Assessment Run 76 2026

Cytokeratin 5 (CK5)

Purpose
Evaluation of the technical performance and the level of analytical sensitivity and specificity of the immunohistochemical (IHC) assays used by NordiQC participants for CK5. The focus of the assessment was use in the diagnostic work-up of prostate and lung samples (identifying mesothelioma and differentiating squamous cell carcinoma and adenocarcinoma). Relevant normal and neoplastic clinical tissues were selected to represent a broad range of CK5 antigen densities (see below).

Material

The slide to be stained for cytokeratin 5 (CK5) comprised:

1. Prostate hyperplasia, 2. Pancreas, 3. Tonsil, 4. Lung adenocarcinoma,
5. Lung squamous cell carcinoma, 6. Malignant epithelioid mesothelioma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CK5 staining as optimal included:

- A moderate to strong and distinct, cytoplasmic staining reaction in virtually all squamous epithelial cells in the tonsil.
- A weak to moderate, predominantly membranous staining reaction of scattered cuboidal epithelial cells in the pancreatic intercalated ducts.
- A strong and distinct cytoplasmic staining reaction in most basal cells in the hyperplastic prostate glands.
- An at least weak to moderate cytoplasmic staining reaction of virtually all neoplastic cells in the lung squamous cell carcinoma.
- An at least weak to moderate staining reaction in most neoplastic cells in the mesothelioma.
- No staining of neoplastic cells in the lung adenocarcinoma.

KEY POINTS FOR CK5 IMMUNOASSAYS

- RTU systems based on mAb clone **XM26** and rmAb clone **SP27** gave the highest pass rates of 97% and 98%, respectively.
- The mAb clone D5/16 B4 was less successful with an overall pass rate of 42%. Fortunately, this could be corrected to obtain an optimal result:
  - Roche/Ventana users can substitute with the RTU product based on rmAb clone SP27.
  - Dako/Agilent users can modify the protocol settings to increase sensitivity by using EnVision Flex+.
- Pancreas is the preferred control to monitor analytical sensitivity.

Participation

Number of laboratories registered for CK5, run 76	398
Number of laboratories returning slides	330 (83%)

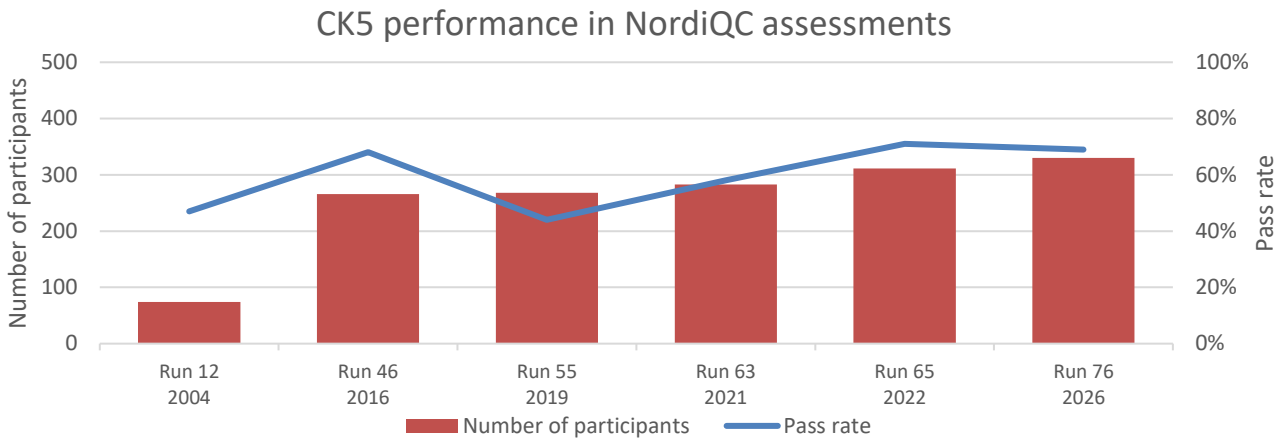
At the date of assessment, 83% of the participants had returned the circulated NordiQC slides.
In this assessment, run 76, general issues with the Danish postal service affected the distribution and return of slides to/from participants, resulting in a lower number of returned slides compared to previous 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
330 laboratories participated in this assessment. 226 (69%) achieved a sufficient mark (optimal or good) – see Table 1a (page 3). Tables 1b and 1c summarizes the antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining reactions were:
- Less successful performance of mouse monoclonal Ab (mAb) clone D5/16 B4 – both as concentrate and in Ready-To-Use (RTU) systems
- Use of less sensitive detection systems

Performance history
This was the sixth NordiQC assessment of CK5. A slight reduction of the pass rate was observed compared to the latest run (Run 65, 2022) as seen in Graph 1.

Graph 1. **Proportion of sufficient results for CK5 in the six NordiQC runs performed**



Controls
Tonsil and pancreas can be recommended as positive tissue control[1]. In tonsil, virtually all squamous epithelial cells throughout all cell layers must show a moderate to strong cytoplasmic staining reaction, whereas virtually all lymphocytes should be negative. In pancreas, scattered cuboidal epithelial cells of intercalated ducts must show a weak to moderate predominantly membranous staining reaction.

Conclusion
The mAbs clones **XM26**, **D5/16 B4** and the rmAb clone **SP27** were the most widely used Abs. The mAb clone XM26 was significantly more successful compared to mAb clone D5/16 B4 with pass rates of 94% and 42% overall, respectively. The mAb clone D5/16 B4 typically provided a too low analytical sensitivity. The RTU systems from Ventana/Roche based on rmAb clone SP27 and Leica Biosystems based on mAb clone XM26, both gave overall high pass rates of 98% and 100%, respectively, when applied on BenchMark and Bond platforms.
Irrespective of the clone applied, efficient HIER (preferable in an alkaline buffer), careful calibration of the primary antibody and use of a sensitive 3-step polymer/multimer detection system were the most important prerequisites for an optimal staining result.

[1] Torlakovic 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.0000000000000163. Review. PubMed PMID: 25474126.

Table 1a. **Overall results for CK5, run 76**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	102	56	23	21	2	77%	55%
Ready-To-Use antibodies	228	99	48	78	3	65%	44%
Total	330	155	71	99	5		
Proportion		47%	22%	30%	1%	69%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for CK5, run 76**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff[1]	OR[2]
mAb clone **D5/16 B4***	1	BioSB	1	-	-	-	48%	19%
	3	Cell Marque	1	1	1	-		
	23	Dako/Agilent	3	7	11	2		
mAb clone **XM26**	3	Abcam	1	1	1	-	93%	72%
	1	Biocare Medical	1	-	-	-		
	3	Diagnostic Biosystems	2	1	-	-		
	61	Leica Biosystems	45	12	4	-		
mAb clone **3E2F1**	1	Thermo Fisher Scientific	-	-	1	-	-	-
rmAb clone **SP27**	1	Abcam	1	-	-	-	-	-
rmAb clone **EP1601Y**	1	Cell Marque	1	-	-	-	-	-
rmAb clone **EP24/EP67***	1	PathnSitu	-	-	1	-	-	-
rmAb clone **EP24**	1	Epitomics	-	1	-	-	-	-
rmAb clone **EP42**	1	Epitomics	-	-	1	-	-	-
rmAb clone **ZR280**	1	Zeta Corporation	-	-	1	-	-	-
Total	102		56	23	21	2		
Proportion			55%	22%	21%	2%	77%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
*) Cytokeratin 5 and 6.

Table 1c. **Ready-to-Use antibodies and assessment marks for CK5, run 76**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff[1]	OR[2]
mAb clone **D5/16 B4* 790-4554[3]**	22	Ventana/Roche	3	3	16	-	27%	14%
mAb clone **D5/16 B4* 790-4554[4]**	40	Ventana/Roche	6	16	17	1	55%	15%
mAb **D5/16 B4* GA780[3]**	19	Dako/Agilent	-	3	16	-	16%	0%
mAb **D5/16 B4* GA780[4]**	25	Dako/Agilent	2	9	14	-	44%	8%
mAb clone **D5/16 B4* IR780[4]**	15	Dako/Agilent	2	5	7	1	47%	13%
mAb clone **D5/16 B4* 8295-C010**	2	Sakura Finetek	1	1	-	-	-	-
mAb clone **D5/16 B4* 356M-10**	1	Cell Marque	-	-	-	1	-	-
mAb clone **150A8C1 PA018**	1	Abcarta	1	-	-	-	-	-
mAb clone **15D1 B64011**	1	Guangzhou Biotron	-	1	-	-	-	-
mAb clone **C6H1 CCM-0975**	1	Celnovte	-	-	1	-	-	-
mAb clone **IHC556* IHC556**	1	GenomeMe	1	-	-	-	-	-

mAb clone **XM26** **PA0468**[3]	14	Leica Biosystems	13	1	-	-	100%	93%
mAb clone **XM26** **PA0468**[4]	13	Leica Biosystems	11	1	1	-	92%	85%
mAb clone **XM26** **PM234**	2	Biocare Medical	2	-	-	-	-	-
mAb clone **XM26/LL002**** **BMS023**	2	Zytomed	-	1	1	-	-	-
mAb clone **ZM186** **ZM186**	1	Zeta Corporation	-	-	1	-	-	-
rmAb clone **EP1601Y** **305R-17/18**	2	Cell Marque	-	2	-	-	-	-
rmAb clone **EP1601Y/LL002**** **905H-08**	1	Cell Marque	-	-	1	-	-	-
rmAb clone **EP42** **BSB6599**	1	BioSB	-	-	1	-	-	-
rmAb clone **EP42** **NPAI430**	1	Biocare Medical	-	1	-	-	-	-
rmAb clone **EP24/EP67*** **MAD-000651QD**	1	Master Diagnostica	-	-	1	-	-	-
rmAb clone **SP27** **760-4935**[3]	32	Ventana/Roche	31	-	1	-	97%	97%
rmAb clone **SP27** **760-4935**[4]	24	Ventana/Roche	22	2	-	-	100%	92%
rmAb clone **SP27** **MAD-000491QD**	1	Master Diagnostica	1	-	-	-	-	-
rmAb clone **BY160** **BFM-0482**	1	Bioin Biotechnology	1	-	-	-	-	-
rmAb clone **BP6021** **BX50016**	1	Biolynx Biotechnology	-	1	-	-	-	-
rmAb clone **DY49108** **4922072**	1	Dakewe	-	1	-	-	-	-
rmAb clone **GR301** **GT215202**	1	Gene Tech	1	-	-	-	-	-
rmAb clone **MXR029** **RMA-1064**	1	Fuzhou Maixin	1	-	-	-	-	-
Total	228		99	48	78	3		
Proportion			44%	21%	34%	1%	65%	

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).
*) Cytokeratin 5 and 6.
**) Cytokeratin 5 and 14.

Detailed analysis of CK5, Run 76
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **D5/16 B4**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Cell Conditioning 1 (CC1, Ventana/Roche) (3/14*) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (2/6). The mAb was typically diluted in the range of 1:50-1:100 in combination with a 3-step polymer/multimer detection system. Using these protocol settings, 8 of 13 (62%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **XM26**: Protocols with optimal results were based on HIER using Target Retrieval Solution (TRS) pH 9 (Dako/Agilent) (22/25), CC1 (Ventana/Roche) (17/28), BERS2 (Leica Biosystems) (9/14) or Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:200 in combination with a 3-step polymer/multimer detection system. Using these protocol settings, 58 of 60 (97%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for CK5 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 **D5/16 B4**	-	-	0/1	-	3/9 (33%)	-	2/5 (40%)	0/3
mAb clone **XM26**	2/3	-	20/22 (91%)	-	17/28 (61%)	-	9/13 (69%)	1/1

1) Autostainer Link 48+.
2) BenchMark Ultra, Ultra plus
3) Bond III, Prime

Ready-To-Use antibodies and corresponding systems

mAb clone **D5/16 B4**, product no. **790-4554**, Ventana/Roche, BenchMark Ultra/Ultra plus:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min.) and 16-32 min. incubation of the primary Ab. OptiView (760-700) was used as detection systems. Using these protocol settings, 20 of 26 (77%) laboratories produced a sufficient staining result (optimal or good).
The product was used by 1 laboratory on a non-intended platform. Data was not included in the description above.

mAb clone **D5/16 B4**, product no. **IR780**, Dako/Agilent, Autostainer Link/Classic:
One protocol with an optimal result was based on HIER in PT-Link using TRS pH 9 (3-in-1) (heating time 20 min. at 97°C), 20 min. incubation of the primary Ab and EnVision FLEX+ (K8000/K8002) as detection system. Using these protocol settings, 2 of 4 laboratories produced a sufficient staining result.
The product was used by 8 laboratories on a non-intended platform. Data was not included in the description above.

mAb clone **D5/16 B4**, product no. **GA780**, Dako/Agilent, Omnis:
Protocols with optimal results were based on HIER using TRS pH 9 (heating time 30 min. at 97°C), 20-25 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings, 3 of 7 (43%) laboratories produced a sufficient staining result.
The product was used by 3 laboratories on a non-intended platform. Data was not included in the description above.

mAb clone **XM26**, product no. **PA0468**, Leica Biosystems, Leica Bond III/Prime:
Protocols with optimal results were typically based on HIER using BERS2 pH 9 (efficient heating time 10-20 min. at 95-100°C), 15-20 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 24 of 24 (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 **SP27**, product no. **760-4935**, Ventana/Roche, BenchMark Ultra/Ultra plus:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 24-64 min.) and 16-32 min. incubation of the primary Ab. UltraView (760-500) +/- amplification kit or OptiView (760-700) were used as detection systems. Using these protocol settings, 52 of 53 (98%) 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.

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 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 CK5 for the most commonly used RTU IHC systems**

RTU systems	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Ventana Benchmark mAb clone **D5/16 B4, UltraView 790-4554**	6% (1/16)	0% (0/16)	20% (3/15)	7% (1/15)
Ventana Benchmark mAb clone **D5/16 B4, OptiView 790-4554**	83% (5/6)	50% (3/6)	79% (19/24)	21% (5/24)
Dako Omnis mAb clone **D5/16 B4, GA780**	16% (3/19)	0% (0/19)	50% (11/22)	9% (2/22)
Dako Autostainer mAb clone **D5/16 B4, IR780**	-	-	43% (3/7)	14% (1/7)
Leica Bond mAb clone **XM26, PA0468**	100% (14/14)	93% (13/14)	100% (11/11)	91% (10/11)
Ventana Benchmark rmAb clone **SP27, UltraView 760-4935**	100% (12/12)	100% (12/12)	100% (8/8)	100% (8/8)
Ventana Benchmark rmAb clone **SP27, OptiView 760-4935**	95% (19/20)	95% (19/20)	100% (14/14)	86% (12/14)

* 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 integrated.

Comments

In this assessment and in concordance with the previous NordiQC assessments of CK5, the prevalent feature of an insufficient result was a too weak or false negative staining reaction of cells and structures expected to be demonstrated. This pattern was observed in 87% of the insufficient results (91/104). The remaining 13% (13/104) insufficient results were characterized by either a false positive staining reaction (n=2) or poor signal-to-noise ratio/excessive background (n=11). Virtually all laboratories were able to demonstrate CK5 in high-level antigen expressing structures such as the squamous epithelial cells of tonsil and the majority of basal cells in the hyperplastic prostate glands, whereas demonstration of CK5 in low-level antigen expressing structures as the neoplastic cells in the mesothelioma and especially the cuboidal epithelial cells of intercalated ducts in pancreas was significantly more challenging and required a carefully calibrated protocol.

31% (102/330) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for CK5. The well-established mAb clones D5/16 B4 and XM26 for CK5/6 and CK5, respectively, were the two most widely used Abs (see Table 1b).
Within a LD assay, mAb clone **XM26** was by far the most successful of the two, and optimal results could be obtained on all four main IHC platforms from Dako/Agilent, Leica Biosystems and Ventana/Roche (see Table 2). In concordance with previous assessments the main prerequisites for sufficient and optimal staining results with mAb clone XM26 were efficient HIER typically in an alkaline buffer, careful calibration of the titre of the primary Ab and the use of a sensitive detection system, preferably a 3-step polymer/multimer based detection system. The proportion of sufficient staining results for the use of 3-step polymer/multimer based detection systems and 2-step polymer/multimer based systems was 98% and 69%, respectively. In this assessment, one laboratory used a combined pretreatment with HIER and Protease 3 (Ventana/Roche) with an optimal result. In the latest CK5 assessment (run 65, 2022), 24 laboratories used the combined pretreatment, 71% (17/24) with an optimal result.

The mAb clone **D5/16 B4** in a LD assay had a low pass rate on all IHC platforms. The overall proportion of sufficient staining results was 48% (13/27), and optimal staining results were only obtained by 5 laboratories (19%). These were based on HIER in an alkaline buffer and 3-step polymer/multimer based detection systems. The main vendor, Dako/Agilent, of the mAb provides the product in an ascites format. It is well-known that an aberrant MAG (Mouse ascites Golgi) reaction can be seen in tissues of blood type

A patients[2]. As CK5 is localized in the cytoplasmic compartment, similar to the MAG reaction, there is a risk of false positivity and diagnostic misclassification. However, MAG reaction was not observed in this assessment.

Ready-To-Use (RTU) antibodies were used by 69% (228/330) of the laboratories.
The **Ventana/Roche RTU system 760-4935**, based on rmAb clone **SP27**, was most successful and provided an overall pass rate of 98% (53/54), 94% optimal (n=51). 41% (22/54) of the laboratories modified the protocol settings. Mostly minor changes in HIER time and incubation time of primary Ab and applying UltraView with UltraView Amplification were seen.
A reduced number of positive cells in prostate was observed for eight laboratories (see Fig. 6b). This staining pattern was not seen for other Abs and these slides were all from the same TMA (in total eight TMAs were constructed and used for this assessment). The staining results were otherwise optimal, and this reduction of staining in prostate did not affect the assessment score, as it might be related to the material. Also, the data analysis did not reveal any explanations for this potentially reduced sensitivity. The rmAb clone SP27 has in NordiQC studies[3] shown positive reaction in 23% of lung adenocarcinomas being negative for other CK5 antibodies as well as p40. The significance of this is uncertain, but must be taken into account in subclassification of NSCLC and emphasizes the importance of diagnostic panels.

The **Leica Biosystems RTU system PA0468**, based on mAb clone **XM26**, also provided a high pass rate. Using the recommended protocol settings, the proportion of sufficient staining results was 100% (14/14) of which 93% (13/14) were assessed as optimal. A pass rate of 100% (11/11), 91% optimal, was seen when modifying the protocol settings. Only minor changes for HIER time and/or incubation time of the primary Ab were made. An excessive background staining was observed in tonsil (see Fig. 1a) for most laboratories. This was accepted, when only seen in tonsil, in line with previous CK5 assessments.

The **Dako/Agilent RTU systems IR780** and **GA780**, based on mAb clone **D5/16 B4**, for Autostainer and Omnis, respectively, both provided a low proportion of sufficient and optimal staining results similar to the observations and data generated in runs 55, 63 and 65. For **GA780** on the Omnis platform, an overall pass rate of 34% (14/41) was seen. The performance of the RTU system used as "plug-and-play" was inferior to the performance obtained by laboratory modified protocol settings as shown in Table 3. The insufficient results were characterized by too weak or false negative test results. The most successful modification was based on use of FLEX+ as detection system and not FLEX as recommended. If using FLEX+ as detection system, a pass rate of 83% (10/12) was seen, compared to 14% (4/29) if using FLEX. For **IR780** on the Autostainer, all laboratories modified the protocol settings. Eight laboratories used the IR780 on other platforms.

The **Ventana/Roche RTU system 790-4554**, based on mAb clone **D5/16 B4**, performed significantly better than the corresponding Dako/Agilent RTU systems with an overall pass rate of 46% (28/61), but still inferior to the other Ventana/Roche RTU system based on rmAb clone SP27. If using a 3-step detection system, a relatively high pass rate of 81% (25/31) was obtained, 26% optimal (n=8), compared to 10% (3/30) using a less sensitive 2-step detection system, 3% optimal (n=1).

Summary
This was the sixth NordiQC assessment of CK5 (see Graph 1). A pass rate of 69% was obtained which is slightly reduced compared to the last assessment (run 65, 2022) with a pass rate of 71%.
The relatively low pass rate of CK5 seems to be influenced by the extended use of the less successful mAb clone D5/16 B4, giving an overall pass rate of 42%. The main vendors Dako/Agilent and Ventana/Roche both offer RTU systems based on this clone and are used by 37% of the participants (121/330). Use of a sensitive 3-step detection system were the most important prerequisites for an optimal staining result. Ventana/Roche also provides an RTU based on the more successful rmAb clone SP27 which along with mAb clone XM26 from Leica Biosystems showed superior performance compared to mAb clone D5/16 B4.

[2] Kliman HJ, Feinberg RF, Schwartz LB, Feinman MA, Lavi E, Meaddough EL. A mucin-like glycoprotein identified by MAG (mouse ascites Golgi) antibodies. Menstrual cycle-dependent localization in human endometrium. Am J Pathol. 1995;146(1):166–81.
[3] Thomsen, C., Nielsen, O., Nielsen, S., Røge, R., & Vyberg, M. (2020). NordiQC Assessments of Keratin 5 Immunoassays. Applied Immunohistochemistry & Molecular Morphology, 28(7), 566-570. https://doi.org/10.1097/PAI.0000000000000855

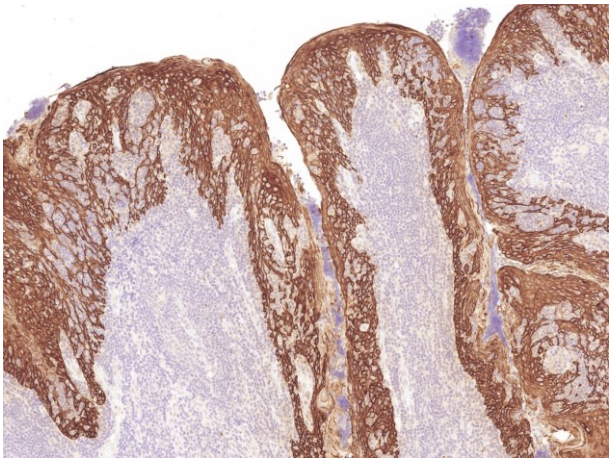


Fig. 1a
Optimal CK5 staining of the tonsil using the mAb clone XM26 in an RTU format (PA0468, Leica Biosystems) using the vendor recommended protocol settings on the Bond III. A strong cytoplasmic staining reaction is seen in virtually all squamous epithelial cells in the tonsil. Also compare with Figs. 2a-6a, same protocol.

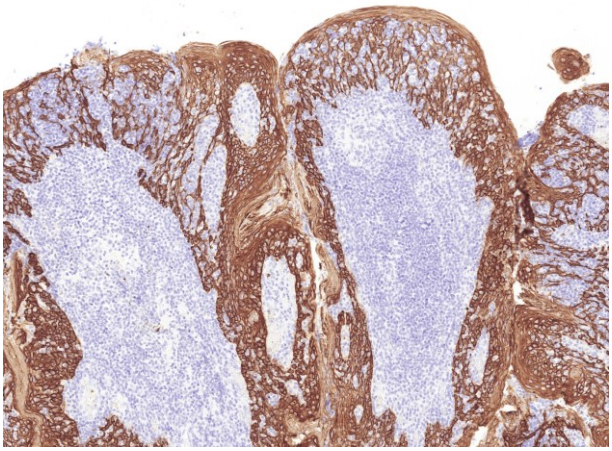


Fig. 1b
CK5 staining of the tonsil using the mAb clone D5/16 B4 in an RTU format (GA780, Dako/Agilent) using the vendor recommended protocol settings on the Dako Omnis. A strong cytoplasmic staining reaction is seen in virtually all squamous epithelial cells in the tonsil as expected and obtained in Fig. 1a, - same field. However, the protocol provided an overall too low analytical sensitivity due to the use of a less sensitive 2-step detection system, compare with Figs. 2b-5b, same protocol.

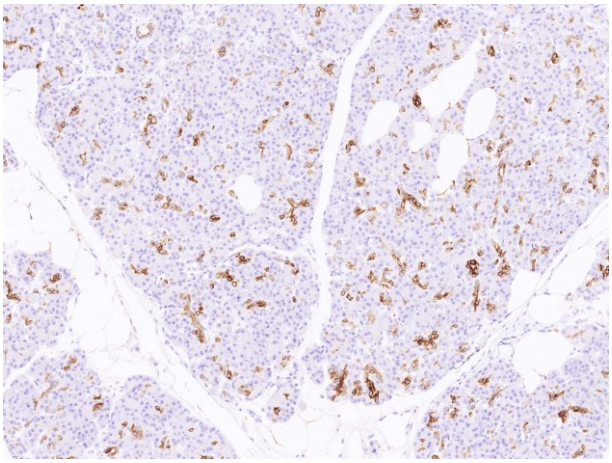


Fig. 2a
Optimal CK5 staining of pancreas with low-level CK5 expression using same protocol as in Fig. 1a. Scattered cuboidal epithelial cells of intercalated ducts display a weak to moderate predominantly membranous staining reaction. No background staining.

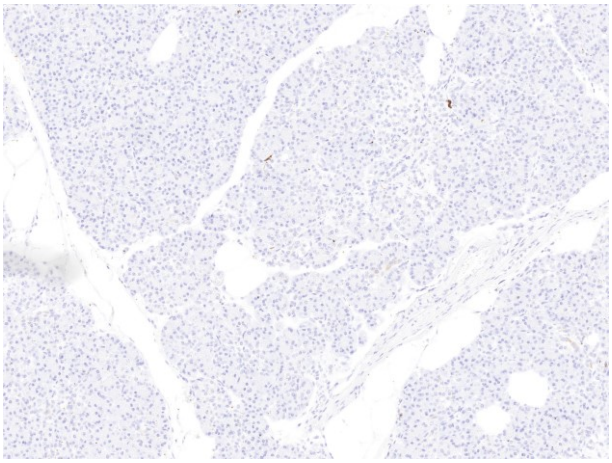


Fig. 2b
Insufficient CK5 staining of the pancreas with low-level CK5 expression using same protocol as in Fig. 1b – same field as in Fig. 2a. No staining reaction is seen in the epithelial cell of the intercalated ducts giving a false negative result – compare with Fig. 2a.

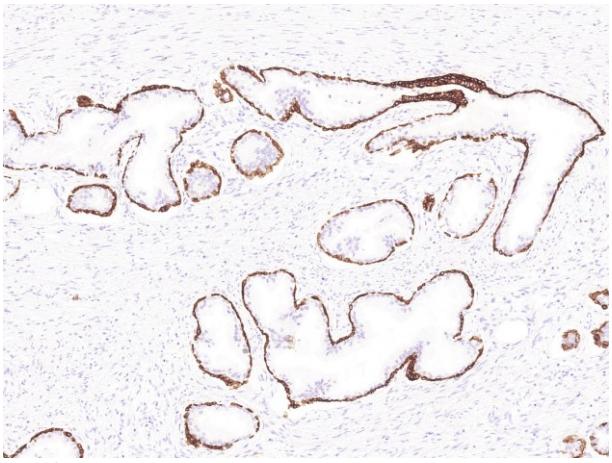


Fig. 3a
Optimal CK5 staining of the prostate hyperplasia using same protocol as in Figs. 1a-2a. A strong and distinct cytoplasmic staining reaction is seen in most basal cells in the hyperplastic prostate glands. No background staining.

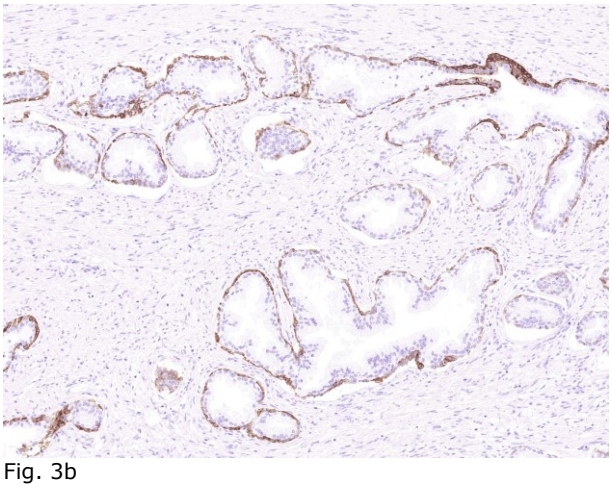


Fig. 3b
Insufficient CK5 staining of the prostate hyperplasia using same protocol as in Figs. 1b-2b – same field as in Fig. 3a. A reduced number of positive basal cells in the hyperplastic prostate glands show a weak staining reaction, compromising the diagnostic utility of the test in prostate samples.

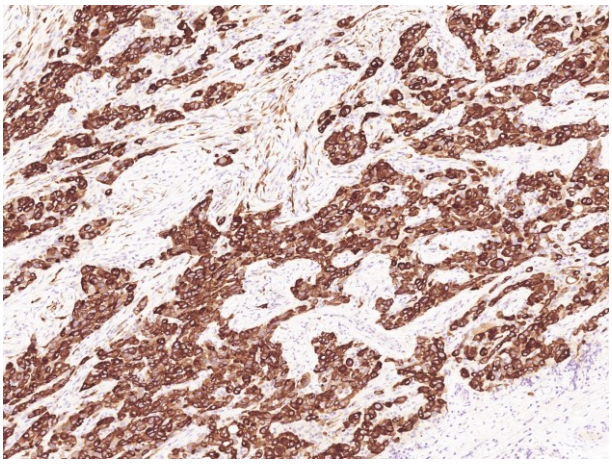


Fig. 4a
Optimal CK5 staining of the mesothelioma with a moderate to high level of CK5 expression using same protocol as in Figs. 1a-3a. All the neoplastic cells show a moderate to strong, distinct cytoplasmic staining reaction.

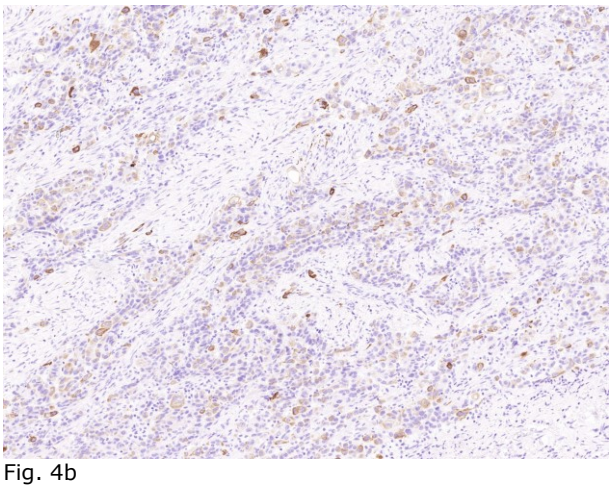


Fig. 4b
Insufficient CK5 staining of the mesothelioma using the same insufficient protocol as in Figs. 1b-3b – same field as in Fig. 4a. The intensity and proportion of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 4a.

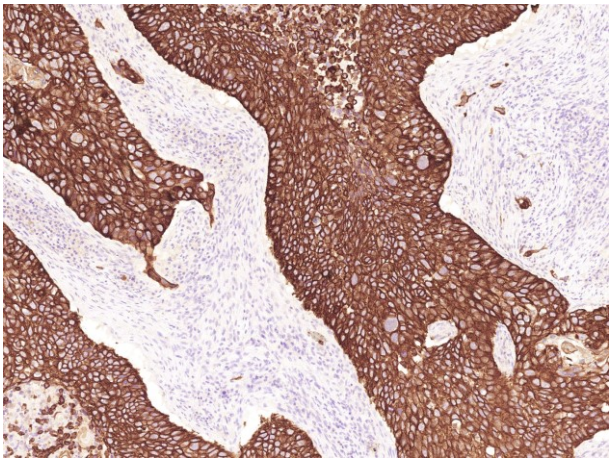


Fig. 5a
Optimal CK5 staining of the lung squamous cell carcinoma with high-level CK5 expression using same protocol as in Figs. 1a-4a. All the neoplastic cells show a strong and distinct cytoplasmic staining reaction.

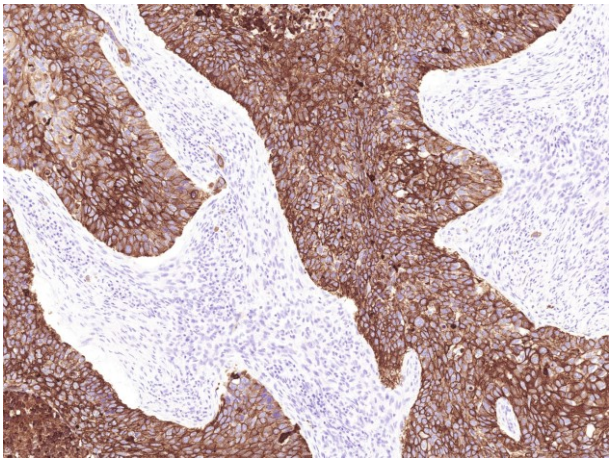


Fig. 5b
CK5 staining of the lung squamous cell carcinoma with high-level CK5 expression using the same insufficient protocol as in Figs. 1b-4b – same field as in Fig. 5a. Virtually all neoplastic cells show a moderate to strong cytoplasmic staining reaction

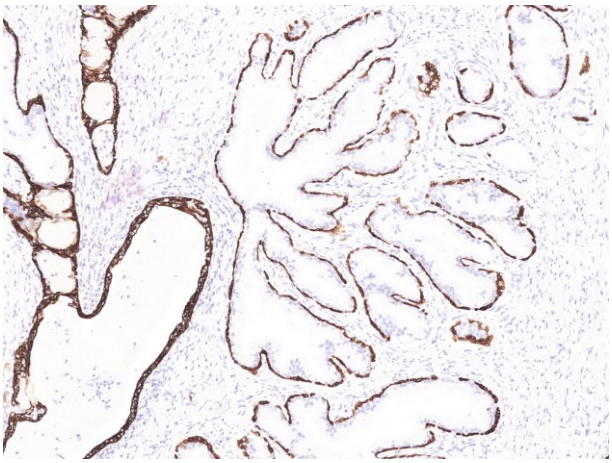


Fig. 6a
Optimal CK5 staining of the prostate hyperplasia using same protocol as in Figs. 1a-5a, but photo taken from a different slide.
A strong and distinct cytoplasmic staining reaction is seen in most basal cells in the hyperplastic prostate glands.

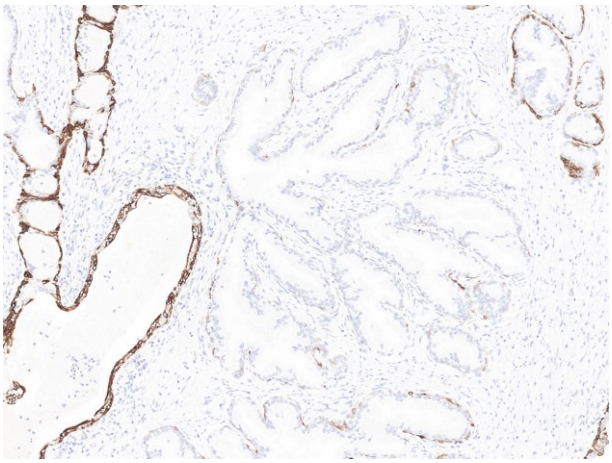


Fig. 6b
CK5 staining of prostate using the rmAb clone SP27 in an RTU format (760-4935, Ventana/Roche) using the vendor recommended protocol settings on the Ventana BenchMark Ultra - same field as in Fig. 6a. No staining reaction is seen in majority of the basal cell in the hyperplastic prostate glands. This reduced sensitivity was seen only in this TMA block and most likely related to the tissue.

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