# Assessment Run 38 2013
# CDX2

**Material**
The slide to be stained for CDX2 comprised:


1. Appendix, 2.Thyroid gland, 3. Pancreas, 4. Colon adenocarcinoma,
5. Lung adenocarcinoma, 6. Pancreas adenocarcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CDX2 staining as optimal included:

- A strong, distinct nuclear staining reaction of virtually all epithelial cells in the appendix
- A moderate to strong, distinct nuclear staining reaction of virtually all neoplastic cells in the colon adenocarcinoma
- An at least weak to moderate, distinct nuclear staining reaction of scattered neoplastic cells in the pancreas adenocarcinoma
- An at least weak to moderate and distinct nuclear staining reaction of the majority of duct epithelial cells in the pancreas
- A maximally weak cytoplasmic reaction in cells with strong nuclear staining. All other cells should be negative

200 laboratories participated in this assessment. 146 (73 %) achieved a sufficient mark (optimal or good). Antibodies (Abs) used and marks are summarized in table 1.

Table 1. **Abs and assessment marks for CDX2, run 38**

| Concentrated Antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **AMT28** | 9 | Leica/Novocastra | 0 | 3 | 3 | 3 | 33 % | - |
| mAb clone **CDX2-88** | 15<br>4 | Biogenex<br>Biocare | 2 | 3 | 2 | 12 | 26 % | 50 % |
| mAb clone **DAK-CDX2** | 31 | Dako | 6 | 9 | 9 | 7 | 48 % | 80 % |
| rmAb clone **EP25** | 2<br>1 | Epitomics<br>Zhongshan | 0 | 2 | 0 | 1 | - | - |
| rmAb clone **EPR2764Y** | 23<br>3<br>3<br>1<br>1<br>1<br>1 | Cell Marque<br>Thermo/Neomarkers<br>Zytomed<br>Epitomics<br>Gene Tech<br>Immunologic<br>Nordic Biosite | 20 | 11 | 2 | 0 | 94 % | 94 % |
| Ready-To-Use Antibodies | | | | | | | | |
| mAb clone **AMT28** **PA0535** | 3 | Leica | 0 | 1 | 2 | 0 | - | - |
| mAb clone **AMT28** **NCL-CDX2** | 1 | Novocastra | 0 | 0 | 1 | 0 | - | - |
| mAb clone **CDX2-88** **PM226** | 2 | Biocare | 1 | 0 | 1 | 0 | - | - |
| mAb clone **CDX2-88** **PH22 6AA** | 1 | Menarini | 0 | 1 | 0 | 0 | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| mAb clones **CDX2-88 ZA-0520** | 1 | Zhongshan | 0 | 0 | 1 | 0 | - | - |
| mAb clone **DAK-CDX2 IS/IR080** | 44 | Dako | 11 | 27 | 6 | 0 | 86 % | 95 % |
| rmAb clone **EPR2764Y 760-4380** | 48 | Ventana/Cell Marque | 35 | 10 | 2 | 1 | 94 % | 95 % |
| rmAb clone **EPR2764Y 235R-18** | 3 | Cell Marque | 2 | 1 | 0 | 0 | - | - |
| rmAb clone **EPR2764Y GT201902** | 1 | Gene Tech | 0 | 0 | 0 | 1 | - | - |
| rmAb clone **EPR2764Y MAD-000343QD** | 1 | Master Diagnostica | 1 | 0 | 0 | 0 | - | - |
| Total | 200 | | 78 | 68 | 29 | 25 | - | |
| Proportion | | | 39 % | 34 % | 15 % | 12 % | 73 % | |

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

The following protocol parameters were central to obtain optimal staining:

**Concentrated Antibodies**
mAb clone **CDX2-88**: The 2 protocols with optimal results were both based on heat induced epitope retrieval (HIER) using either Bond Epitope Retrieval Solution 2 (BERS2; Leica) (1/2) or Tris-EDTA/EGTA pH 9 (1/5) 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 2 of 4 (50 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **DAK-CDX2**: Protocols with optimal results were all based on HIER using either TRS pH 9 (3-in-1) (Dako) (5/11) or Tris-EDTA/EGTA pH 9 (1/5) as retrieval buffer. The mAb was typically diluted in the range of 1:10-1:30 depending on the total sensitivity of the protocol employed. Using these protocol settings 8 of 10 (80 %) laboratories produced a sufficient staining (optimal or good).

rmAb clone **EPR2764Y**: Protocols with optimal results were all based on HIER using either TRS pH 9 (3-in-1) (Dako) (2/4), TRS low pH 6.1 (Dako) (1/1), BERS 2 (Leica) (4/5), Cell Conditioning 1 (CC1; Ventana) (10/16), Dewax and HIER buffer H (Thermo) (1/1) or Citrate pH 6 (2/2) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 29 of 31 (94 %) laboratories produced a sufficient staining (optimal or good).

Table 2 summarizes the overall proportion of optimal staining results for the three most frequently used concentrated Abs and IHC stainer platforms.

Table 2. **Optimal results for CDX2 using concentrated antibodies on the 3 main IHC systems***

| Concentrated antibodies | **Dako Autostainer Link / Classic** | | **Ventana BenchMark XT / Ultra** | | **Leica Bond III / Max** | |
|---|---|---|---|---|---|---|
| | 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 **CDX2-88** | 50 %<br>1/2** | - | 0 %<br>0/8 | - | 50 %<br>1/2 | - |
| mAb clone **DAK-CDX2** | 67 %<br>6/9 | - | 0 %<br>0/7 | - | - | - |
| rmAb clone **EPR2764Y** | 60 %<br>3/5 | 100 %<br>1/1 | 63 %<br>10/16 | - | 80 %<br>4/5 | 0 %<br>0/2 |

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms.

** Number of optimal results/number of laboratories using this buffer.

**Ready-To-Use Antibodies**
mAb clone **CDX2-88** (product.no. PM226; Biocare): The protocol with an optimal result was based on HIER in a Pressure Cooker using Diva Decloaker pH 6.2, 30 min. incubation of the primary Ab and MACH4 (M4U534) as detection system.

mAb clone **DAK-CDX2** (product.no. IS/IR080, Dako): Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 10-20 min. at 95-99°C), 15-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 39 of 41 (95 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **EPR2764Y** (prod. no. 760-4380, Ventana/Cell Marque): Protocols with optimal results were all based on HIER using mild, standard or extended Cell Conditioning 1, 12-48 min. incubation of the primary Ab and UltraView (760-500) (+/- amplification kit) or OptiView (760-700) as detection system. Using these protocol settings 42 of 44 (95 %) laboratories produced a sufficient staining (optimal or good).

The most frequent causes of insufficient stainings were:

- Too low concentration of the primary antibody.
- Less successful performance of the mAb clones CDX2-88 and AMT28.
- Poor performance of the mAb clones DAK-CDX2 and CDX-88 on the Ventana BenchMark platform.
- Insufficient HIER – too short efficient HIER time.

In this assessment and in concordance with the previous NordiQC runs for CDX2, the prevalent feature of an insufficient staining was a too weak or completely false negative staining reaction of the cells expected to be demonstrated. Virtually all laboratories were able to demonstrate CDX2 in high-level CDX2 expressing cells in the appendix and the colon adenocarcinoma, whereas low-level CDX2 expressing cells in the pancreas adenocarcinoma and the epithelial cells of the pancreatic ducts could only be demonstrated with an optimal protocol. This staining pattern was seen in 74 % of the insufficient results (40 of 54 laboratories). The remaining 26 % of insufficient result was characterized by a too weak staining in combination with e.g. an excessive background staining, impaired morphology or excessive counterstaining.

The rmAb clone EPR2764Y gave the highest proportion of optimal results, as seen in table 1 and an optimal result could be obtained on the 3 most widely used IHC platforms, as shown in table 2.
The relatively new Abs for CDX2, mAb clone DAK-CDX2 and rmAb EPR2764Y gave a higher pass rate and proportion of optimal results compared to the more established mAb clones CDX2-88 and AMT28. This was seen for both the concentrated formats and the corresponding RTU systems.
For the mAb clone DAK-CDX2 the proportion of optimal results was highly influenced by the IHC stainer platform. If the Ab was applied on the Dako Autostainer system and used as a concentrate in the range of 1:10-30, HIER in TRS high pH 9, 67 % of the laboratories obtained an optimal staining result.
In comparison no optimal results were obtained by the 7 laboratories using the same clone and similar protocol settings on the Ventana BenchMark platform, see table 2. Several parameters could contribute to this significant difference e.g. sensitivity of the detection systems used and/or impact of other reagents.

In general. RTU systems for the mAb clone DAK-CDX2 (Dako) and the rmAb clone EPR2764Y (Ventana) both provided a high pass rate. However it has to be emphasized that RTU formats have to be used within a system. In this assessment, all laboratories (n=4) using the Dako RTU format of the mAb clone DAK-CDX2 on a Ventana IHC platform provided an insufficient staining result.

**Controls**
In this assessment and as observed in the previous runs for CDX2, pancreas is highly recommendable as positive controlThe vast majority of the ductal epithelial cells must show an at least weak to moderate but distinct nuclear staining reaction. If these cells are identified, CDX2 can be demonstrated in low expressing neoplasias[1]. Appendix and colon are not recommended as positive controls, since the epithelial cells express high levels of CDX2 and cannot be used to evaluate whether the protocol applied has a high or low sensitivity.
In order to monitor the specificity, no nuclear or cytoplasmic staining must be seen in lymphocytes, endothelial cells and muscle cells.

**Performance history**
This was the 4th assessment of CDX2 in NordiQC. A constant increase in the pass rate has been observed in the two latest runs as listed in table 3.

Table 3**. Proportion of sufficient results for CDX2 in the four NordiQC runs performed**

| | Run 22 2008 | Run 27 2009 | Run 33 2011 | Run 38 2013 |
|---|---|---|---|---|
| Participants, n= | 56 | 93 | 148 | 200 |
| Sufficient results | 64 % | 46 % | 51 % | 73 % |

Several parameters may contribute to the improved pass rate: Fewer laboratories are using the less successful mAb clones CDX-88 and AMT28. In this run 18 % of the laboratories used one of these clones compared to 33 % and 73 % in run 33 and 27, respectively. Also, the tailored recommendations for protocol improvement given to laboratories with an insufficient mark seemed to have a positive impact: 73 laboratories who had received recommendations in run 33 also submitted stains for this run. 48 laboratories followed the recommendations, and out of these, 39 (81 %) improved to a sufficient result. 20 laboratories did not change their protocol, and out of these only 5 (20 %) improved. 5 laboratories changed their entire system and of these 3 obtained a sufficient result.

**Conclusion**

The mAb clone **DAK-CDX2** and the rmAb clone **EPR2764Y** can both be used to obtain optimal staining for CDX2. The two Abs gave high pass rates both as concentrates and RTU systems. The rmAb clone EPR2764Y could produce optimal results on all the 3 main IHC systems ( (Dako, Ventana and Leica), while the performance of the mAb clone DAK-CDX2 seems to be influenced by the stainer platform, giving a significantly lower pass rate on the Ventana Benchmark platform compared to the pass rate on the Dako Autostainer platform. Pancreas is an appropriate positive control for CDX2: A weak to moderate, distinct nuclear reaction in the majority of the duct epithelial cells in the pancreas must be seen.

1. Demonstration of CDX2 is highly antibody dependant. Appl Immunohistochem Mol Morphol. 2013 Jan;21(1):64-72. Borrisholt M, Nielsen S, Vyberg M

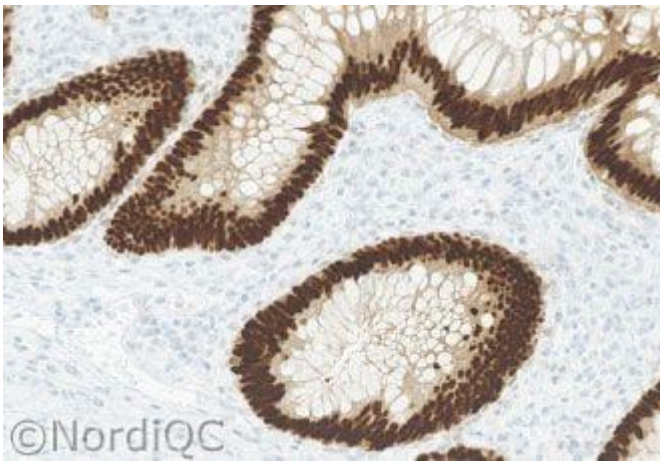



Fig. 1a
Optimal CDX2 staining of the appendix using the rmAb clone EPR2764Y as a concentrate optimally
calibrated and with HIER in an alkaline buffer and performed on the BenchMark ULTRA, Ventana.
A strong nuclear staining reaction is seen in virtually all the epithelial cells. In the cytoplasmic compartment a weak staining reaction is seen. No background staining is seen.
Also compare with Figs. 2a and 2b – same protocol.

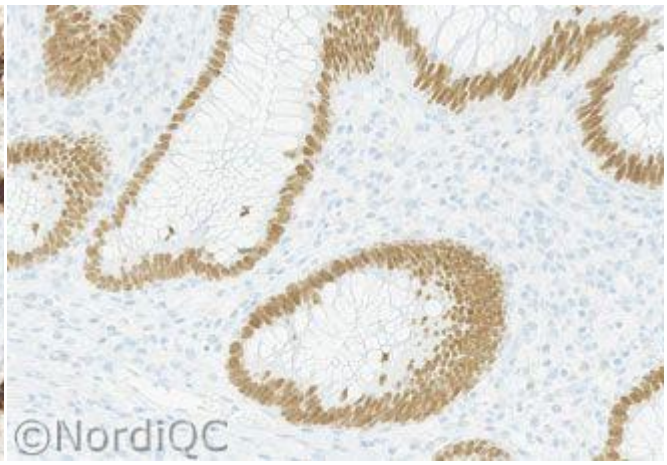
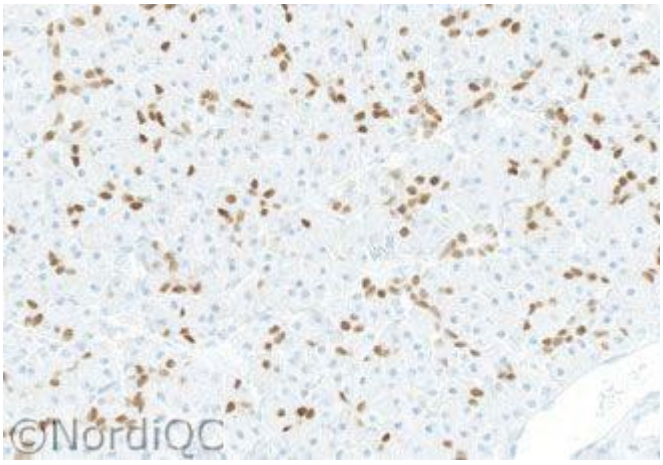



Fig. 1b
CDX2 staining using an insufficient protocol based on the mAb clone DAK-CDX2 with
HIER in an alkaline buffer and performed on the BenchMark ULTRA, Ventana.
A moderate nuclear staining reaction is seen in virtually all the epithelial cells – same field as in Fig. 1a.
However also compare with Figs. 2b and 3b – same protocol.



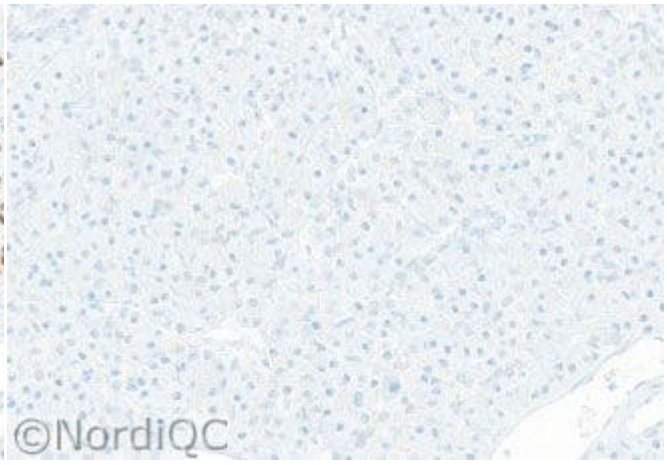



Fig. 2a
Optimal CDX2 staining of the normal pancreas using same
protocol as in Fig. 1a.
A moderate to strong nuclear staining reaction is seen in virtually all the ductal epithelial cells.
A high signal-to-noise ratio is observed.

Fig. 2b
Insufficient and false negative CDX2 staining of the normal pancreas using same protocol as in Fig. 1b.
No nuclear staining reaction is seen in the ductal epithelial cells - same field as in Fig. 2a.
Also compare with Fig. 3b – same protocol.

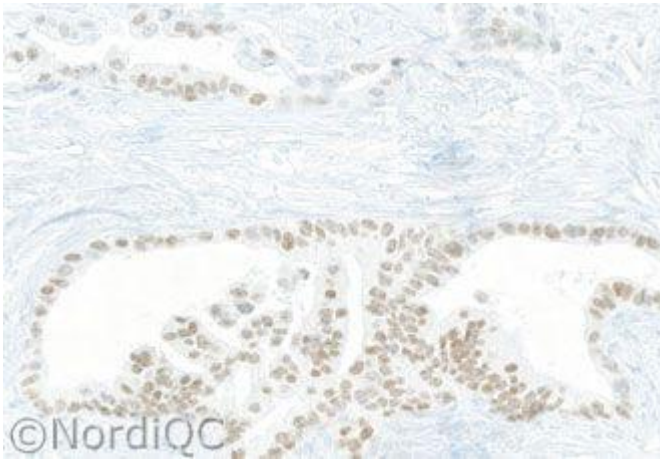



Fig. 3a
Optimal CDX2 staining of the pancreas adenocarcinoma using same protocol as in Figs. 1a and 2b.
The vast majority of the neoplastic cells show a weak to moderate and distinct nuclear staining reaction.

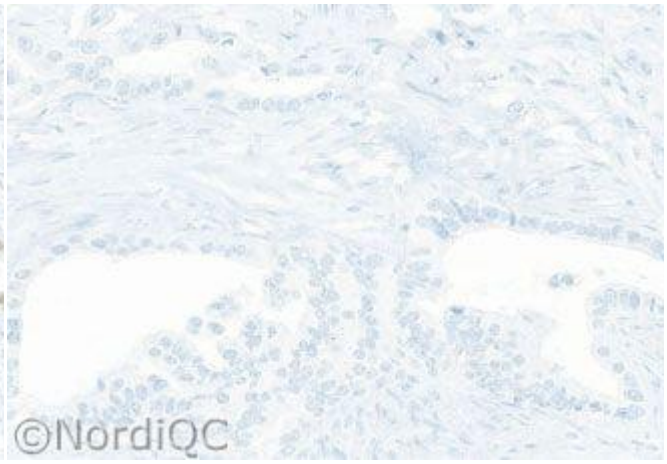



Fig. 3b
Insufficient and false negative CDX2 staining of the pancreas adenocarcinoma using same protocol as in Figs. 1b and 2b.
No nuclear staining reaction is seen in the neoplastic cells - same field as in Fig. 3a.

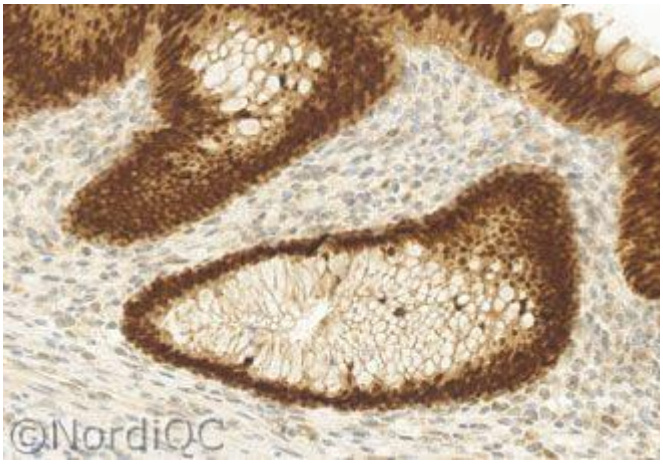



Fig. 4a
Insufficient CDX2 staining of the appendix using the mAb clone CDX-88.
The epithelial cells show a strong nuclear staining reaction, but an aberrant nuclear and cytoplasmic staining reaction is seen in the stromal cells.
The low signal-to-noise ratio most likely is caused by the use of the low affinity Antibody relatively concentrated in combination with a high sensitive protocol based on efficient HIER.
Also compare with Fig. 4b – same protocol.

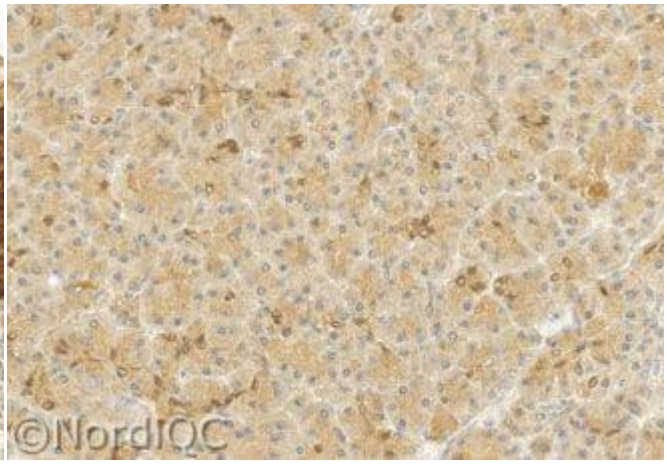



Fig. 4b
Insufficient CDX2 staining of the normal pancreas using the same protocol as in Fig. 4a.
The ductal epithelial cells are demonstrated, but an excessive cytoplasmic staining reaction and a general background staining compromises the interpretation.

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