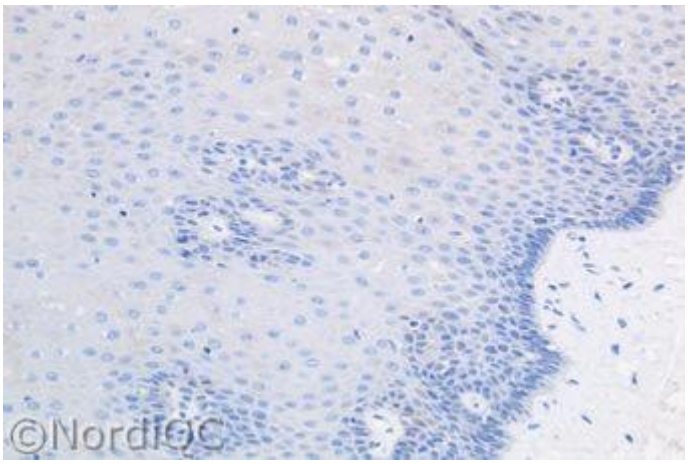


Fig. 4a
Optimal staining for CK-LMW of the esophagus using same protocol as in Fig. 1a – 3a. No staining is seen in the squamous epithelial cells. This pattern was seen with the mAb clones DC10 & C51 reacting with CK type 18. Also compare with the result obtained the mAb clone CAM 5.2 in Fig. 4b.

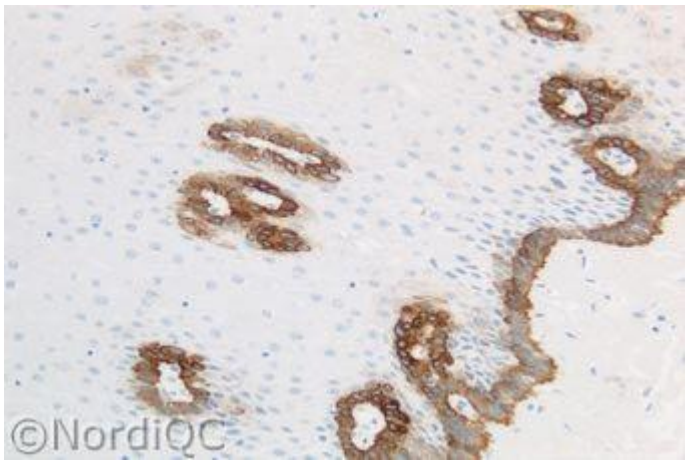


Fig. 4b
Optimal staining for CK-LMW of the esophagus using the mAb clone CAM 5.2 with proteolysis and applied as Ready-To-Use. The basal epithelial cells expressing CK type 8 show a distinct cytoplasmic reaction, while all other squamous epithelial cells are negative.

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