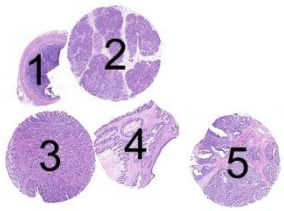
Assessment Run 27 2009
CDX2

The slide to be stained for CDX2 comprised:
1. Appendix, 2. Pancreas, blood type A, 3. Colon adenocarcinoma, 4. Ovarian mucinous cystadenoma, 5. Colon adenocarcinoma.
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a CDX2 staining as optimal included:

- A strong, distinct nuclear staining of virtually all the epithelial cells in the appendix
- A moderate to strong, distinct nuclear staining of virtually all the neoplastic cells in the two colon adenocarcinomas
- An at least weak to moderate, distinct nuclear staining in scattered cells in the ovarian mucinous cystadenoma.
- An at least weak to moderate and distinct nuclear reaction in the majority of the duct epithelial cells in the pancreas.

A weak cytoplasmic reaction in cells with strong nuclear staining was accepted. All other cells should be negative.

93 laboratories participated in this assessment. 46 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CDX2, run 27**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CDX2-88**	35 3	BioGenex Biocare	7	6	9	16	36 %	50 %
mAb clone **AMT28**	21	Novocastra	3	4	5	9	33 %	60 %
mAb clone **DAK-CDX2**	4	Dako	2	2	0	0	-	-
mAb clone **SFI-2**	1	Master Diagnostica	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone **DAK-CDX2 IS080/IR080**	15	Dako	13	2	0	0	100 %	100 %
mAb clone **CDX2-88, AM392**	6	BioGenex	0	0	0	6	-	-
mAb clone **CDX2-88, IP226**	1	Biocare	0	0	0	1	-	-
mAb clone **CDX2-88, E087**	2	Linaris	0	1	1	0	-	-
rmAb clone **EPR2764Y, 760-4380**	4 1	Ventana Cell Marque	1	2	1	1	60 %	100 %
Total	93		26	17	16	34	-	-
Proportion			28 %	18 %	17 %	37 %	46 %	-

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

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

Concentrated Abs
mAb clone **CDX2-88**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) in either Tris-EDTA/EGTA pH 9 (2/11)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (2/6), Bond Epitope Retrieval Solution 2 (Bond, Leica) (2/4) or Citrate pH 6 (1/4) as the retrieval buffer. The mAb was typically diluted in the range of 1:20– 1:100 depending on the total sensitivity of the protocol

employed. Using these protocol settings 9 out of 18 (50 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

mAb clone **AMT28**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (2/10) or Citrate pH 6 (1/2) as the retrieval buffer. 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 10 (60%) laboratories produced a sufficient staining.

mAb clone **DAK-CDX2**: The protocols giving an optimal result were both based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (1/1) or Cell Conditioning 1 (BenchMark, Ventana) (1/2) as the retrieval buffer. The mAb was typically diluted in the range of 1:20– 1:25 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 3 (100 %) laboratories produced a sufficient staining.

Ready-To-Use Abs
mAb clone **DAK-CDX2** (prod. no IS080/IR080, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 20 or 30 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. 1 lab used standard Cell Conditioning 1 (BenchMark, Ventana), an incubation time of 32 min in the primary Ab and Ultra View (760-500) as the detection system. Using these protocol settings 15 out of 15 (100 %) laboratories produced a sufficient staining.

rmAb clone **EPR2764Y** (prod. no. 760-4380, Ventana/Cell Marque), the protocol giving an optimal result was based on HIER using standard Cell Conditioning 1 standard (BenchMark, Ventana), an incubation time of 32 min in the primary Ab and Ultra View (760-500) as the detection system. Using these protocol settings 2 out of 2 (100 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient stains were:
- Too low concentration of the primary antibody
- Insufficient HIER
- Use of low sensitive detection systems
- Less successful ready-to-use (RTU) mAb clone CDX2-88

In this assessment 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 antigen expressing cells in the appendix and one of the colon adenocarcinomas (specimen no. 3) in the multi-tissue block, whereas the low expressing cells in other colon adenocarcinoma (specimen no. 5), the ovarian mucinous cystadenoma and the epithelial cells of the intercalating ducts in the pancreas could only be demonstrated with an optimal protocol.
In this assessment the two newly launched Abs for CDX2, mAb clone DAK-CDX2 and rmAb EPR2764Y gave a higher pass rate than the old mAb clones CDX2-88 and AMT28. E.g., all 19 protocols based on the mAb clone DAK-CDX2 gave a sufficient result, whereas only 7 out of 21 protocols based on the clone AMT28 gave a sufficient result. The 2 new Abs were primarily applied as RTU formats within a predefined RTU system. The superior performance reflects credit on the producers!
As observed in the previous assessment of CDX2 (run 22, 2008), the mAb clone CDX2-88 from BioGenex frequently gave a false positive cytoplasmic staining in the epithelial cells of the pancreas, blood type A due to the "Mouse Ascites Golgi" (MAG) reaction.
In concordance with previous observations, pancreas is a recommendable positive control for CDX2 provided that a distinct nuclear reaction is seen in the majority of the duct epithelial cells. Virtually all labs obtaining this reaction pattern in the pancreas were assessed as sufficient.

This was the second assessment of CDX2 in NordiQC. The proportion of sufficient results declined from 64 % in run 22, 2008 to 46 % in the current run – see table 2. The lower pass rate is probably due to more challenging tissue material circulated and many laboratories participating for the first time.

Table 2. **Proportion of sufficient results for CDX2 in the two NordiQC runs performed**

	Run 22 2008	Run 27 2009
Participants, n=	56	93
Sufficient results	64 %	46 %

Conclusion
The mAbs clones CDX2-88, AMT28, DAK-CDX2 and the rmAb clone EPR2764Y can all be used to obtain an optimal demonstration of CDX2. The new mAb clone DAK-CDX2 and the rmAb clone EPR2764Y gave in this assessment a superior performance. For all four Abs efficient HIER and a sensitive detection system is mandatory to obtain an optimal staining. Pancreas is an appropriate control: A weak to moderate, distinct nuclear reaction in the majority of the duct epithelial cells in the pancreas must be seen.

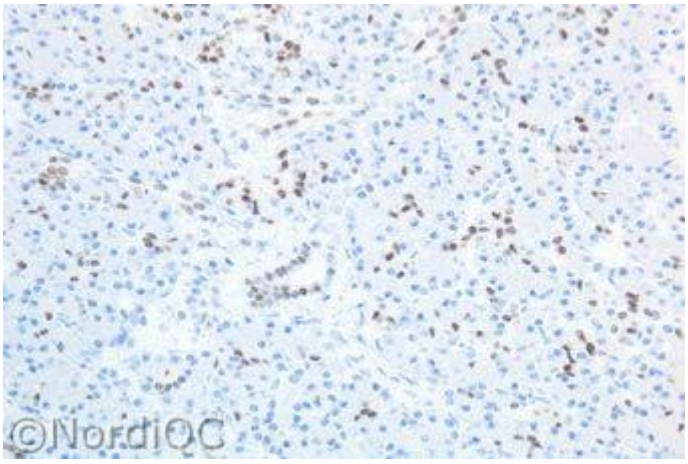


Fig. 1a
Optimal CDX2 staining using the mAb clone DAK-CDX2, optimally calibrated and with HIER in an alkaline buffer. A weak to moderate staining is seen in the majority of the ductal epithelial cells of the pancreas.

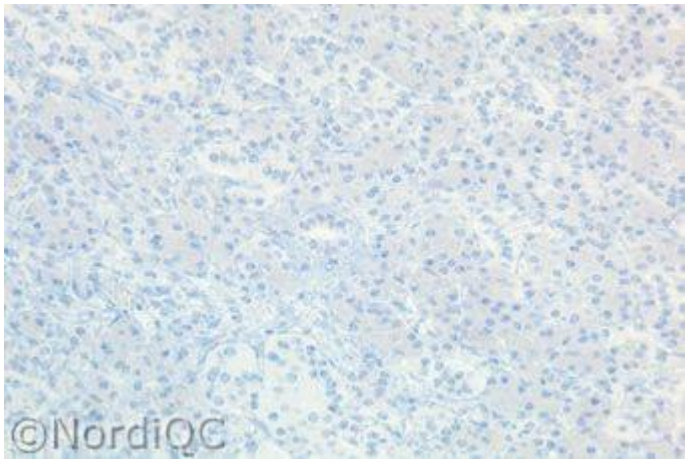


Fig. 1b
CDX2 staining using the mAb clone CDX2-88 too diluted. No nuclear staining is seen in the ductal epithelial cells of the pancreas. Also compare with Figs. 2b left & right – same protocol.

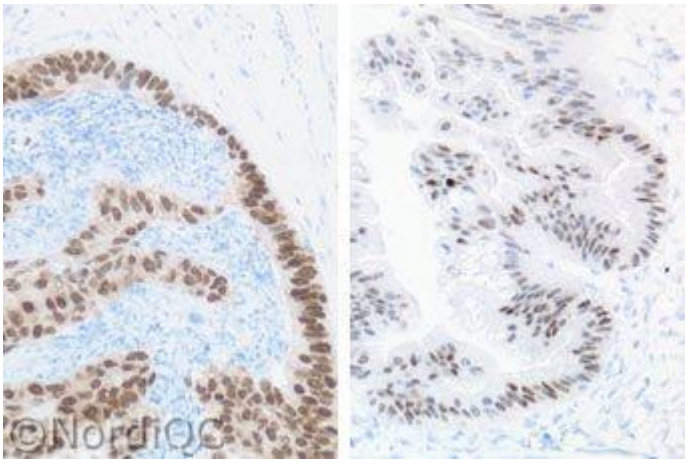


Fig. 2a
Optimal staining for CDX2 using same protocol as in Fig. 1a.
Left: Colon adenocarcinoma tissue no. 5 in the multitissue block: Virtually all the neoplastic cells show a moderate to strong and distinct nuclear reaction.
Right: Ovarian mucinous cystadenoma: The majority of the neoplastic cells show a moderate to strong nuclear reaction.

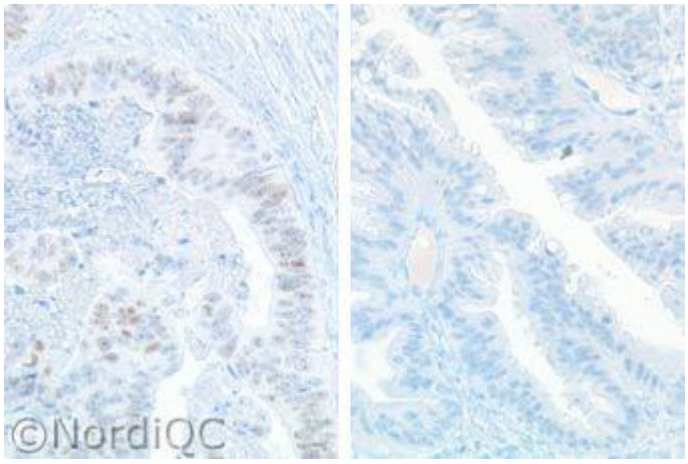


Fig. 2b
Insufficient staining for CDX-2 using same protocol as in Fig. 1b – same fields as in Figs. 2a left & right.
Left: Colon adenocarcinoma tissue no. 5 in the multitissue block: The neoplastic cells show only a weak and diffuse nuclear reaction.
Right: Ovarian mucinous cystadenoma: No nuclear reaction is seen in the neoplastic cells.

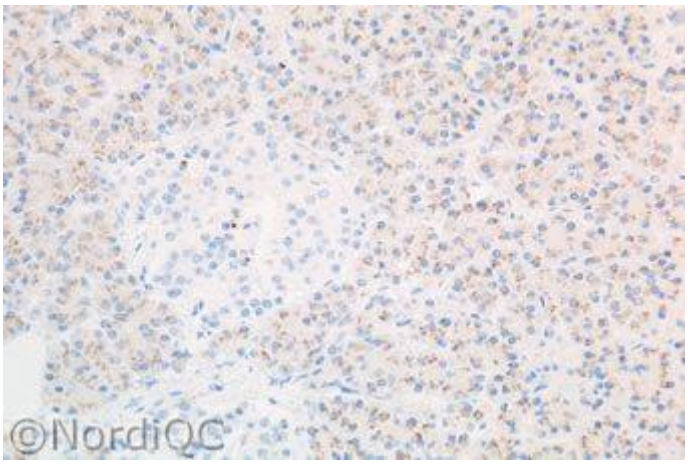


Fig. 3a
Insufficient CDX2 staining using the mAb clone CDX2-88 of the pancreas, blood type A.
No nuclear staining is seen in the ductal epithelial cells while a strong "mouse-anti-Golgi" (MAG) reaction is seen in the majority of the acinic cells. The intensity and proportion of cells showing this aberrant reaction seemed to be highly dependant on the lot of the mAb applied.

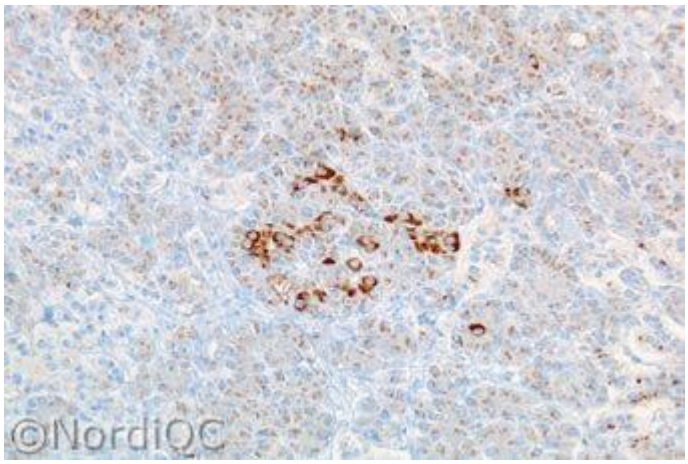


Fig. 3b
Insufficient CDX2 staining using the mAb clone CDX2-88 of the pancreas, blood type A, using efficient HIER and a biotin based detection system. No nuclear staining is seen in the ductal epithelial cells while a MAG reaction is seen in the majority of the acinic cells together with a false positive cytoplasmic reaction due to endogenous biotin in the endocrine cells of the Langerhans islets.

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