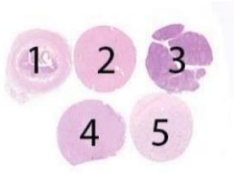
## Assessment Run 69 2023

# Cytokeratin 8/18 (CK8/18)

Updated 03.01.2024

**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. Breast carcinoma, 5. Renal clear cell carcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CK8/18 staining as optimal included:

- A strong, distinct cytoplasmic staining reaction of virtually all appendiceal columnar epithelial cells and bile duct epithelial cells in liver.
- An at least weak to moderate predominantly cytoplasmic but also with a membranous accentuated 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 and membranous staining reaction in the majority of neoplastic cells in the renal clear cell carcinoma.

**Participation**

| | |
|---|---|
| Number of laboratories registered for CK8/18, run 69 | 304 |
| Number of laboratories returning slides | 283 (93%) |

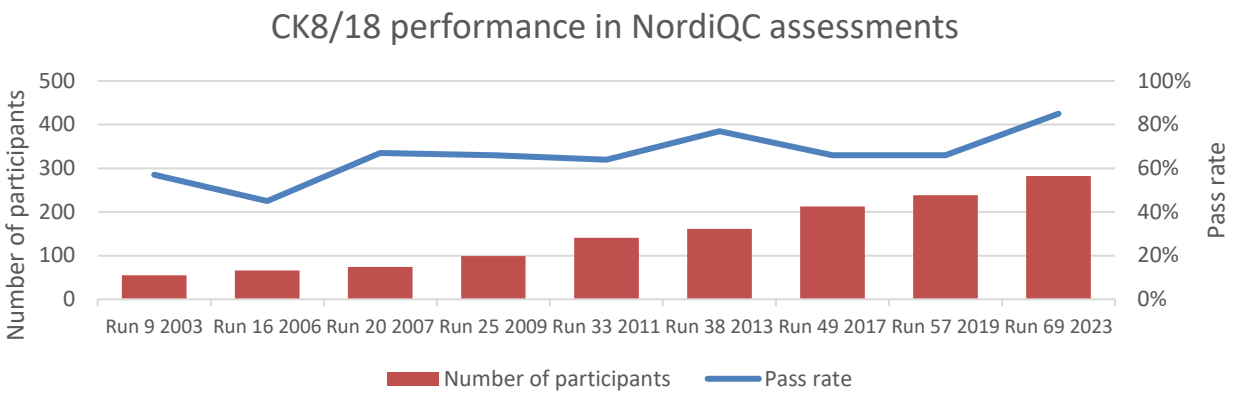
**Results**
At the date of assessment, 93% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

283 laboratories participated in this assessment and 85% achieved a sufficient mark (optimal or good).
One laboratory used an inappropriate antibody and was not included in the statistic.
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 successful primary antibodies targeting only CK18.

**Performance history**
This was the nineth NordiQC assessment of CK8/18. 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 CK8/18 in the nine NordiQC runs performed**




**Conclusion**
Within a laboratory developed (LD) assay, the mAb clone cocktail **B22.1/B23.1** *(CK8/18)*, rmAb clone cocktail **EP17/EP30** *(CK8/18)* and rmAb clone **EP17** *(CK8)* are all highly recommendable Abs for demonstration of CK8/18. Irrespective of selected clone, 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.
The Ventana/Roche Ready-To-Use (RTU) system (760-4344) based on the mAb clone cocktail **B22.1/B23.1** *(CK8/CK18)*, and the Dako/Agilent RTU system (IR/IS094) based on rmAb clone cocktail **EP17/EP30** *(CK8/CK18)* are both highly recommendable Abs for demonstration of CK8/18. Vendor recommended protocol settings and laboratory modified protocol settings both resulted in a high proportion of sufficient staining results.

The CK18 antibody mAb clone DC10 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 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 69**

| Concentrated antibodies | Reactivity | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|---|
| mAb clone **B22.1/B23.1** | *CK8/18* | 16<br>2<br>1 | Cell Marque<br>Bio SB<br>Immunologic | 16 | 1 | 1 | 1 | 90% | 84% |
| mAb clone **5D3** | *CK8/18* | 11<br>2<br>1<br>1<br>1 | Leica Biosystems<br>Biocare<br>Diagnostic BioSystem<br>Epredia<br>Thermo Scientific | 8 | 5 | 2 | 1 | 81% | 50% |
| mAb clone **CAM5.2** | *CK8(7)* | 2<br>2<br>2<br>1 | BD Bioscience<br>Becton Dickinson<br>Zytomed<br>Master Diagnistica | 1 | 2 | 3 | 1 | 43% | 14% |
| mAb clone **DC10** | *CK18* | 6 | Dako/Agilent | 1 | 3 | 2 | - | 67% | 17% |
| mAb clone **ZM125** | *CK8/18* | 2 | Zeta Corporation | - | - | 2 | - | - | - |
| mAb clone **TS1** | *CK8* | 1 | Thermo Scientific | 1 | - | - | - | - | - |
| mAb clone **TS1+DC10** ("homebrew") | *CK8/18* | 1 | Thermo Scientific | - | 1 | - | - | - | - |
| rmAb clones **EP17/EP30** | *CK8/18* | 14<br>2 | Dako/Agilent<br>Epitomics | 15 | 1 | - | - | 100% | 94% |
| rmAb clone **EP17** | *CK8* | 4<br>3<br>1 | Epitomics<br>Cell Marque<br>Bio SB | 8 | - | - | - | 100% | 100% |
| rmAb clone **EP30** | *CK18* | 1 | Cell Marque | 1 | - | - | - | - | - |
| rmAb+mAb clone **IHC559** | *CK8/18* | 1 | GenomeMe | 1 | - | - | - | - | - |
| rmAb clone **QR112** | *unknown* | 1 | Quartett | - | 1 | - | - | - | - |
| Conc total | | 79 | | 52 | 14 | 10 | 3 | 85% | 67% |
| Ready-To-Use antibodies | | | | | | | | Suff.[1] | OR.[2] |
| mAb clone **B22.1/B23.1 760-4344[3]** | *CK8/18* | 20 | Ventana/Roche | 14 | 6 | - | - | 100% | 70% |
| mAb clone **B22.1/B23.1 760-4344[4]** | *CK8/18* | 60 | Ventana/Roche | 44 | 15 | 1 | - | 98% | 73% |
| mAb clone **CAM5,2 790-4555[3]** | *CK8(7)* | - | Ventana/Roche | - | - | - | - | - | - |
| mAb clone **CAM5,2 790-4555[4]** | *CK8(7)* | 4 | Ventana/Roche | 3 | 1 | - | - | - | - |
| rmAb clones **EP17/EP30 IR/IS094[3]** | *CK8/18* | 9 | Dako/Agilent | 7 | 2 | - | - | 100% | 78% |
| rmAb clones **EP17/EP30 IR/IS094[4]** | *CK8/18* | 36 | Dako/Agilent | 33 | 3 | - | - | 100% | 92% |
| mAb clone **DC10 IR/IS618[3]** | *CK18* | - | Dako/Agilent | - | - | - | - | - | - |
| mAb clone **DC10 IR/IS618[4]** | *CK18* | 5 | Dako/Agilent | - | - | 4 | 1 | 0% | 0% |
| mAb clone **DC10 GA618[3]** | *CK18* | 3 | Dako/Agilent | - | - | 3 | - | - | - |
| mAb clone **DC10 GA618[4]** | *CK18* | 8 | Dako/Agilent | 1 | 1 | 6 | - | 25% | 13% |
| mAb clone **5D3 PA0067[3]** | *CK8/18* | 14 | Leica Biosystems | 7 | 4 | 3 | - | 79% | 50% |
| mAb clone **5D3 PA0067[4]** | *CK8/18* | 10 | Leica Biosystems | 4 | 1 | 4 | 1 | 50% | 40% |
| mAb clone **TS1 PA0567** | *CK8* | 1 | Leica Biosystems | - | - | 1 | - | - | - |
| mAb clone **EP17** + mAb clone **DC10 8298-C010[3]** | *CK8/18* | 3 | Sakura Finetek | 3 | - | - | - | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| mAb clone **B22.1/B23.1 818M-97/98** | *CK8/18* | 15 | Cell Marque | 12 | 3 | - | - | 100% | 80% |
| mAb clone **B22.1/B23.1 MAD-000589QD** | *CK8/18* | 3 | Master Diagnostica | - | 2 | 1 | - | - | - |
| mAb clone **B22.1/B23.1 BFM-0385** | *CK8/18* | 1 | Bioin Biotechnology | 1 | - | - | - | - | - |
| mAb clone **C7E10/C6B9 CCM-1012** | *CK8/18* | 1 | Celnovte | 1 | - | - | - | - | - |
| mAb clone **CAM5.2 452M-98** | *CK8(7)* | | Cell Marque | | | | | | |
| mAb clone **CAM5,2 345779** | *CK8(7)* | 2 | Becton Dickinson | - | 1 | 1 | - | - | - |
| mAb clone **MX004+MX035 MAB-1002** | *CK8/18* | 1 | Fuzhou Maixin | - | 1 | - | - | - | - |
| mAb clone **5D3 AM131-5M/AM131-10M** | *CK8/18* | 1 | BioGenex | - | 1 | - | - | - | - |
| mAb clone **5D3 PDM070** | *CK8/18* | 3 | Diagnostic BioSystems | - | - | 3 | - | - | - |
| Unknown Ab clone **459G1A4 PA355** | *CK8/18* | 1 | Abcarta | - | 1 | - | - | - | - |
| rmAb clone **DA047 RMB1A064** | *CK8/18* | 1 | Shenzhen Dartmon Biotechnology | 1 | - | - | - | - | - |
| rmAb clones **BP6005/BP6054 I10862E** | *CK8/18* | 1 | Biolynx Biotechnology | 1 | - | - | - | - | - |
| RTU total | | 203 | | 132 | 42 | 27 | 2 | 86% | 65% |
| Total | | 282 | | 184 | 56 | 37 | 5 | | |
| Proportion | | | | 65% | 20% | 13% | 2% | 85% | |

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 CK8/18, Run 69**
The following protocol parameters were central to obtain optimal staining:

**Concentrated antibodies**
mAb clones **B22.1/B23.1**: Protocols with optimal results were based on HIER using either Cell Conditioning 1 (CC1, Ventana/Roche) (10/12)*, Target Retrieval Solution (TRS) High pH (3-in-1) (Dako/Agilent) (1/1), TRS, High pH (Dako/Agilent) (2/2) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (2/2) as retrieval buffer or Bond Enzyme kit (Leica Biosystems) (1/1). 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, 18 of 19 (95%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **5D3**: Protocols with optimal results were all based on HIER using BERS2 (Leica Biosystems) (4/5), TRS High pH (3-in-1) (Dako/Agilent) (1/3), TRS, High pH (Dako/Agilent) (1/2) or TRIS-EDTA/EGTA pH 9 (2/2) as retrieval buffer. The mAb was diluted in the range of 1:30-1:750 depending on the total sensitivity of the protocol employed. Using these protocol settings, 9 of 9 (100%) laboratories produced a sufficient staining result.

rmAb clones **EP17/EP30**: Protocols with optimal results were based on HIER using either CC1 (Ventana/Roche) (3/4), TRS High pH (3-in-1) (Dako/Agilent) (1/1), TRS High pH (Dako/Agilent) (5/5) or BERS2 (Leica Biosystems) (6/6) as retrieval buffer. The mAb was diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 16 of 16 (100%) laboratories produced a sufficient staining result.

rmAb clone **EP17**: Protocols with optimal results were all based on HIER using CC1 (Ventana/Roche) (7/7) or TRS High pH (3-in-1) (Dako/Agilent) (1/1). The rmAb was diluted 1:50-1:1200. Using these protocol settings, 8 of 8 (100%) laboratories produced an optimal staining.

Table 2. **Proportion of optimal results for CK8/18 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 / Prime** | | |
|---|---|---|---|---|---|---|---|---|---|
| | TRS pH 9.0 | TRS pH 6.1 | TRS pH 9.0 | TRS pH 6.1 | CC1 pH 8.5 | CC2 pH 6.0 + P3 | ER2 pH 9.0 | ER1 pH 6.0 | No Treatment |
| mAb clones **B22.1/B23.1** | 1/1** | 0/1 | 2/2 | - | 10/12 (83%) | - | 2/2 | - | 1/1 |
| mAb clone **5D3** | 1/3 | - | ½ | - | - | 0/1 | 4/5 | - | 0/3 |
| mAb clone **DC10** | - | 1/1 | 0/1 | - | 0/3 | - | 0/1 | - | - |
| mAb clone **CAM5.2** | - | - | 0/3 | - | 0/1 | - | 1/1 | - | - |
| rmAb clones **EP17/EP30** | 1/1 | - | 5/5 (100%) | - | 3/4 ( | - | 6/6 (100%) | - | - |
| rmAb clone **EP17** | 1/1 | - | - | - | 7/7 (100%) | - | - | - | - |

* 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 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 16-40 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.), 4-32 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, 75 of 76 (99%) laboratories produced a sufficient staining result.

mAb clone **CAM5.2**, product no.790-4555 Ventana/Roche, BenchMark Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 24-32 min.) or a combined pre-treatment using Protease 3 (efficient time 8 min.) after HIER in CC1 (efficient heating time 8 min.), 20-32 min. incubation of the primary Ab and OptiView (760-700) as detection system. Using this protocol setting, 3 of 3 (100%) laboratories produced a sufficient staining result.

mAb clone **5D3** product no. PA0067, Leica Biosystems, BOND III:
Protocols with optimal results were typically based on HIER using Bond Epitope Retrieval Solution 1 (BERS1) (efficient heating time 20 min. at 95-100°C), 15 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. One laboratory used Bond Enzyme pretreatment kit (efficient incubation time 5 min.) also with optimal results.
Using this or very similar protocol settings, 12 of 16 (75%) produced a sufficient staining result.

mAb clones **C7E10/C6B9**, product no. CCM-1012 Celnovte, CNT360:
One protocol with an optimal result was based on HIER using TRIS-EDTA / EGTA pH 9 (efficient heating time 20 min. at 100°C), 20 min. incubation of the primary Ab and MicroStacker TM PLUS (SD5600 as detection system.

rmAb clones **EP17/EP30**, product no. IR/IS094, Dako/Agilent, Autostainer+/Autostainer Link:
Optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-20 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, 12 of 12 (100%) laboratories produced sufficient staining results.

rmAb clones **BP6005/BP6054**, product no. I10862E Biolynx Biotechnology, Lynx480:
One protocol with an optimal result was based on HIER using Antigen Retrieval 2 (EDTA) based buffer (efficient heating time 20 min. at 100°C) and 30 min. incubation of the primary Ab. BXV Visalization System (I20032C) was used as detection systems.

rmAb clone **DA047**, product no. RMB1A064 Shenzhen Dartmon Biotechnology, Dartmon - AS330PLUS:
One protocol with an optimal result was based on HIER using Immunohistochemical Antigen Retrieval

Buffer (efficient heating time 20 min. at 100°C) and 30 min. incubation of the primary Ab. Immunochromogenic Reagent (DMRD4044) was used as detection systems.

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 CK8/18 for the most commonly used RTU IHC systems**

| RTU systems | **Recommended protocol settings*** | | **Laboratory modified protocol settings**** | |
|---|---|---|---|---|
| | Sufficient | Optimal | Sufficient | Optimal |
| Dako AS<br>mAb DC10<br>**IR618** | - | - | 0/3 | 0/3 |
| Dako Omnis<br>mAb DC10<br>**GA618** | 0/3 | 0/3 | 25% (2/8) | 13% (1/8) |
| Dako AS48<br>rmAb EP17/EP30<br>**IR/IS094** | 100% (9/9) | 78% (7/9) | 100% (5/5) | 60% (3/5) |
| Leica Bond<br>mAb 5D3<br>**PA0067** | 79% (11/14) | 50% (7/14) | 71% (5/7) | 57% (4/7) |
| VMS Ultra/XT<br>mAb B22.1/B23.1<br>**760-4344** | 100% (20/20) | 70% (14/20) | 98% (59/60) | 73% (44/60) |
| VMS Ultra/XT<br>mAb CAM5,2<br>**790-4555** | - | - | 4/4 | 3/4 |

* 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 this assessment and in concordance with the previous NordiQC CK8/18 (CK-LMW) assessments, the prevalent feature of an insufficient staining result was a too weak or completely false negative staining reaction of cells expected to be demonstrated. This pattern was seen in 93% of the insufficient results (39 of 42 laboratories). The remaining insufficient results were characterized by a poor signal-to-noise ratio and/or a false positive staining reaction compromising interpretation. 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 renal clear cell carcinoma and hepatocytes were more challenging and required an optimally calibrated IHC system. Virtually all laboratories using antibodies against CK8 or CK8/18 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. 4). 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] in this tumour entity.

28% (79 of 282) of the laboratories used concentrated Ab formats within LD assays for CK8/18. Compared to the latest run where a total of 40% used the concentrated Abs. The mAb clone cocktail B22.1/B23.1 was the most widely used Ab and could be used to obtain optimal staining results on all four main IHC platforms. The concentrate seems very robust and provided a high pass-rate 90% sufficient and 84% optimal using both 2- and 3-layer detection systems.
The mAb clone 5D3 as a concentrate was used by 16 laboratories with an overall pass rate of 81%, 50% being optimal (see Table 1). The concentrate format was used by 3 laboratories without pretreatment on the Bond platforms, none with optimal results, however using HIER in alkaline buffer gave a pass rate of 100% (5 of 5). In concordance with previous CK8/18 (CK-LMV) assessments, Leica Biosystems, 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.

Used in a concentrated Ab format within LD assays for CK8/18, the rmAb clone cocktail EP17/EP30 and rmAb clone EP17 grouped together provided a pass pate of 100% (24 of 24) of which 96% 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 an optimal staining result, whereas neither choice of detection system (2-step or 3-step) nor IHC stainer platform seemed to influence performance.

RTU antibodies were used by 72% (203 of 282) of the laboratories. The Ventana/Roche 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 80 laboratories. An overall pass rate of 99% was seen, 73% optimal. Preforming the protocol according to vendor recommendations provided by Ventana/Roche, using UltraView as detection system a pass rate of 100% was provided with 70% being optimal. Modifications of the protocol settings were mostly related to prolonging HIER, increasing the primary Ab incubation time and/or changing to a 3-layer detection system. Comparing the use of 2- or 3-layer detection systems within the modified protocols UltraView provided a pass rate of 96% (26 of 27), 52% (n=14) being optimal, whereas using OptiView as detection systems produced a pass rate of 100% (33 of 33), 91% (n=30) being optimal.

The Dako/Agilent RTU system for both Autostainer and Omnis platform based on the DC10 clone (IR/IS/GA618) was used by a total of 16 laboratories with an overall low pass rate of 13% and only 6% optimal.
In the present CK8/18 assessment, the mAb clone DC10 also showed a poor performance with an overall pass rate of 27% (6 of 22) pooling the data for both concentrated and RTU formats. The inferior performance is illustrated in Figs. 1b-6b. The main challenge was to demonstrate a sufficient staining reaction in the breast ductal carcinoma. As mentioned above, 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]. As an RTU system with the main purpose of finding adenocarcinoma of unknown origin, this product cannot be recommended.

For the Dako/Agilent Autostainer system the rmAb clone cocktail EP17/EP30 (IR/IS094) displayed a high pass rate of 100%, however only used by 9 laboratories as intended on the Autostainer platform and following vendor recommendations. 14 laboratories modified the protocol settings on the Autostainer and a total of 31 transferred the product to other platforms, 27 to the Dako Omnis all with optimal results. The basic protocol parameters on the Dako Omnis were based on HIER in TRS High pH for 30 min., incubation in primary Ab for 20-30 min. and EnVision FLEX as the detection system (20 min. incubation).
In that context, the ideal RTU format of a primary Ab is used within a system with precise information on vendor recommended protocol settings, equipment, reagents and results expected. Therefore, it is not advisable to use a RTU format of the primary Ab on a system/platform for which it has not been developed and validated, although it might produce optimal results (see Table 1). However in this situation the migitation of IR/IS094, rmAb clone cocktail EP17/EP30 to Dako Omnis seems to be superior to the vendor validated product GA618, mAb clone DC10 for Dako Omnis. Different local/national regulatory guidelines should be applied to verify/validate the end results when implementing IR/IS094 for Dako Omnis.

The Leica Biosystem RTU for the Leica Bond IHC platform based on mAb clone 5D3 (PA0067) had an overall pass rate of 67% (16 of 24). If the RTU system was applied by the vendor recommended protocol settings using HIER in BERS1 for 10 min. and protocol F (Ab incubation 15 min.) a pass rate of 19% was seen, being reduced compared to the 100% obtained in the recent run 57. At present no clear indication causing the reduced pass rate in this assessment was found.

This was the nineth NordiQC assessment of CK8/18 (CK-LMW) (see Graph 1) and the pass rate increased to 85% being the highest level ever. Both concentrated formats and RTU systems provided an equal high pass rate with the use of robust primary antibodies. In this assessment, and in concordance to previous runs, challenges was mainly seen using antibodies only targeting CK18, and these should be very carefully used as a diagnostic marker in work up for the unknown primary tumor.

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.

**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). 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. 3a). 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.

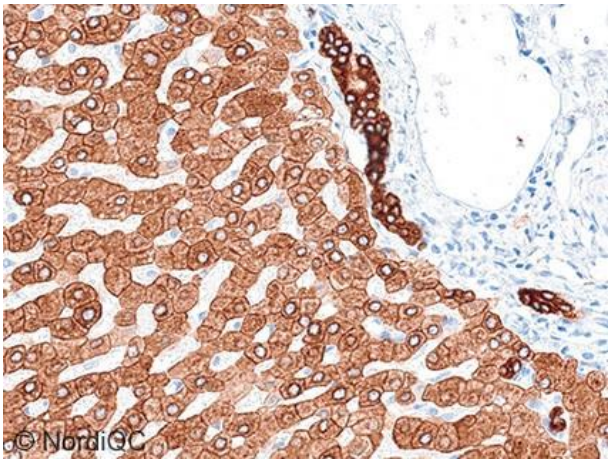



Fig. 1a (x200)
Optimal CK8/18 staining reaction of the liver using the Dako/Agilent RTU format of rmAb clone cocktail EP17/EP30 (IR/IS094) by incubation of 20 min. in primary Ab, HIER in TRS High pH 9 for 20 min., a 2-step polymer based detection kit (EnVision FLEX) and performed on Dako Autostainer. 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 Figs. 2a-5a, same protocol.

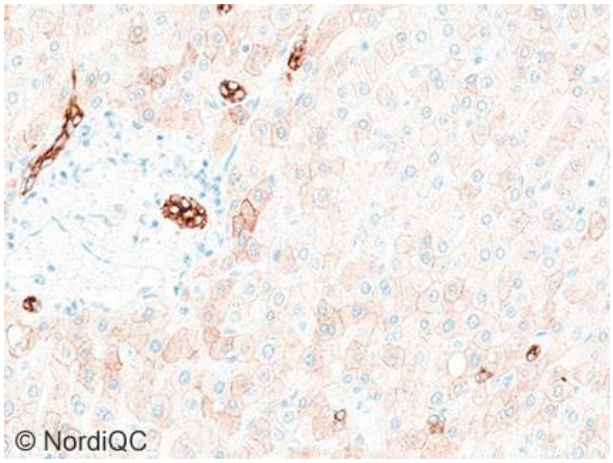



Fig. 1b (x200)
Insufficient staining reaction of the liver using the mAb clone CAM5.2 as a concentrate diluted 1:50 with a primary Ab incubation of 32 min, HIER in CC1 pH 8.5 for 32 min., a 3-step multimer based detection kit (OptiView) and performed on Ventana Benchmark Ultra. Only the bile duct epithelial cells are distinctively demonstrated, while the vast majority of hepatocytes are only faintly positive. Compare with Figs. 2b-5b, same protocol.

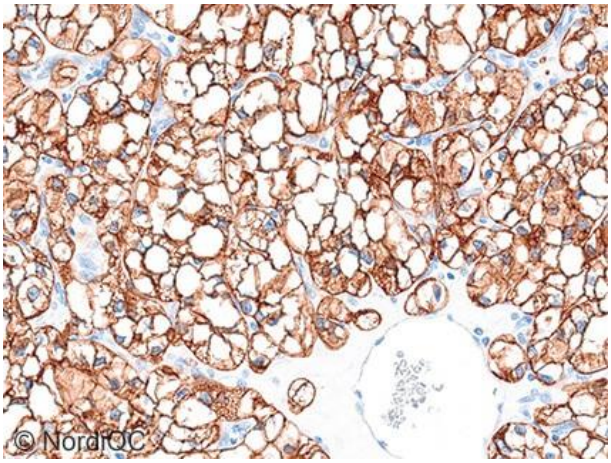



Fig. 2a (x200)
Optimal CK8/18 staining reaction of the renal clear cell carcinoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a distinct, moderate to strong staining reaction.

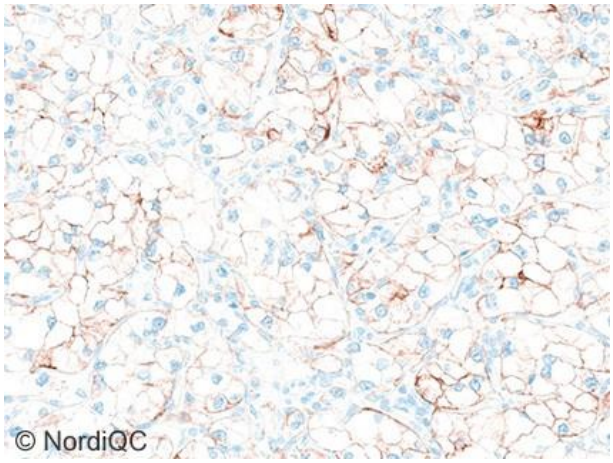



Fig. 2b (x200)
Insufficient CK8/18 staining reaction of the renal clear cell carcinoma using same protocol as in Fig. 1b. Only scattered neoplastic cells show a weak and equivocal staining reaction.

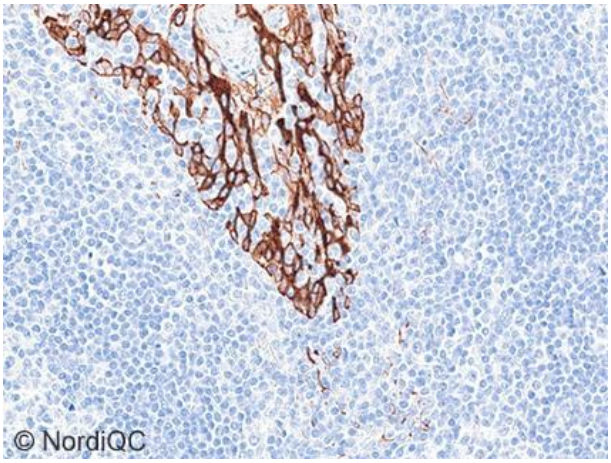



Fig. 3a (x100)
Optimal CK8/18 staining reaction of the tonsil using same protocol as in Figs. 1a - 5a. The majority of squamous epithelial cell display a moderate to strong cytoplasmic staining reaction. Scattered dendritic cells show a weak to moderate cytoplasmic staining reaction.

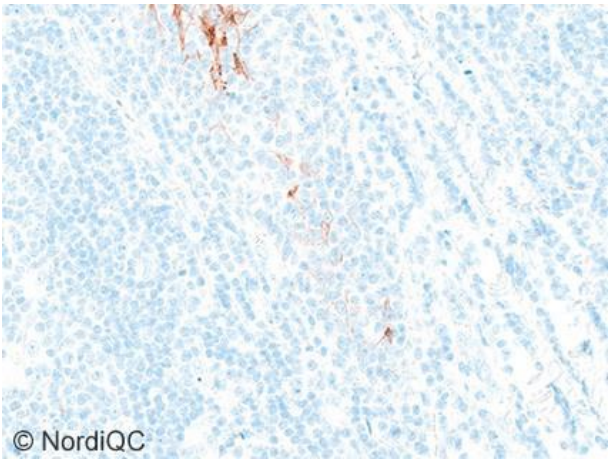



Fig. 3b (x100)
Insufficient CK8/18 staining reaction of the tonsil using the same protocol as in Figs. 1b – 5b. The majority of squamous epithelial cells display a too weak staining reaction. Compare with Fig. 3a.

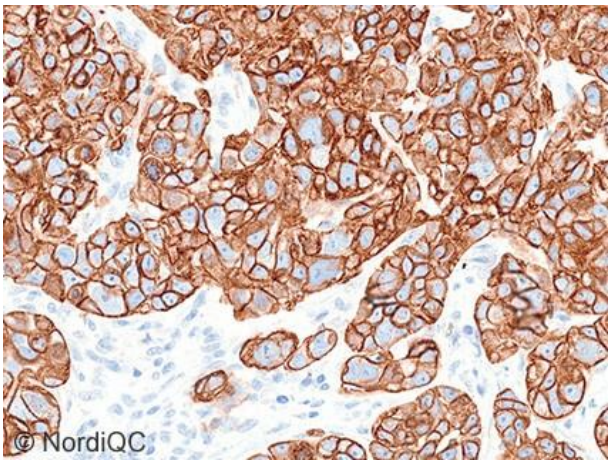



Fig. 4a (x200)
Optimal CK8/18 staining reaction of the breast ductal carcinoma using same protocol as in Figs. 1a-5a. Virtually all neoplastic cells show a strong and distinct cytoplasmic staining reaction.

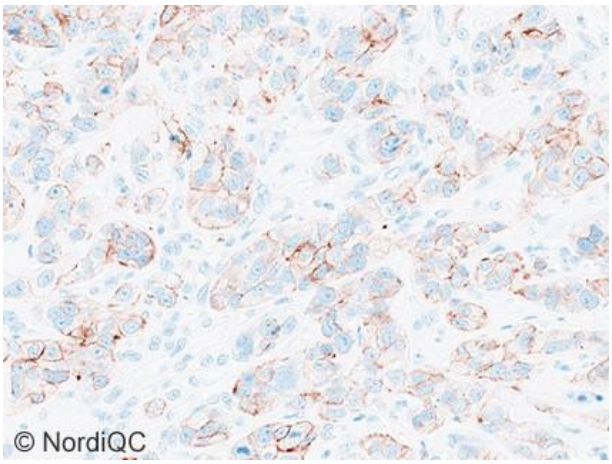



Fig. 4b (x200)
Insufficient CK8/18 staining reaction of the breast ductal carcinoma using same protocol as in Figs. 1b-5b. Virtually all neoplastic cells are only faintly demonstrated. Compare with Fig. 4a.

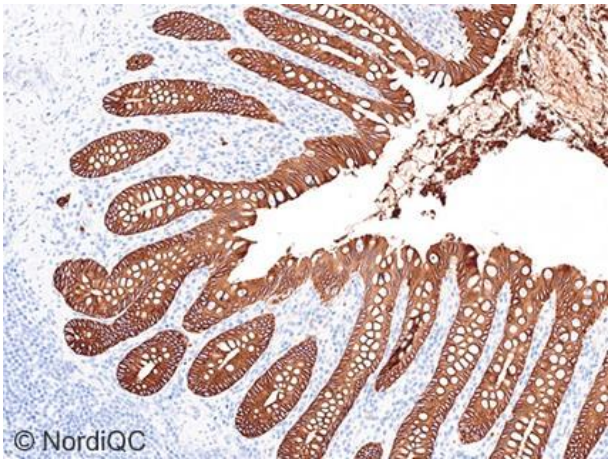



Fig. 5a (x100)
Optimal CK8/18 staining reaction of the appendix using same protocol as in Figs. 1a-5a.
Virtually all columnar epithelial cells show a strong and distinct, predominantly membranous but also cytoplasmic staining reaction.


Fig. 5b (x100)
Insufficient CK8/18 staining reaction of the appendix using same protocol as in Figs. 1b-5b. Virtually all surface epithelial cells show a strong cytoplasmic staining reaction, while most crypt cells display only weak staining reaction.

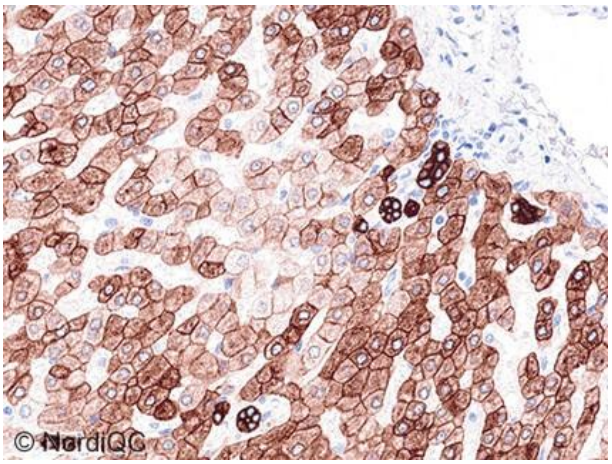



Fig. 6a (x200)
Sufficient CK8/18 staining reaction of the liver using an insufficient protocol with the Dako/Agilent RTU format IR/IS618 based on the mAb clone DC10 by incubation of 20 min. in primary Ab, HIER in TRS High pH 9 for 25 min., a 2-step polymer based detection kit (EnVision FLEX) and performed on Dako Autostainer. Same field as in Fig. 1a. The staining is slightly weaker compared to Fig. 1a but would still be considered optimal. However, compare with Figs. 6b-8b, same protocol, the compiled staining result is insufficient.

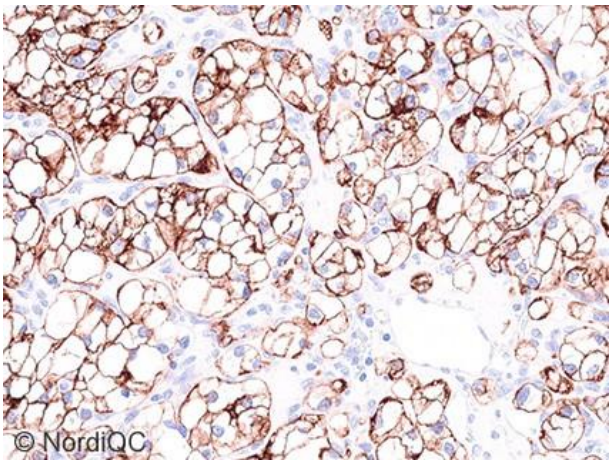



Fig. 6b (x200)
Sufficient staining reaction for CK8/18 in the renal clear cell carcinoma using same – overall -insufficient protocol as in Fig. 6a. Virtually all the neoplastic cells show a distinct, moderate to strong staining reaction. Compare to Figs. 2a and b – same field as Fig. 2a.
However, compare with Figs. 6a-8b, same protocol, the compiled staining result is insufficient.

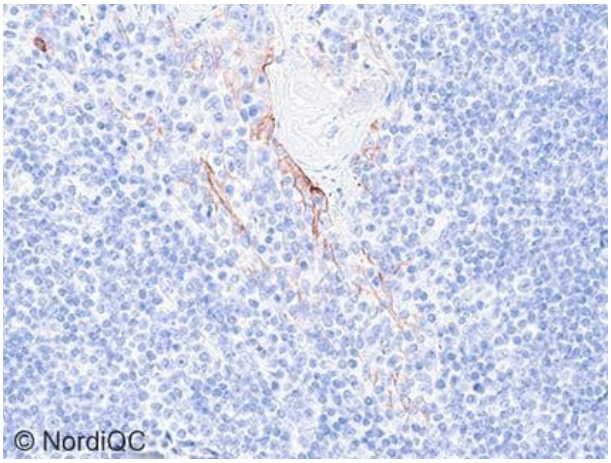



Fig. 7a (x100)
Insufficient CK8/18 staining reaction of the tonsil using the same protocol as in Figs. 6a-8b. The majority of squamous epithelial cells display a too weak staining reaction. Compare with Fig. 3a – same field.

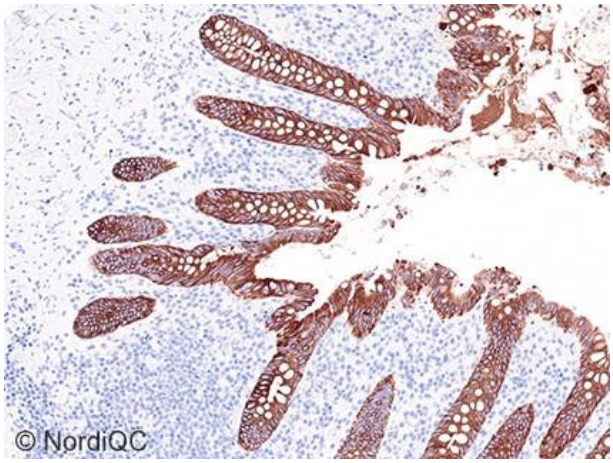



Fig. 7b (x200)
Sufficient staining reaction for CK8/18 of the appendix using same insufficient protocol as in Figs. 6a-8b. Virtually all columnar epithelial cells display a strong and distinct, predominantly membranous but also cytoplasmic staining reaction.

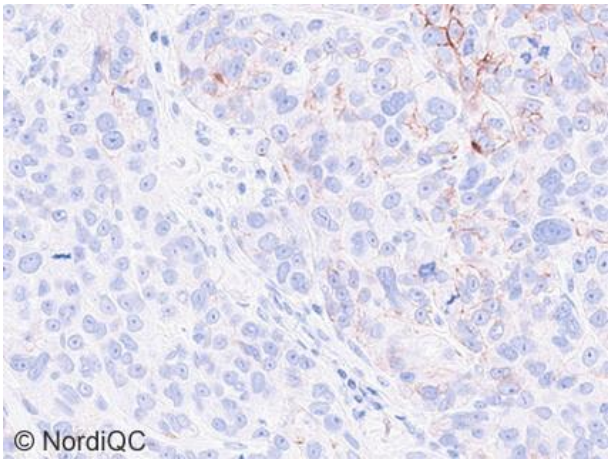



Fig. 8a (x200)
Insufficient CK8/18 staining reaction of the breast ductal carcinoma using same protocol as in Figs. 6a-8b. Despite producing optimal staining reaction in liver (and the renal cell carcinoma), virtually all neoplastic cells are unstained. This tumour belongs to the approx. 25% of breast 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. 4a – same field.

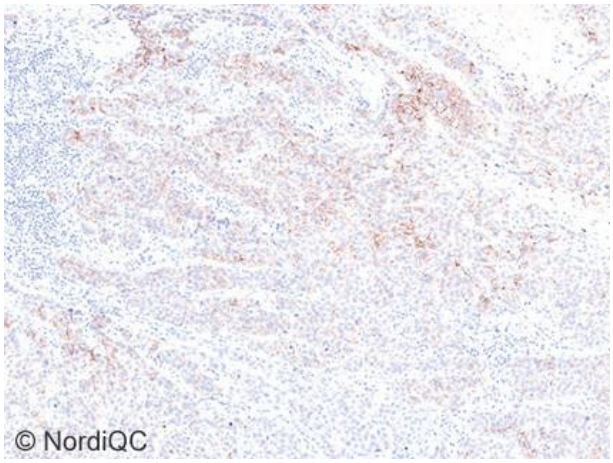



Fig 8b (x100)
Same area as Fig 8a, but in a higher magnification. Only some areas of the breast ductal carcinoma display a weak to moderate staining reaction whereas others are completely negative.

TJ/LE/SN 30.11.2023

| Version | Description of change and reason | Date | Authorized by |
|---|---|---|---|
| 2 | Table 1 and corresponding text for ready-to-use prod. from Celnovte changed due to wrong clone name and product number in version 1. | 03.01.2024 | LE/SN |