

Purpose

Evaluation of the technical performance and the level of analytical sensitivity and specificity of the immunohistochemical (IHC) assays used by NordiQC participants for CDX2. The focus of the assessment was identification of intestinal differentiation and colorectal origin in the characterization of cancers of unknown primary origin. Relevant normal and neoplastic clinical tissues were selected to represent a broad range of CDX2 antigen densities (see below).

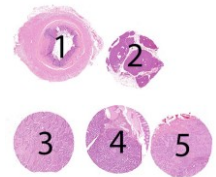
Material

The slide to be stained for CDX2 comprised:

1. Appendix, 2. Pancreas, 3. Lung adenocarcinoma, 4-5. Colon adenocarcinomas

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CDX2 staining as optimal included:



- A strong, distinct nuclear staining reaction of virtually all epithelial cells in the appendix.
- An at least weak to moderate, distinct nuclear staining reaction of virtually all ductal and intercalated duct epithelial cells in the pancreas.
- An at least moderate, predominantly nuclear staining reaction of the majority of the neoplastic cells in the colon adenocarcinoma, tissue core no. 4.
- A strong, distinct nuclear staining reaction of virtually all neoplastic cells in the colon adenocarcinoma, tissue core no. 5.
- No staining reaction in the lung adenocarcinoma.

A weak to moderate cytoplasmic reaction in cells with strong nuclear staining was accepted.

KEY POINTS FOR CDX2 IMMUNOASSAYS

- The rmAb clones EP25 and EPR2764Y and mAb clone DAK-CDX2 were used by 85% of all participants either as concentrated antibodies or as RTU formats, providing an overall pass rate of 97%.
- The RTU systems 760-4380 (EPR2764Y), Ventana/Roche, IR/GA080 (DAK-CDX2), Dako/Agilent and PA0375 (EP25), Leica Biosystems provided superior performance using RTU systems compared to laboratory developed assays based on concentrated formats of same clones.
- Pancreas is recommended as a positive tissue control because it displays low Level of CDX2 expression.

Participation

Number of laboratories registered for CDX2, run 76	488
Number of laboratories returning slides	404 (83%)

At the date of assessment, 83% of the participants had returned the circulated NordiQC slides. In this assessment, run 76, general issues with the Danish postal service affected the distribution and return of slides to/from participants, resulting in a lower number of returned slides compared to previous assessments.

Slides received after the assessment were not included in this report. However, all returned slides were assessed, and participating laboratories with insufficient results received advice.

Results

404 laboratories participated in this assessment and 391 laboratories (97%) achieved a sufficient mark (optimal or good), see Table 1a (see page 3). Tables 1b and 1c summarises the antibodies (Abs) used and assessment marks (see page 3 and 4).

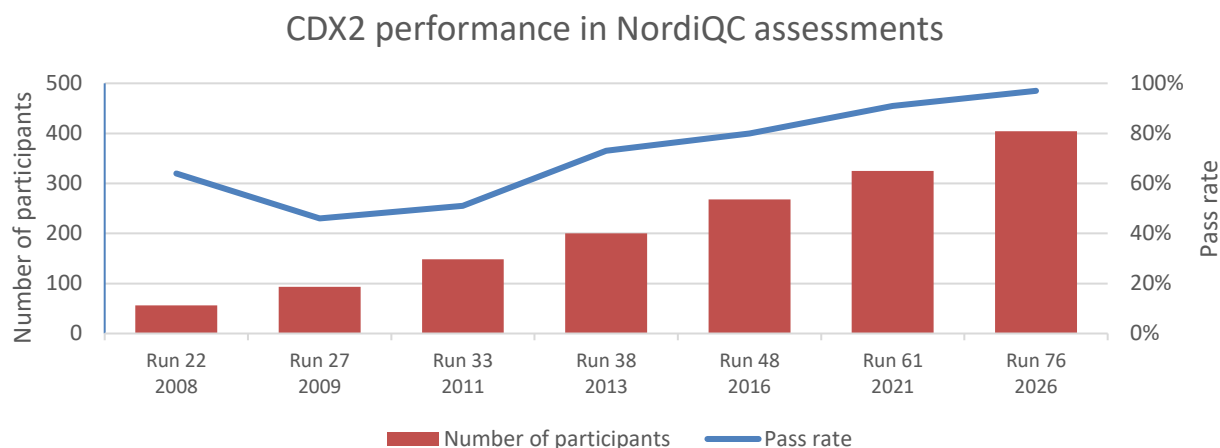
The most frequent causes of insufficient staining reactions were:

- Too low concentration of the primary antibody.
- Inefficient HIER (too short HIER time or use of citric based buffer).
- Use of less sensitive detection systems.

Performance history

This was the seventh NordiQC assessment of CDX2. The pass rate increased to 97% in this assessment compared to the previous run 61 where the pass rate was 91% (see Graph 1).

Graph 1. **Proportion of sufficient results for CDX2 in the seven NordiQC runs performed.**



Controls

Pancreas is recommended as positive tissue control displaying a low level of CDX2 antigen. Virtually all ductal and intercalated duct epithelial cells must show an at least weak to moderate, distinct nuclear staining reaction. Appendix and colon are not recommended as primary positive tissue controls, since the epithelial cells express high levels of CDX2, and thus are not an ideal indicator for the appropriate level of analytical sensitivity.

Appendix or tonsil can both be used as negative tissue controls for CDX2. To assess assay specificity, no nuclear or cytoplasmic staining should be seen in endothelial cells or smooth muscle cells in any of the tissues. The vast majority of lymphocytes should be negative, although weak nuclear staining reaction may be observed in scattered lymphatic cells, as seen in this assessment in the lung adenocarcinoma. The recommendations of the mentioned tissue controls for IHC are concordant with the guidelines published by the International Ad Hoc Expert Committee¹.

¹Torlakovic EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. *Appl Immunohistochem Mol Morphol*. 2015 Jan;23(1):1-18. doi: 10.1097/PAI.000000000000163. Review. PubMed PMID: 25474126.

Conclusion

The mouse monoclonal antibody (mAb) clone **DAK-CDX2** and the rabbit monoclonal antibodies (rmAb) clones **EPR2764Y** and **EP25** are all recommendable for demonstration of CDX2. Optimal results were obtained using efficient HIER, preferably in an alkaline buffer, in combination with careful calibration of the antibody titer adjusted to the total sensitivity of the protocol applied. Assays based on the mAb clone DAK-CDX2, provided a higher pass rate with the use of a sensitive 3-step detection system (e.g., EnVision Flex+). The RTU systems from Dako/Agilent (GA080), Leica (PA0375) and Ventana/Roche (760-4380) provided superior results for demonstration of CDX2, and using vendor recommended protocol settings, almost all obtained sufficient results.

Table 1a. **Overall results for CDX2, run 76**

	n	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
Concentrated antibodies	47	32	7	7	1	83%	68%
Ready-To-Use antibodies	357	309	43	5	-	99%	87%
Total	404	341	50	12	1		
Proportion		84%	12%	3%	1%	97%	

1) Proportion of sufficient stains (optimal or good).

2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for CDX2, run 76**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone DAK-CDX2	6	Dako/Agilent	1	1	3	1	33%	17%
rmAb clone EPR2764Y	29	Cell Marque	23	4	2	-	93%	79%
	2	Zytomed Systems	1	1	-	-		
	2	Abcam	2	-	-	-		
	1	Zytomics	1	-	-	-		
	1	Thermo Scientific	1	-	-	-		
rmAb clone EP25	1	Biocare Medical	-	-	1	-	-	-
	1	PathNSitu	-	1	-	-		
rmAb clone QR045	2	Quartett	2	-	-	-	-	-
rmAb clone ZR215	1	Zeta Corporation	-	-	1	-	-	-
rmAb clone IHC302	1	GenomeMe	1	-	-	-	-	-
Total	47		32	7	7	1		
Proportion			68%	15%	15%	2%	83%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).

2) Proportion of Optimal Results (≥5 assessed protocols).

Table 1c. **Ready-To-Use antibodies and assessment marks for CDX2, run 76**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
rmAb clone EPR2764Y, 760-4380³	57	Ventana/Roche	53	3	1	-	98%	93%
rmAb clone EPR2764Y, 760-4380⁴	106	Ventana/Roche	97	9	-	-	100%	92%
mAb clone DAK-CDX2, GA080³	53	Dako/Agilent	50	3	-	-	100%	94%
mAb clone DAK-CDX2, GA080⁴	34	Dako/Agilent	25	9	-	-	100%	74%
mAb clone DAK-CDX2, IR080³	4	Dako/Agilent	3	1	-	-	-	-
mAb clone DAK-CDX2, IR080⁴	13	Dako/Agilent	8	3	2	-	84%	62%
rmAb clone EP25, PA0375³	29	Leica Biosystems	26	3	-	-	100%	90%
rmAb clone EP25, PA0375⁴	10	Leica Biosystems	6	4	-	-	100%	60%
rmAb clone EP25, 8285-C010	3	Sakura Finetek	1	2	-	-	-	-
rmAb clone EPR2764Y, 235R-17/18	33	Cell Marque	29	3	1	-	97%	88%
rmAb clone EPR2764Y, BRB028	3	Zytomed Systems	2	1	-	-	-	-
rmAb clone EP25, GT201902	1	Gene Tech	1	-	-	-	-	-
rmAb clone EP25, MAD000645	2	Master Diagnostica/ Vitro S.A	1	1	-	-	-	-
rmAb clone EP25, HAR028	1	PathNsitu	-	1	-	-	-	-
mAb clone BC39, API3184	1	Biocare Medical	-	-	1	-	-	-
rmAb clone 353G7A8, PA207	1	Abcarta	1	-	-	-	-	-
rmAb clone 353G7A8, B61011	1	Guangzhou Biotron	1	-	-	-	-	-
rmAb clone BY155, BFM-0477	1	Bioin Biotechnology	1	-	-	-	-	-
rmAb clone BP6023, BX50018	1	Biolyx Biotechnology	1	-	-	-	-	-
rmAb clone C2X5, CCR-0825	1	Celnovte	1	-	-	-	-	-
rmAb clone DY49022, 4911852	1	Dakewe	1	-	-	-	-	-
rmAb clone MXR024, RMA-1056	1	Fuzhou Maixin	1	-	-	-	-	-
Total	357		309	43	5	0		
Proportion			87%	12%	1%	0%	99%	

1) Proportion of sufficient stains (optimal or good) (≥ 5 assessed protocols).

2) Proportion of Optimal Results (≥ 5 assessed protocols).

3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥ 5 assessed protocols).

4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥ 5 assessed protocols).

Detailed analysis of CDX2, Run 76

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

rmAb clone **EPR2764Y**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Cell Conditioning 1 (CC1, Ventana/Roche) (14/15)*, Cell Conditioning 2 (CC2, Ventana/Roche) (1/2), TRS pH 9 (3-in-1) (Dako/Agilent) (5/5), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (7/12) or Tris-EDTA pH 9 (1/1) as retrieval buffer. The rmAb was typically diluted in the range of 1:50-1:400. Using these protocol settings, 30 of 32 (94%) laboratories produced a sufficient staining result.

* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **DAK-CDX2**: One protocol with an optimal result was based on HIER using BERS2 (Leica Biosystems) (1/3) as retrieval buffer. The mAb was diluted in the range of 1:50 and Bond Refine (Leica Biosystems) was used as detection system.

Table 2. **Proportion of optimal results for CDX2 for the most commonly used antibody concentrates on the four main IHC systems by optimal settings as listed above.**

Concentrated antibodies	Dako/Agilent Autostainer ¹		Dako/Agilent Omnis		Ventana/Roche BenchMark ²		Leica Biosystems Bond ³	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone DAK-CDX2	-	-	-	-	-	-	1/1	-
rmAb clone EPR2764Y	-	-	4/4	-	93% (13/14)	1/2	54% (6/11)	-

1) Autostainer Classical, Link 48.

2) BenchMark GX, XT, Ultra, Ultra plus

3) Bond III, Prime, Max

Ready-To-Use antibodies and corresponding systems

rmAb clone **EPR2764Y**, product no. **760-4380**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus: Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 24-56 min.) and 12-48 min. incubation of the primary Ab. UltraView (760-500) +/- amplification kit or OptiView (760-700) were used as detection systems. Using these protocol settings, 148 of 149 (99%) laboratories produced a sufficient staining result (optimal or good).

The product was used by 2 laboratories on a non-intended platform. Data was not included in the description above

mAb clone **DAK-CDX2**, product no. **IR080**, Dako/Agilent, Autostainer+/Autostainer Link:

Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20-25 min. at 97-99°C), 20 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Using these protocol settings, 5 of 5 (100%) laboratories produced a sufficient staining result.

The product was used by 7 laboratories on a non-intended platform. Data was not included in the description above

mAb clone **DAK-CDX2**, product no. **GA080**, Dako/Agilent, Omnis:

Protocols with optimal results were typically based on HIER using TRS pH 9 (efficient heating time 20-30 min. at 97°C), 15-30 min. incubation of the primary Ab and Envision FLEX or Flex+ (GV800+GV821) as detection system. Using these protocol settings, 82 of 82 (100%) laboratories produced a sufficient staining result.

The product was used by 2 laboratories on a non-intended platform. Data was not included in the description above

rmAb clone **EP25** product no. **PA0375**, Leica Biosystems, Bond III/MAX/Prime:

Protocols with optimal results were typically based on HIER using BERS2 (efficient heating time 20 min. at 99-100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 33 of 33 (100%) produced a sufficient staining result.

Table 3 (see page 6) summarises 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 accordingly 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 CDX2 for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
VMS BenchMark rmAb EPR2764Y 760-4380 UltraView DAB	98% (56/57)	93% (53/57)	97% (36/37)	83% (31/37)
VMS BenchMark rmAb EPR2764Y 760-4380 OptiView DAB			100% (66/66)	97% (64/66)
Dako Omnis mAb DAK-CDX2 GA080	100% (53/53)	94% (50/53)	100% (32/32)	75% (24/32)
Dako AS mAb DAK-CDX2 IR080	(4/4)	(3/4)	67% (4/6)	33% (2/6)
Leica Bond rmAb EP25 PA0375	100% (29/29)	90% (26/29)	100% (10/10)	60% (6/10)

* 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 runs for CDX2, the prevalent feature of an insufficient result was a too weak or completely false negative staining reaction of cells expected to be demonstrated. This staining pattern was seen in 92% (12/13) of the insufficient results. Virtually all laboratories were able to demonstrate CDX2 in high-level antigen expressing cells of the appendix and the colon adenocarcinoma, tissue core no. 5, whereas low-level CDX2 expressing cells of the colon adenocarcinoma, tissue core no. 4, and the epithelial cells of the intercalated pancreatic ducts were more challenging and could only be demonstrated with an optimally calibrated protocol.

The rmAb clones EP25 and EPR2764Y and the mAb clone DAK-CDX2 were the most widely used antibodies for demonstration of CDX2 and were applied by 85% (345/404) of the laboratories (see Table 1b and 1c). Assays based on these robust primary Abs contributed to the overall high pass rate of 97% obtained in this assessment and emphasize the importance of selecting high-performing antibodies with regard to analytical sensitivity and specificity.

Used as concentrated format within laboratory developed (LD) assays, the **rmAb clone EPR2764Y** provided 93% (33/35) sufficient results of which 79% (28/35) were assessed as optimal. As described in the previous report (Run 61), efficient HIER in an alkaline buffer and careful calibration of the primary Ab were the two most central parameters for optimal results. 57% (20/35) applied a 3-step multimer/polymer-based detection system for the demonstration of CDX2 with high level of optimal results (95%, 19/20).

The remaining 43% (15/35) used a 2-step detection system providing a pass rate of 93% (14/15) but only 60% (9/15) were optimal.

Twelve protocols were based on Bond Refine (Leica Biosystems) as the detection system. By design, this system only enhances the reaction of mouse primary antibodies, as the linker (the post-primary reagent/molecule) between the primary antibody and the polymer is a rabbit anti-mouse antibody. When a rabbit primary antibody is applied, the system essentially functions as a two-step polymer detection system. As a result, protocol optimization on the Bond platform needed a careful calibration. Achieving optimal performance required sufficient HIER time (20–30 min.) and careful calibration of the antibody titer, typically in the range of 1:50–1:150.

The **mAb clone DAK-CDX2** used within as a LD assay could also produce optimal results. However, compared with the rmAb clone EPR2764Y, the clone was less successful, as a lower proportion of sufficient and optimal results was observed, despite the use of similar protocol settings, although generally with lower antibody titers (1:20–100).

As mentioned in the last report (Run 61), the performance of the mAb clone DAK-CDX2 is influenced by the stainer platform and especially challenged on BenchMark (Ventana/Roche). For this platform, only 12% (2/17) of the assays based on DAK-CDX2 provided a sufficient result during the last two runs (compiled data). One protocol was optimal. These observations indicate that for CDX2 assays it is advisable to substitute the mAb clone DAK-CDX2 (e.g. with EPR2764Y) on the BenchMark platform.

In total, 88% (357/404) of the laboratories used an RTU format. The most widely used RTU systems for CDX2 were the Ventana/Roche 760-4380, Leica Biosystems PA0375, Dako/Agilent IR080 and GA080, based on the rmAb clones EPR2764Y, EP25 and the mAb clone DAK-CDX2, respectively. Applied on the fully automated platforms, BenchMark (Ventana/Roche); Bond (Leica Biosystems) or Omnis (Dako/Agilent), these products provided superior performance. Following vendor recommended protocol settings, almost all (138/139) produced a sufficient result (see Table 3). A high proportion of protocols, based on laboratory modified protocol settings, could also provide a significant proportion of sufficient and optimal results, typically adjusting incubation time in primary Ab, HIER time/temperature and choice of detection system.

The **Ventana RTU system 760-4380** (BenchMark), based on the rmAb clone EPR2764Y, was in this assessment used by 40% (163/404) of the participants, providing an overall pass rate of 99% (162/163). Applying vendor recommended protocol settings (32 min. incubation of the primary Ab, HIER in CC1 for 64 min. and UltraView as detection kit), a significant proportion of the results were assessed as optimal (see Table 3). This observation (supported by the data seen for the concentrated formats of the same Ab), emphasize that the rmAb clone EPR2764Y is very robust, as the use of the less sensitive detection system (UltraView) still demonstrated excellent performance.

This product has been developed by Cell Marque for the Ventana BenchMark platforms, and Cell Marque have their own product line (**235R-17/18**) of the same Ab, providing 97% (32/33) sufficient results of which 88% (29/33) were optimal (see Table 1c). Assays with optimal results were performed primarily on the BenchMark platforms, but also on the Leica Bond and Autostainer platforms. The one laboratory with an insufficient result, used OptiView with amplification as detection system - compromising the interpretation.

The **Dako/Agilent RTU system GA080** (Omnis) based on mAb clone **DAK-CDX2** provided an overall pass rate of 100% (87/87). Applying vendor recommended protocol settings (25 min. incubation of the primary Ab, HIER in TRS High for 30 min. and EnVision FLEX+ as detection kit), 94% (50/53) of the submitted results were assessed as optimal. Especially, the choice of the detection system impacted the overall performance of the RTU system and out of the 8 laboratories using only the 2-step detection system (EnVision Flex, Dako/Agilent) only 50% (2/4) were optimal. Protocols with inferior results were typically due to reduced primary antibody incubation time or use of the antibody on a non-intended platform.

The **Dako/Agilent RTU system IR080** (Autostainer) based on the mAb clone **DAK-CDX2**, provided a lower pass rate of 88% (15/17) compared to RTU systems performed on the fully automated platforms e.g., Dako Omnis. Both vendor recommended (20 min. incubation of the primary Ab, HIER in TRS High for 20 min. and EnVision FLEX as detection kit) and laboratory modified protocol settings could produce optimal results. Reduced incubation time of the primary Ab or too short HIER used in combination with the less sensitive detection system EnVision Flex, were the main reasons for insufficient results. Seven laboratories applied the IR080 to non-intended platforms - all provided sufficient staining results.

The **Leica Biosystems RTU system PA0375** based on the rmAb clone **EP25** (Bond) provided a high pass rate of 100% (39/39). Both vendor recommended (15 min. incubation of the primary Ab, HIER in BERS2 for 20 min. and Refine as detection system) and laboratory modified protocol settings, provided optimal results. However, vendor recommended protocol settings provided a superior number of optimal results (90%) compared to laboratory modified protocol settings (60%).

Summary

This was the seventh NordiQC assessment of CDX2. The pass rate has consistently increased over the last three runs (see Graph 1) despite an increased number of new participants. Several parameters contributed to the high proportion of sufficient results: 1) The extended use of robust primary Abs (e.g. EPR2764Y), 2) The superior performance of the RTU systems developed for fully automated platforms from the three major vendors, and in total applied by 76% (307/404) of the laboratories, 3) Participants updating protocols based on recommendations from the NordiQC organization in past runs (typically recommending to perform HIER in an alkaline buffer, careful calibration of the primary Ab and the use of a 3-step multimer/polymer detection system).

Importantly, protocols must stain accordingly to the expected antigen level, and pancreas is the central immunohistochemical critical assay performance control (ICAPC) to guide the level of analytical sensitivity (see below).

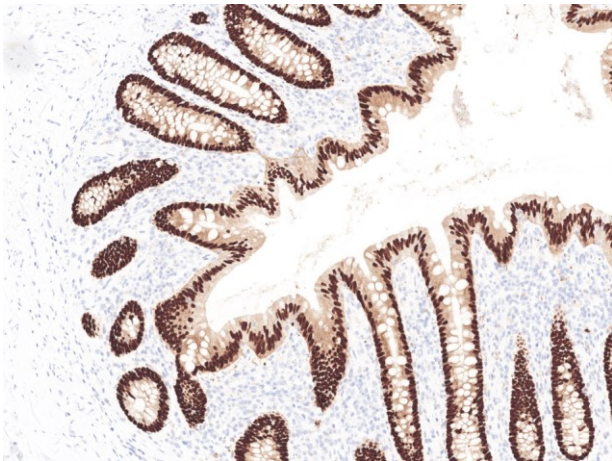


Fig. 1a (x100)
Optimal staining for CDX2 of the appendix using the rmAb clone EPR2764Y as RTU format (Ventana, 760-4380) on BenchMark Ultra following the recommendations given by the vendor (see description above) - same protocol used in Figs. 2a - 5a. Virtually all epithelial cells show a strong nuclear staining reaction. A weak cytoplasmic reaction in cells with nuclear staining reaction was accepted.

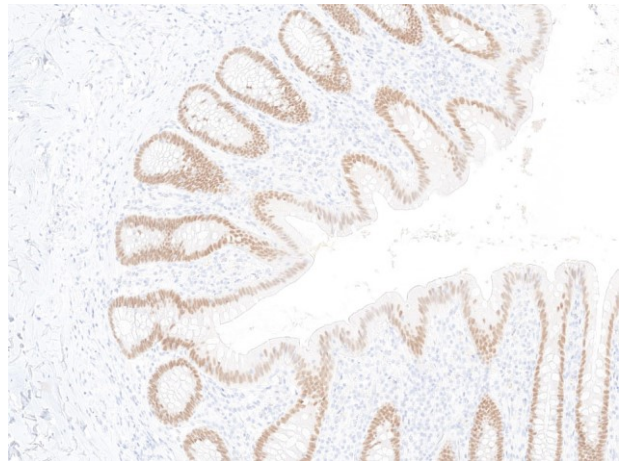


Fig. 1b (x100)
Staining for CDX2 of the appendix using the mAb clone DAK-CDX2 as a concentrated format (1:20) (M3636, Dako/Agilent) within a LD assay on BenchMark Ultra, applying same protocol settings as for Fig 1a - same protocol used in Figs. 2b - 5b. Although the nuclei of epithelial cells are demonstrated, the intensity is significantly reduced - compare with Fig. 1a, same field.

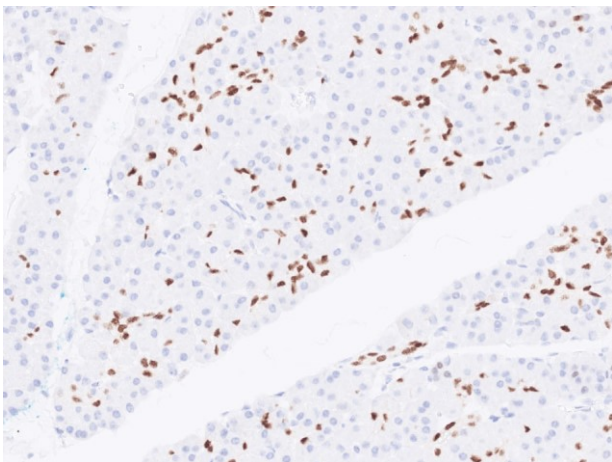


Fig. 2a (x200)
Optimal CDX2 staining of the pancreas using same protocol as in Fig. 1a. Virtually all the ductal and intercalated epithelial cells display a moderate to strong and distinct nuclear staining reaction.

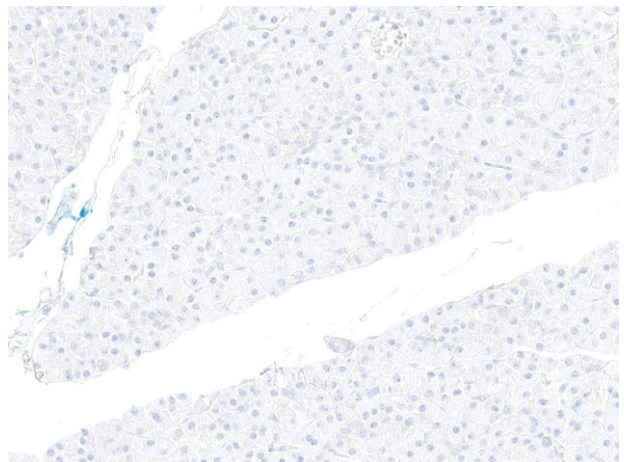


Fig. 2b (x200)
Insufficient CDX2 staining of the pancreas using same protocol as in Fig. 1b. The ductal/intercalated duct epithelial cells are negative - compare with Fig. 2a, same field.

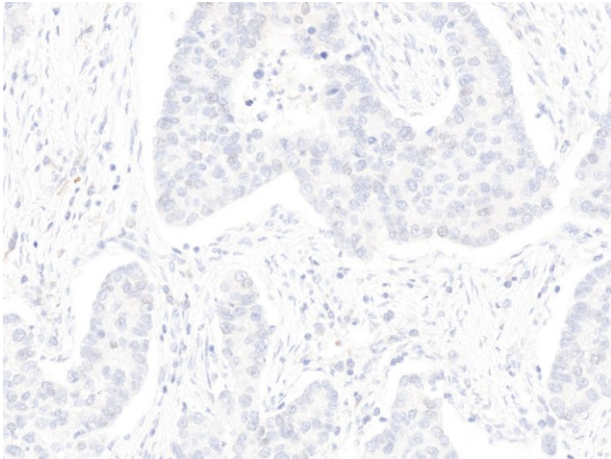


Fig. 3a (x200)
Optimal CDX2 staining of the lung adenocarcinoma, using same protocol as in Figs. 1a - 2a. All neoplastic cells are, as expected, negative.

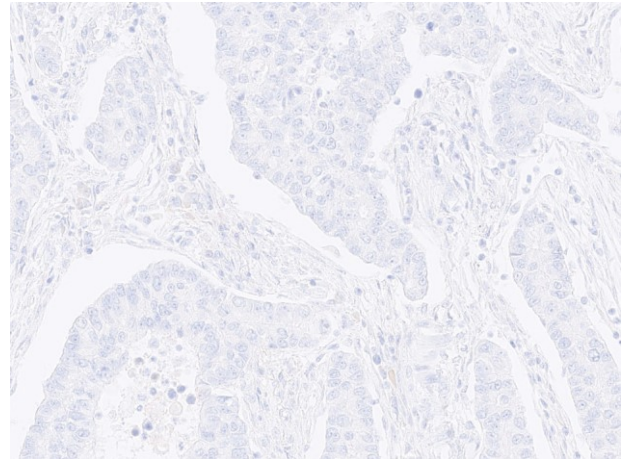


Fig. 3b (x200)
CDX2 staining of the lung adenocarcinoma using the same protocol as in Figs. 1b-2b. The neoplastic cells are negative, as expected. However, overall, the protocol provided a too low level of analytical sensitivity, risking misdiagnosis of colon adenocarcinomas - compare with Figs. 1b-5b.

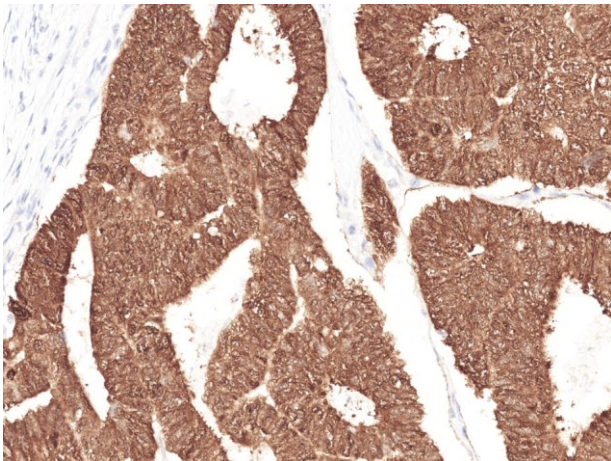


Fig. 4a
Optimal CDX2 staining of the colon adenocarcinoma, tissue core 4, using same protocol as in Figs. 1a - 3a. The majority of the neoplastic cells show a moderate to strong, and distinct nuclear staining reaction. A coexisting cytoplasmic staining reaction is seen and expected due to the high CDX2 expression level.

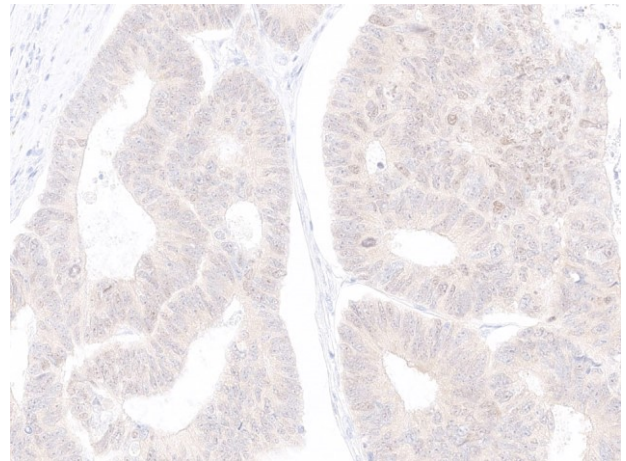


Fig. 4b
Insufficient CDX2 staining of the colon adenocarcinoma, tissue core 4, using same protocol as in Figs. 1b - 3b. A too weak nuclear staining reaction was seen in the majority of the neoplastic cells. The cytoplasmic staining is slightly stronger than the nuclear staining and could be misinterpreted as an unspecific reaction. Compare with Fig. 4a for an optimal result, same field.

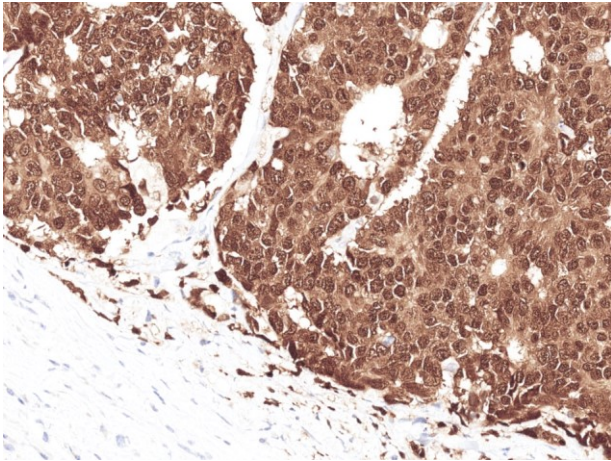


Fig. 5a
Optimal CDX2 staining of the colon adenocarcinoma, tissue core 5, using same protocol as in Figs. 1a - 4a. All the neoplastic cells show a strong and distinct nuclear staining reaction. A cytoplasmic staining reaction in cells with nuclear staining reaction was expected.

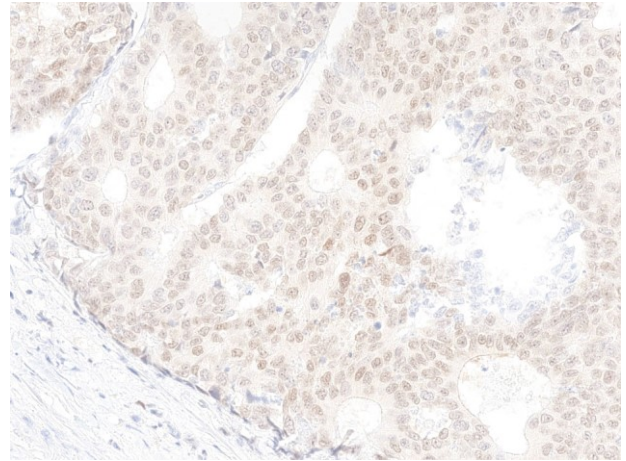


Fig. 5b (x200)
Insufficient CDX2 staining of the colon adenocarcinoma, tissue core 5, using same protocol as in Figs. 1b - 4b. Virtually all the neoplastic cells are demonstrated but display a significantly reduced and too weak nuclear staining reaction – compare with Fig. 5a, same field.

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