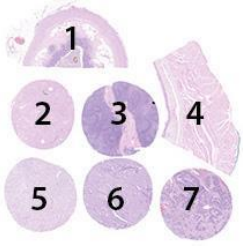
## Assessment Run 57 2019

# Cytokeratin 8/18 (CK8/18)

**Purpose**
Evaluation of the technical performance and level of analytical sensitivity and specificity of the IHC assays for CK8/18 performed by the NordiQC participants, identifying non-squamous carcinomas including adenocarcinoma of unknown origin (e.g. breast carcinoma, renal clear cell carcinoma and small cell lung carcinoma). Relevant clinical tissues, both normal and neoplastic, were selected to include a wide spectrum of CK8/18 antigen densities (see below).

**Material**
The slide to be stained for CK8/18 comprised:




1. Appendix, 2. Liver, 3. Tonsil, 4. Esophagus, 5. Renal clear cell carcinoma, 6. Breast ductal carcinoma, 7. Small cell lung carcinoma (SCLC).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CK8/18 staining as optimal included:

- A strong, distinct cytoplasmic staining reaction of virtually all appendiceal columnar epithelial cells and bile duct epithelial cells.
- An at least weak to moderate predominantly membranous staining reaction of virtually all hepatocytes.
- A moderate to strong cytoplasmic staining reaction in the majority of squamous epithelial cells in tonsil. In tonsil, cytokeratin-positive interstitial reticulum cells (CIRCs) with dendritic/reticular pattern is expected to show a weak to moderate cytoplasmic staining reaction.
- A moderate to strong, distinct cytoplasmic staining reaction of virtually all neoplastic cells in the breast ductal carcinoma.
- An at least weak to moderate cytoplasmic staining reaction in the majority of neoplastic cells in the renal clear cell carcinoma and SCLC.

**Participation**

| | |
|---|---|
| Number of laboratories registered for CK8/18, run 57 | 250 |
| Number of laboratories returning slides | 240 (96%) |

**Results**
240 laboratories participated in this assessment. 2 submitted slides stained with an inappropriate primary antibody for an epitope like CK-PAN or CK-HMW. Of the remaining 238 laboratories, 66% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks given (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful primary antibodies (all CK18 antibodies)
- Less successful performance of the mAb clone 5D3 on the Ventana BenchMark platform
- Too low concentration of the primary Ab
- Use of less sensitive detection systems
- Inappropriate epitope retrieval

**Performance history**
This was the eighth NordiQC assessment of CK8/18 (CK-LMW). The pass rate was similar compared to the previous run 49, 2017 as shown in Table 2.

Table 2. **Proportion of sufficient results for CK8/18 (CK-LMW) in the eight NordiQC runs performed**

| | Run 9 2003 | Run 16 2006 | Run 20 2007 | Run 25 2009 | Run 33 2011 | Run 38 2013 | Run 49 2017 | Run 57 2019 |
|---|---|---|---|---|---|---|---|---|
| Participants, n= | 55 | 66 | 74 | 99 | 141 | 161 | 213 | 238 |
| Sufficient results | 57% | 45% | 67% | 66% | 64% | 77% | 66% | 66% |

**Conclusion**
Within a laboratory developed (LD) assay, the mAb clone cocktail **B22.1/B23.1** *(CK8/CK18)*, rmAb clone cocktail **EP17/EP30** *(CK8/CK18)* and rmAb clone **EP17** *(CK8)* are all highly recommendable Abs for demonstration of CK8/CK18. Irrespective of selected clone, Heat Induced Epitope Retrieval (HIER) at high pH, use of a sensitive 2- or 3-step polymer/multimer based detection system and appropriate calibration of the titer of the primary antibody were crucial for an optimal performance. Using one of these three Abs, within a LD assay, a pass rate of 86% was seen, which was significantly higher than the overall LD assay pass rate of 50% obtained in this assessment.
The Ventana Ready-To-Use (RTU) system (760-4344) based on the mAb clone cocktail **B22.1/B23.1** *(CK8/CK18)*, and the Dako RTU system (IR094) based on rmAb clone cocktail **EP17/EP30** *(CK8/CK18)* are both highly recommendable Abs for demonstration of CK8/CK18. Vendor recommended protocol settings and laboratory modified protocol settings both results in a high proportion of sufficient staining results.

The CK18 antibodies (mAb clones DC10, C51, and CY90) all failed to convincingly demonstrate positive staining reaction in the breast ductal carcinoma. Bearing in mind that partial or complete loss of CK18 expression has been observed in ~25% of breast carcinomas depending on their histological type[1], the use of the individual CK18 in identifying non-squamous carcinomas including adenocarcinoma of unknown origin cannot be recommended.

Liver is recommended as primary positive tissue control. Virtually all hepatocytes must show an at least moderate cytoplasmic staining reaction, typically with membranous accentuation, while the epithelial cells lining the bile ducts must show a strong cytoplasmic staining reaction. No staining should be seen in the connective tissue and lymphocytes in the portal rooms. Tonsil is a recommended additional tissue control, serving both as positive and negative tissue control. The majority of squamous epithelial cells must display a moderate to strong cytoplasmic staining reaction, whereas no staining reaction should be seen in lymphocytes. In tonsil, cytokeratin-positive interstitial reticulum cells (CIRCs) with dendritic/reticular pattern will show a weak to moderate cytoplasmic staining reaction. Appendix cannot be recommended as positive tissue control as the epithelial cells only express high levels of CK8/18

Table 1. **Antibodies and assessment marks for CK8/18, run 57**

| Concentrated antibodies | reactivity | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|---|
| mAb clone **34betaH11** | *CK8* | 1 | Diagnostic BioSystems | 0 | 0 | 1 | 0 | - | - |
| mAb clone **5D3** | *CK8/18* | 16<br>3<br>2<br>2<br>1<br>1 | Leica/Novocastra<br>Diagnostic BioSystems<br>Biocare<br>Thermo Scientific<br>DCS<br>Monosan | 5 | 6 | 4 | 10 | 44% | - |
| mAb clones **B22.1/B23.1** | *CK8/18* | 11<br>1<br>1<br>1 | Cell Marque<br>Bio SB<br>Immunologic<br>Menarini | 8 | 3 | 2 | 1 | 79% | 92% |
| mAb clone **C51** | *CK18* | 1 | Zymed | 0 | 0 | 0 | 1 | - | - |
| mAb clone **CAM5.2** | *CK8(7)* | 3 | Zytomed | 0 | 1 | 2 | 0 | - | - |
| mAb clone **CY90** | *CK18* | 1<br>1 | Nordic Mubio<br>Sigma | 0 | 0 | 1 | 1 | - | - |
| mAb clone **DC10** | *CK18* | 11<br>3<br>1<br>1 | Agilent/Dako<br>Thermo Scientific<br>Biocare<br>Immunologic | 0 | 0 | 9 | 7 | 0% | - |
| mAb clone **TS1** | *CK8* | 2<br>1 | Thermo Scientific<br>Leica/Novocastra | 0 | 2 | 1 | 0 | - | - |
| mAb clone **TS1+DC10** ("homebrew") | *CK8/18* | 1 | Thermo + Dako | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **BSR15** | *CK8* | 1 | Nordic Biosite | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **EP17** | *CK8* | 4<br>1 | Epitomics<br>Cell Marque | 4 | 0 | 1 | 0 | - | - |
| rmAb clones **EP17/EP30** | *CK8/18* | 9<br>1 | Dako/Agilent<br>Cell Marque | 9 | 1 | 0 | 0 | 100% | - |
| Ready-To-Use antibodies | | | | | | | | | |
| mAb clone **35betaH11 760-2637** | *CK8* | 2 | Roche/Ventana | 0 | 0 | 0 | 2 | - | |
| mAb clones **5D3 PA0067** | *CK8/18* | 6 | Leica/Novocastra | 1 | 5 | 0 | 0 | - | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| mAb clones **5D3 PA0067** [3] | *CK8/18* | 2 | Leica/Novocastra | 0 | 0 | 1 | 1 | - |
| mAb clone **5D3 PM056** | *CK8/18* | 2 | Biocare | 0 | 0 | 1 | 1 | - |
| mAb clone **5D3 AM131** | *CK8/18* | 1 | BioGenex | 0 | 0 | 1 | 0 | - |
| mAb clones **B22.1/B23.1 760-4344** | *CK8/18* | 65 | Roche/Ventana | 28 | 31 | 5 | 1 | 91% |
| mAb clones **B22.1/B23.1 BSB 5415** | *CK8/18* | 1 | Bio SB | 0 | 1 | 0 | 0 | - |
| mAb clones **B22.1/B23.1 818M-97/98** | *CK8/18* | 1 | Cell Marque | 0 | 0 | 0 | 1 | - |
| mAb clones **B22.1/B23.1 MAD-000589QD** | *CK8/18* | 1 | Master Diagnostica | 1 | 0 | 0 | 0 | - |
| mAb clone **CAM5.2 345777/349205** | *CK8 (7)* | 13 | BD Bioscience | 2 | 2 | 5 | 4 | 31% |
| mAb clone **CAM5.2 790-4555** | *CK8 (7)* | 5 | Roche/Ventana | 1 | 2 | 2 | 0 | - |
| mAb clone **CAM5.2 452M-98** | *CK8 (7)* | 1 | Cell Marque | 0 | 0 | 0 | 1 | - |
| mAb clone **CAM5.2 BSB 2058** | *CK8 (7)* | 1 | Bio SB | 0 | 0 | 0 | 1 | - |
| mAb clone **DC10 IR618/IS618** | *CK18* | 5 | Agilent/Dako | 0 | 1 | 3 | 1 | - |
| mAb clone **DC10 GA618** | *CK18* | 9 | Agilent/Dako | 0 | 2 | 6 | 1 | - |
| mAb clone **MX029 MAB-0718** | *CK8/18* | 1 | Maixin | 1 | 0 | 0 | 0 | - |
| mAb clone **TS1 PA0567** | *CK8 (7)* | 1 | Leica/Novocastra | 0 | 0 | 1 | 0 | - |
| rmAb clone **EP17** + mAb clone **DC10 8298-C010** | *CK8/18* | 1 | Sakura Finetek | 1 | 0 | 0 | 0 | |
| rmAb clones **EP17/EP30 IR094** | *CK8/18* | 15 | Agilent/Dako | 13 | 2 | 0 | 0 | 100% |
| rmAb clones **EP17/EP30 IR094** [4] | *CK8/18* | 16 | Agilent/Dako | 16 | 0 | 0 | 0 | 100% |
| rmAb clones **EP17/EP30 IR094** [5] | *CK8/18* | 4 | Agilent/Dako | 2 | 2 | 0 | 0 | - |
| Unknown clone, conflicting data submitted [6] | *?* | 3 | Agilent/Dako? | 3 | 0 | 0 | 0 | - |
| Total | | 238 | | 97 | 61 | 46 | 34 | - |
| Proportion | | | | 41% | 25% | 20% | 14% | 66% |

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Leica Bond systems, but used by laboratories on different platforms (e.g. Ventana BenchMark)
4) RTU system developed for the semiautomatic Dako Autostainer system but used by laboratories on the fully automated Dako Omnis platform.
5) RTU system developed for the semiautomatic Dako Autostainer system but used by laboratories on different platforms (e.g. Leica Bond, BioCare IntelliPath or Ventana BenchMark)
6) Conflicting data submitted. Most of the submitted data indicate the use of mAb clone DC10 IR618/IS618 or GA618, but the submitted lot no. for the RTU's and staining pattern of the submitted slides strongly supports the use of the rmAb clones EP17/EP30 IR094.

### Detailed analysis of CK8/18, run 57

The following protocol parameters were central to obtain optimal staining:

### Concentrated antibodies

mAb clone **5D3**: Protocols with optimal results were all based on HIER using Bond Epitope Retrieval Solution 2 (BERS2, Leica) (5/9)* as retrieval buffer. The mAb was diluted in the range of 1:50-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 6 of 7 (86%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clones **B22.1/B23.1**: Protocols with optimal results were all based on HIER using either Cell Conditioning 1 (CC1, Ventana) (5/9), Target Retrieval Solution (TRS) High pH (3-in-1) (Dako) (1/2), TRS, High pH (Dako) (1/1) or BERS2 (Leica) (1/1) as retrieval buffer. The mAb was diluted in the range of 1:50-1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings, 11 of 12 (92%) laboratories produced a sufficient staining result.

rmAb clone **BSR15**: One protocol with an optimal result was based on HIER using Tris-EGTA/EDTA pH 9 (efficient heating time 20 min. at 98°C), 30 min. incubation of the primary Ab, diluted 1:100 and Biosite Histo Plus HRP Polymer anti-Rabbit kit (KDB-10046, Nordic Biosite) as detection system.

rmAb clone **EP17**: Protocols with optimal results were all based on HIER using CC1 (Ventana) (4/5). The rmAb was diluted 1:100. Using these protocol settings, 4 of 4 (100%) laboratories produced an optimal staining.

rmAb clones **EP17/EP30**: Protocols with optimal results were all based on HIER using either CC1 (Ventana) (5/5), TRS High pH (Dako) (3/3) or BERS2 (Leica) (1/1) as retrieval buffer. The mAb was diluted in the range of 1:50-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 9 of 9 (100%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for CK8/18 for the most commonly used antibodies as concentrate on the 4 main IHC systems***

| Concentrated antibodies | **Dako Autostainer Link / Classic** | | **Dako Omnis** | | **Ventana BenchMark XT / Ultra** | | **Leica 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 **5D3** | 0/4** | - | 0/2 | - | 0/5 (0%) | - | 5/9 (56%) | - |
| mAb clones **B22.1/B23.1** | 1/1 | - | 1/1 | - | 5/10 (50%) | - | 1/1 | - |
| mAb clone **DC10** | 0/1 | - | 0/2 | - | 0/9 (0%) | - | 0/4 | - |
| rmAb clones **EP17/EP30** | 0/1 | - | 3/3 | - | 5/5 (100%) | - | 1/1 | - |

* 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 **5D3** product no. PA0067, Leica/Novocastra, BOND III:
One protocol with an optimal result was based on HIER using Bond Epitope Retrieval Solution 1 (BERS1) (efficient heating time 20 min. at 99-100°C), 15 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using this or very similar protocol settings, 6 of 6 (100%) produced a sufficient staining result.

mAb clones **B22.1/B23.1**, product no. 760-4344 Roche/ Ventana, BenchMark Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 16-64 min.) or a combined pre-treatment using Protease 1 or 3 (efficient time 4 min.) after HIER in CC1 (efficient heating time 8-32 min.), 12-32 min. incubation of the primary Ab and UltraView (760-500) with or without amplification (760-080) or OptiView (760-700) as detection system. Using these protocol settings, 52 of 54 (96%) laboratories produced a sufficient staining result.

mAb clones **B22.1/B23.1**, product no. MAD-000589QD Master Diagnostica, Thermo Autostainer:
One protocol with an optimal result was based on HIER using EDTA / EGTA pH 8 (efficient heating time 20 min. at 95°C), 10 min. incubation of the primary Ab and Master Plus (MAD-000237QD) as detection system.

mAb clone **CAM5.2**, product no.790-4555 Roche / Ventana, BenchMark Ultra:
One protocol with an optimal result was based on a combined pre-treatment using Protease 3 (efficient time 8 min) after HIER in CC1 (efficient heating time 8 min.), 24 min. incubation of the primary Ab and OptiView (760-700) as detection system. Using this protocol setting, 1 of 1 (100%) laboratories produced a sufficient staining result.

rmAb clones **EP17/EP30**, product no. IR094, Dako, Autostainer+/Autostainer Link:
Optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-30 min. at 97-98°C), 20-30 min. incubation of the primary Ab and EnVision FLEX (K8000/K8012) as detection systems. Using these protocol settings, 15 of 15 (100%) laboratories produced sufficient staining results.

Table 4. **Comparison of pass rates for vendor recommended and laboratory modified RTU protocols**

| RTU systems | **Vendor recommended protocol settings*** | | **Laboratory modified protocol settings**** | |
|---|---|---|---|---|
| | Optimal | Sufficient | Optimal | Sufficient |
| Dako AS48<br>rmAb EP17/EP30<br>**IR/IS094** | 7/8 (88%) | 8/8 (100%) | 6/7 (86%) | 7/7 (100) |
| Leica Bond<br>mAb 5D3<br>**PA0067** | 1/5 (20%) | 5/5 (80%) | 0/1 (0%) | 1/1 (100%) |
| VMS Ultra/XT<br>mAb B22.1/B23.1<br>**760-4344** | 1/11 (9%) | 11/11 (100%) | 27/54 (50%) | 20/54 (89%) |

* 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 >25%, detection kit
Only protocols performed on the specified vendor IHC stainer are included.

**Comments**
In this assessment and in concordance with the previous NordiQC CK8/18 (CK-LMW) assessments, the prevalent feature of an insufficient staining was a too weak or completely false negative staining reaction of cells expected to be demonstrated. This pattern was seen in 96% of the insufficient results (77 of 80 laboratories). The remaining insufficient results were characterized by a poor signal-to-noise ratio and/or a false positive staining reaction compromising interpretation (see Fig. 8). Too weak staining was typically characterized by reduced staining reaction both in regard to intensity and proportion of cells expected to be demonstrated (see Figs. 1 and 2). Virtually all laboratories successfully demonstrated epithelial cells of appendix and bile ducts which all have high expression levels of CK8/18. The neoplastic cells of the SCLC, renal clear cell carcinoma and hepatocytes were more challenging and required an optimally calibrated IHC system. Virtually all laboratories using CK8 or CK8/18 antibodies successfully demonstrated CK8/18 in the majority of neoplastic cells of the breast carcinoma, whereas the vast majority of laboratories using CK18 antibodies failed (see Fig. 5). Partial or complete loss of CK18 expression has been observed in ~25% of breast carcinomas depending on their histological type. Thus, the use of the individual CK18 as a diagnostic marker for breast cancer cells might lead to false-negative findings due to down-regulation of this protein[1].

40% (95 of 238) of the laboratories used concentrated Ab formats within LD assays for CK8/18. The mAb clone 5D3 was the most widely used Ab and could be used to obtain optimal staining results as shown in Tables 1 and 3. However, the overall pass rate and proportion of optimal results for clone 5D3 were low and major IHC-platform related differences were seen. On the Leica Bond platform, protocols based on HIER in alkaline buffer using a 3-step polymer-based detection system provided a pass rate of 78% (7 of 9), 56% optimal. In contrast, the mAb clone 5D3 consistently gave insufficient results with protocols performed on BenchMark XT/Ultra (n=5), despite applying comparable protocol settings. In concordance with previous CK8/18 (CK-LMV) assessments, Leica, the main supplier of mAb clone 5D3, provides misleading guidelines concerning the epitope retrieval: For the concentrated format of 5D3, proteolytic pre-treatment is still recommended, while the data sheet for the corresponding RTU format PA0067 states HIER must be used.
The mAb clone cocktail B22.1/B23.1 used in a concentrated Ab format within LD assays for CK8/18 provided an overall high proportion of sufficient results (79%). Focusing on the B22.1/B23.1 cocktail supplied by Cell Marque (n=11), the proportion of sufficient results increased to 91%. Efficient HIER in an alkaline buffer, carefully calibration of the primary Ab and use of a sensitive 3-step multimer/polymer detection system were the most important parameters for an optimal staining result.
Used in a concentrated Ab format within LD assays for CK8/18 rmAb clone cocktail EP17/EP30 and rmAb clone EP17 grouped together provided a pass pate of 93% (14 of 15) of which 87% was optimal. Both Abs seemed to have higher analytical sensitivity for CK8/18 compared to the well-established mAb clones 5D3, CAM5.2 and DC10. Efficient HIER in alkaline buffer and careful calibration of the primary Ab were the central parameters for optimal staining, whereas neither choice of detection system (2-step or 3-step) nor IHC stainer platform seemed to influence performance.
In the present CK8/18 assessment, the mAb clone DC10 performed poorly (see Figs. 3 – 6). Used in a concentrated Ab format within LD assays for CK8/18, no sufficient staining results were seen using mAb clone DC10. All LD assays failed to demonstrate a sufficient staining reacting in the breast ductal carcinoma. Partial or complete loss of CK18 expression has been observed in ~25% of breast carcinomas depending on their histological type. Thus, the use of the individual CK18 as a diagnostic marker for breast cancer cells might lead to false-negative findings due to down-regulation of this protein[1].

RTU antibodies were used by 60% (143 of 238) of the laboratories. The Ventana RTU system for the BenchMark IHC platform based on mAb clone cocktail B22.1/B23.1 (760-4344) was the most widely used RTU system applied by 65 laboratories. An overall pass rate of 91% was seen, 43% optimal. If the protocols were performed accordingly to the recommendations provided by Ventana, using UltraView as detection system, only one of eleven (9%) protocols provided optimal results. Laboratory modified protocol settings provided a pass rate of 89%, 50% optimal. Excluding laboratory modified protocol settings that reduced analytical sensitivity the pass rate reached 94% (46 of 49), 55% optimal.

For the Dako RTU system for Autostainer based on rmAb clone cocktail EP17/EP30 (IR094), an overall pass rate of 100% was observed. Optimal results could both be obtained using the protocol recommendations given by Dako and by laboratory modified protocol settings typically adjusting HIER time and/or incubation time of the primary Ab. 16 laboratories used the RTU format on Omnis with protocol settings similar to the Dako recommendations for Autostainer but modified to "Omnis RTU" settings using HIER for 30 min. in TRS High pH and 15-25 min. incubation of the primary Ab and polymer conjugate. All 16 laboratories obtained an optimal mark.

The Ventana RTU system for the BenchMark IHC platform based on mAb clone CAM5.2 (790-4555) was only used by 5 laboratories and with a pass rate of 60% the proportion of sufficient and in particular optimal results was inferior to the other Ventana RTU system based on mAb clone cocktail B22.1/B23.1 (760-4344).

The RTU format mAb clone CAM5.2, prod. no. 345779/349205, Becton Dickinson (BD) was applied by 13 laboratories compared to 35 laboratories in the previous run (Run 49, 2017). In concordance with previous CK8/18 (CK-LMV) assessments, the pass rate was low. An overall pass rate of 31% was observed and only 15% optimal. It must be emphasized that this RTU format is not developed for a particular IHC system/platform and must be used within a LD assay identifying best practice protocol settings focusing on choice of epitope retrieval method, detection system etc. The protocols that provided optimal results were based on proteolytic pre-treatment, alone or in combination with HIER which is in compliance with previous NordiQC assessments for CK8/18 (CK-LMW) indicating these epitope retrieval methods may be the preferred methods for mAb clone CAM5.2. However, in this and the previous assessment (Run 49, 2017) a total 11 of 16 protocols based on proteolytic pre-treatment gave insufficient staining results. This suggests that optimizing IHC-protocols based on mAb clone CAM5.2 is very challenging.

The Leica RTU system for the Leica Bond IHC platform based on mAb clone 5D3 (PA0067) had a pass rate of 100%, where the majority of laboratories followed vendor recommended protocol settings.

This was the eighth NordiQC assessment of CK8/18 (CK-LMW) (see Table 2). Despite significant increase in use of antibodies with a high analytical sensitivity (e.g. mAb clone cocktail B22.1/B23.1, rmAb clone cocktail EP17/30 and rmAb clone EP17), identical pass rate was obtained compared to the previous run 49, 2017. More challenging tissue material circulated may in part explain the status quo. The breast carcinoma included in the present multiblock belongs to the large minority of breast carcinomas (~25%) with partial or total loss of CK18 expression[1]. Consequently, the total pass rate for all CK18 antibodies was only 9% (3 of 33) compared to 74% (39 of 53) in run 49, 2017.

**Controls**

In this assessment and as observed in previous NordiQC assessments, liver is recommendable as positive tissue control for CK8/18. Virtually all hepatocytes must show an at least moderate cytoplasmic staining reaction, typically with membranous accentuation, while the epithelial cells lining the bile ducts must show strong cytoplasmic staining reaction. No staining should be seen in the connective tissue and lymphocytes in portal rooms (see Figs. 1a and 3a). Tonsil is a recommended additional tissue control, serving as both positive and negative tissue control. The majority of squamous epithelial cells must display a moderate to strong cytoplasmic staining reaction, whereas in lymphocytes no staining reaction should be seen. In tonsil, cytokeratin-positive interstitial reticulum cells (CIRCs) with dendritic/reticular pattern will show a weak to moderate cytoplasmic staining reaction (see Fig. 6a). Appendix cannot be recommended as positive tissue control as the epithelial cells express high levels of CK8/18 and thus cannot be used to monitor the consistency of the IHC protocol and the analytical sensitivity to demonstrate CK8/18 in low-level expressing cells and neoplasias.

Basal squamous cells of the esophagus will display a weak to moderate cytoplasmic staining reaction if using Abs towards CK8 as e.g. the rmAb clone EP17.

1. Woelfle U, Sauter G, Santjer S, Brakenhoff R, Pantel K. Down-Regulated Expression of Cytokeratin 18 Promotes Progression of Human Breast Cancer. Clin Cancer Res. American Association for Cancer Research; 2004 Apr 15;10(8):2670–4.

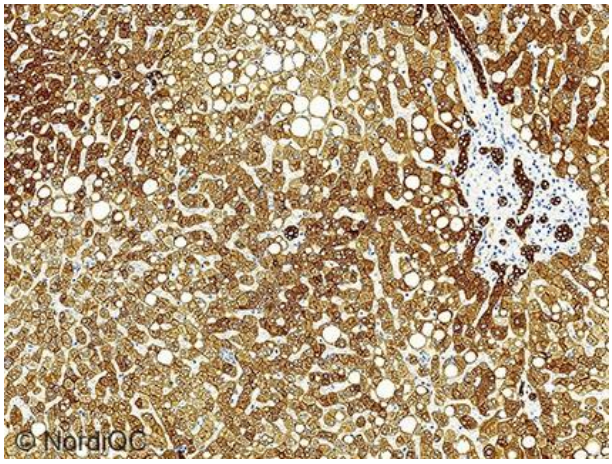



Fig. 1a
Optimal CK8/18 staining of the liver using the mAb clone cocktail B22.1/B23.1 as a concentrate in an optimally calibrated LD assay, performed on the Leica Bond III. The vast majority of hepatocytes show a distinct, moderate staining reaction with a membrane enhancement, while the columnar epithelial cells of the bile ducts show a strong cytoplasmic staining reaction. Compare with Fig. 2a, same protocol.

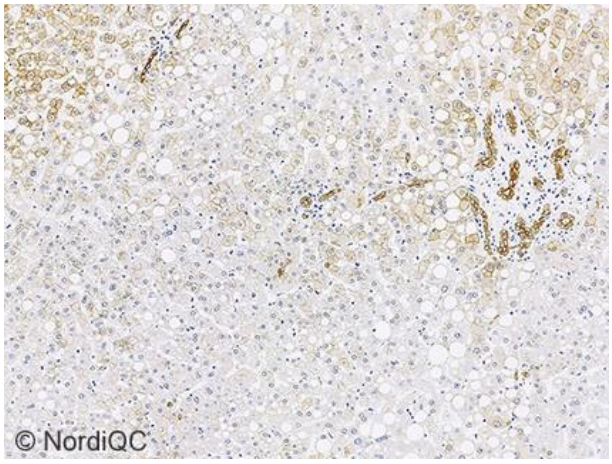



Fig. 1b
Insufficient CK8/18 staining of the liver using the mAb clone CAM5.2 for CK 8(7) as a concentrate in a LD assay based on proteolytic pre-treatment in Bond Enzyme Pretreatment kit, performed on the Leica Bond III. Same field as in Fig. 1a. Only the bile duct epithelial cells are distinctively demonstrated, while the vast majority of hepatocytes are negative or only faintly positive. Compare with Fig. 2b, same protocol.

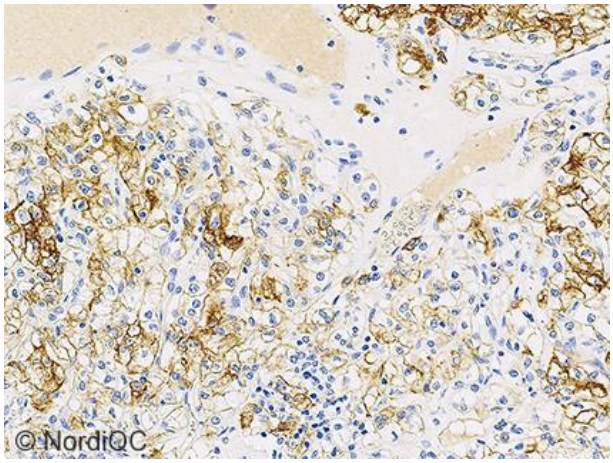



Fig. 2a
Optimal CK8/18 staining of the renal clear cell carcinoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a distinct, moderate to strong staining reaction.

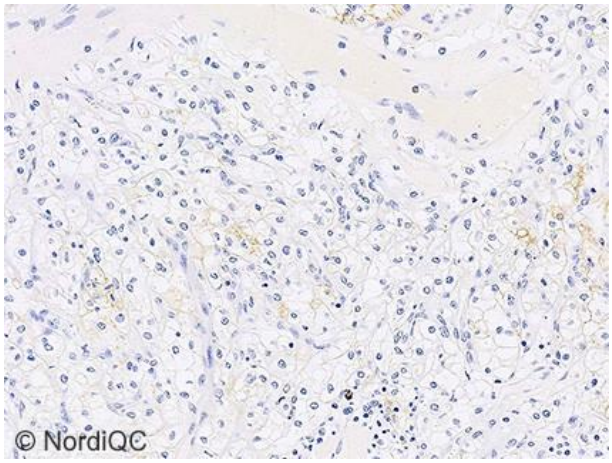



Fig. 2b
Insufficient CK8/18 staining of the renal clear cell carcinoma using same protocol as in Fig. 1b. - same field as in Fig. 2a. Only scattered neoplastic cells show a weak and equivocal staining reaction.

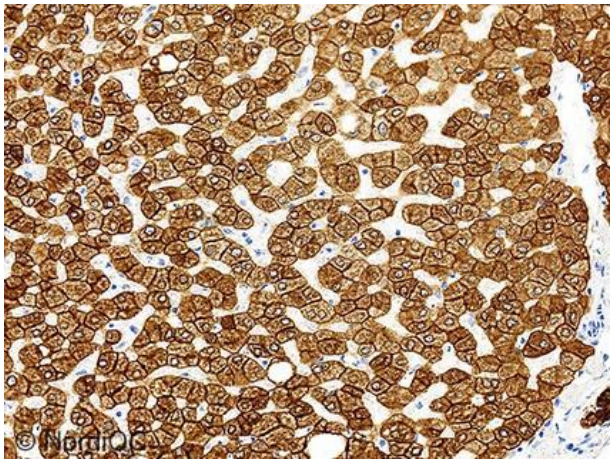



Fig. 3a
Optimal CK8/18 staining of the liver using the rmAb clone cocktail EP17/EP30 as a concentrate in an optimally calibrated LD assay, performed on the Dako Omnis. The vast majority of hepatocytes show a distinct, moderate staining reaction with a membrane enhancement. Compare with Figs. 4a – 6a, same protocol.

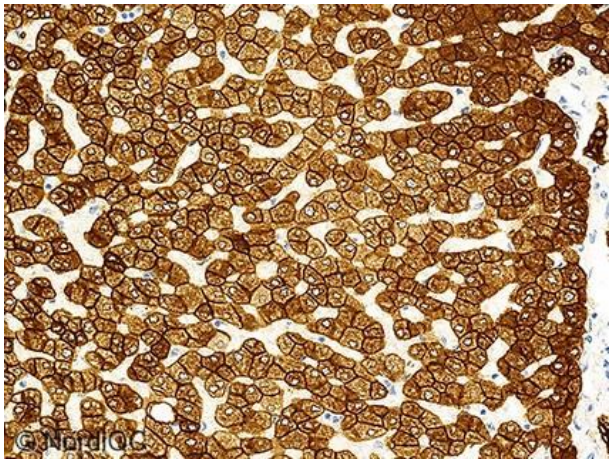



Fig. 3b
Optimal CK8/18 staining of the liver using an insufficient protocol based on the mAb clone DC10. Clone DC10 reacts with CK18 and in this (DC10) CK18-protocol the vast majority of hepatocytes show a distinct, moderate staining reaction with a membrane enhancement. Compare with Figs. 4b – 6b, same protocol. Compare also with Fig. 3a – same field.

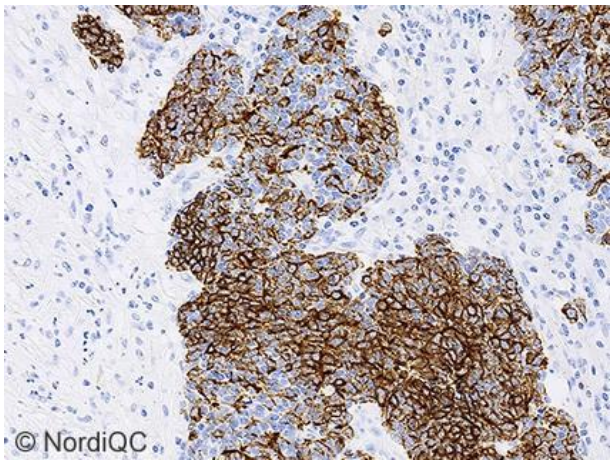



Fig. 4a
Optimal CK8/18 staining of the SCLC using same protocol as in Fig. 3a. The majority of neoplastic cells show a weak to strong and distinct staining reaction. A dot-like cytoplasmic staining reaction is observed in the weakly positive neoplastic cells.

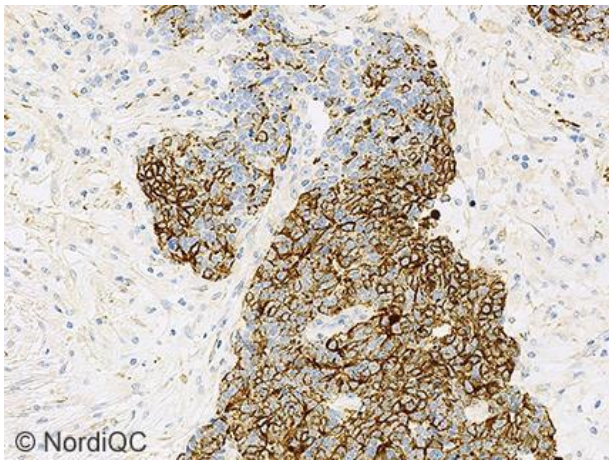



Fig. 4b
Optimal CK8/18 staining of the SCLC using the same DC10-protocol as in Fig. 3b. The majority of neoplastic cells show a weak to strong and distinct staining reaction. A dot-like cytoplasmic staining reaction is observed in the weakly positive neoplastic cells. Compare with Figs. 3b, 5b and 6b, same protocol. Compare also with Fig. 4a – same field.

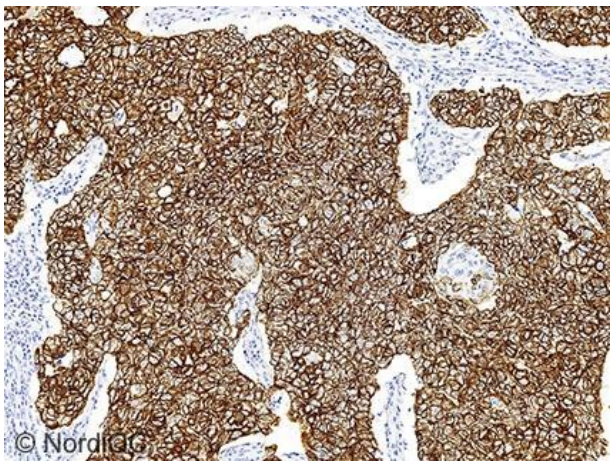



Fig. 5a
Optimal CK8/18 staining of the breast ductal carcinoma using same protocol as in Figs. 3a - 4a. Virtually all neoplastic cells show a strong and distinct cytoplasmic staining reaction. The use of a cocktail of CK8 and CK18 antibodies secures optimal staining reaction despite the apparent loss of CK18 in the tumour. Compare with Fig. 5b – same field.

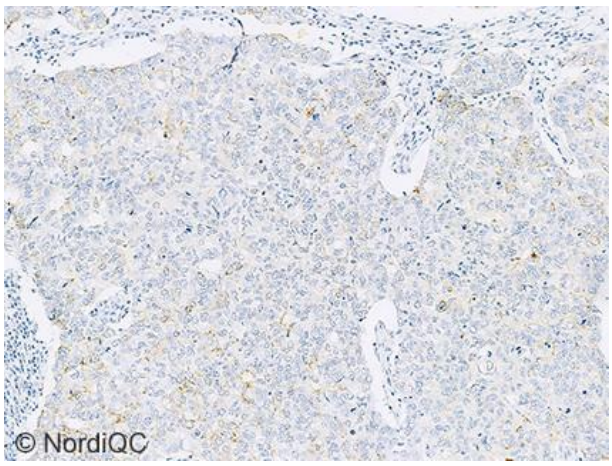



Fig. 5b
Insufficient CK8/18 staining of the breast ductal carcinoma using same protocol as in Figs. 3b – 4b. Despite producing optimal staining in liver (and the SCLC), virtually all neoplastic cells are unstained. This tumour belongs to the approx. 25% of breast carcinomas that shows partial or complete loss of CK18 expression, making CK18 antibodies like clone DC10 less suitable identifying non-squamous carcinomas including adenocarcinoma of unknown origin. Compare with Fig. 5a – same field.

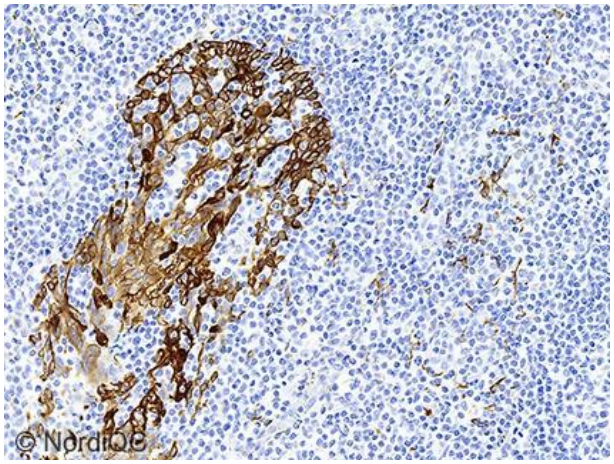



Fig.6a
Optimal CK8/18 staining of the tonsil using same protocol as in Figs. 3a - 5a. The majority of squamous epithelial cell display a moderate to strong cytoplasmic staining reaction. Scattered dendritic cells show a weak to moderate cytoplasmic staining reaction.

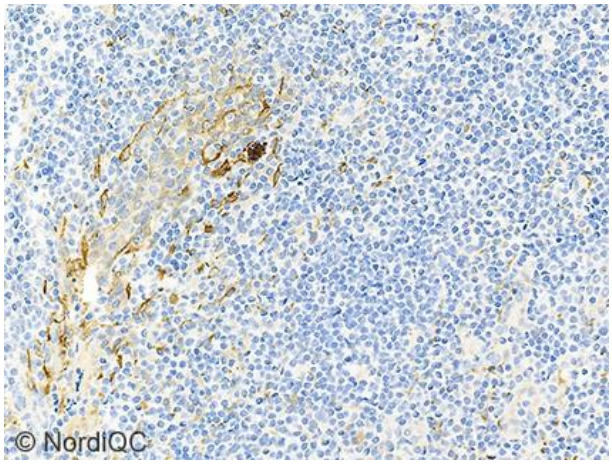



Fig. 6b
Insufficient CK8/18 staining of the tonsil using the same DC10-protocol as in Figs. 3b – 5b. The majority of squamous epithelial cells display a too weak staining reaction. Compare with Figs. 3b - 5b, same protocol. Compare also with Fig. 6a – same field.

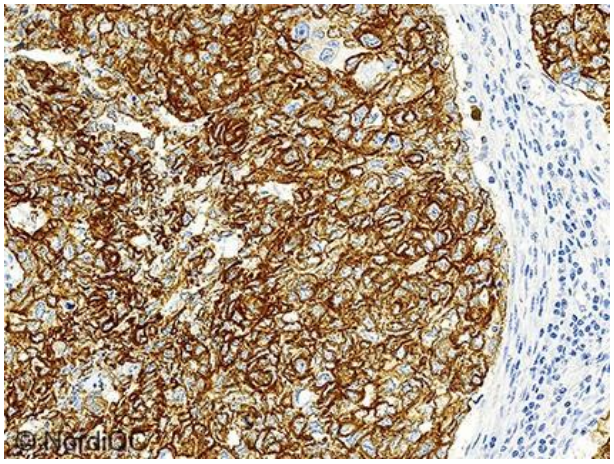



Fig. 7a
Optimal CK8/18 staining of the breast ductal carcinoma using same protocol as in Figs. 1a and 2a. Virtually all neoplastic cells show a strong and distinct cytoplasmic staining reaction.

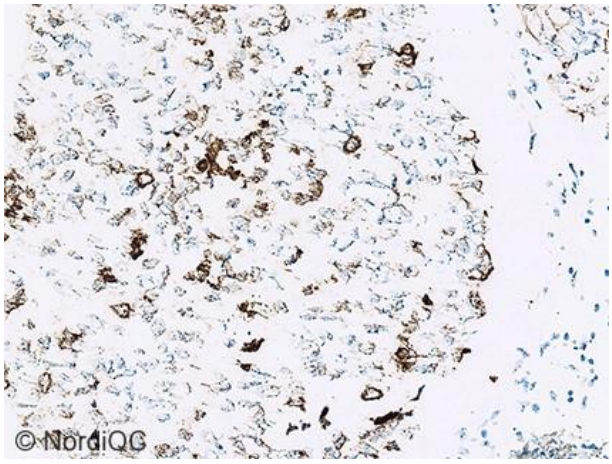



Fig. 7b
Insufficient CK8/18 staining of the breast ductal carcinoma and like in Fig. 7a using the mAb clone cocktail B22.1/B23.1 as a concentrate in a LD assay, but performed on the BenchMark Ultra in a protocol based on combined HIER and protease retrieval. The use of strong protease (Protease 1, Ventana) for 8 min. following HIER in CC1 for 32 min, results in impaired morphology.

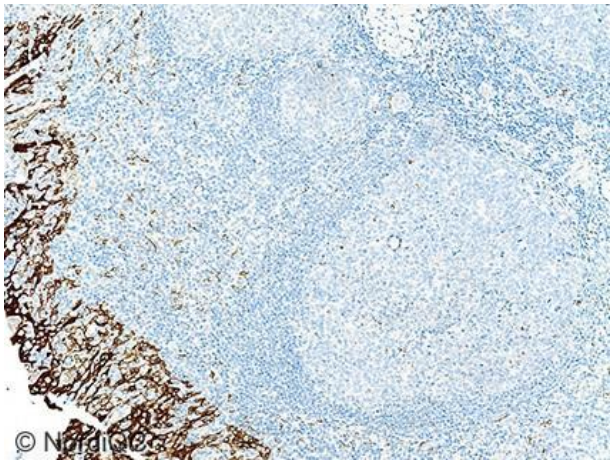



Fig. 8a
Optimal CK8/18 staining of the tonsil using the rmAb clone cocktail EP17/EP30 as a concentrate in an optimally calibrated LD assay, performed on the Ventana BenchMark Ultra. The majority of squamous epithelial cells display a moderate to strong cytoplasmic staining reaction. Scattered dendritic cells show a weak to moderate cytoplasmic staining reaction.

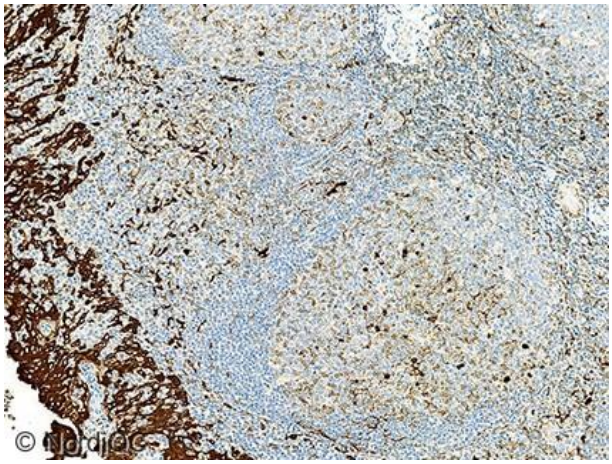



Fig. 8b
Insufficient CK8/18 staining of the tonsil using the rmAb clone EP17 as a concentrate in a LD assay, performed on the Ventana BenchMark Ultra. Too high concentration of primary Ab in combination with a detection system with a very high analytical sensitivity (OptiView with tyramide amplification) results in false positive staining reaction in many lymphocytes. Compare with Fig. 8a – same field.

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