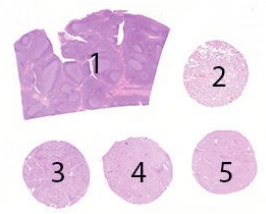
Assessment Run 74 2025
p40 (ΔNp63)

Updated 16.07.2025

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of the IHC assays for p40 performed by the NordiQC participants for the differentiation between lung squamous cell carcinoma and lung adenocarcinoma. Relevant clinical tissues, both normal and neoplastic, were selected to include a wide spectrum of p40 antigen densities (see below).

Material
The slide to be stained for p40 comprised:



1. Tonsil, 2. Placenta, 3-4. Lung squamous cell carcinoma,
5. Lung adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing p40 staining as optimal included:

- A moderate to strong, distinct nuclear staining reaction of virtually all squamous epithelial cells in the tonsil.
- An at least weak to moderate, distinct nuclear staining reaction of dispersed cytotrophoblastic cells in the placenta.
- A moderate to strong, distinct nuclear staining reaction of virtually all neoplastic cells in the lung squamous cell carcinoma, tissue core no. 3.
- An at least weak to moderate staining reaction in 70-100% of the neoplastic cells in the lung squamous cell carcinoma, tissue core no. 4.
- No staining reaction of the neoplastic cells in the lung adenocarcinoma.
- No staining reaction of other cells including lymphocytes in the tonsil.

KEY POINTS FOR p40 IMMUNOASSAYS
- The mAb clone **BC28** is recommendable both as a concentrated Ab and a RTU.
- New RTU systems based on rmAb clones **DAK-p40** and **SP225** were successful.
- Polyclonal Abs were less successful and should be avoided.
- Placenta is recommended as primary critical positive tissue control.

Participation

Number of laboratories registered for p40, run 74	442
Number of laboratories returning slides	412 (93%)

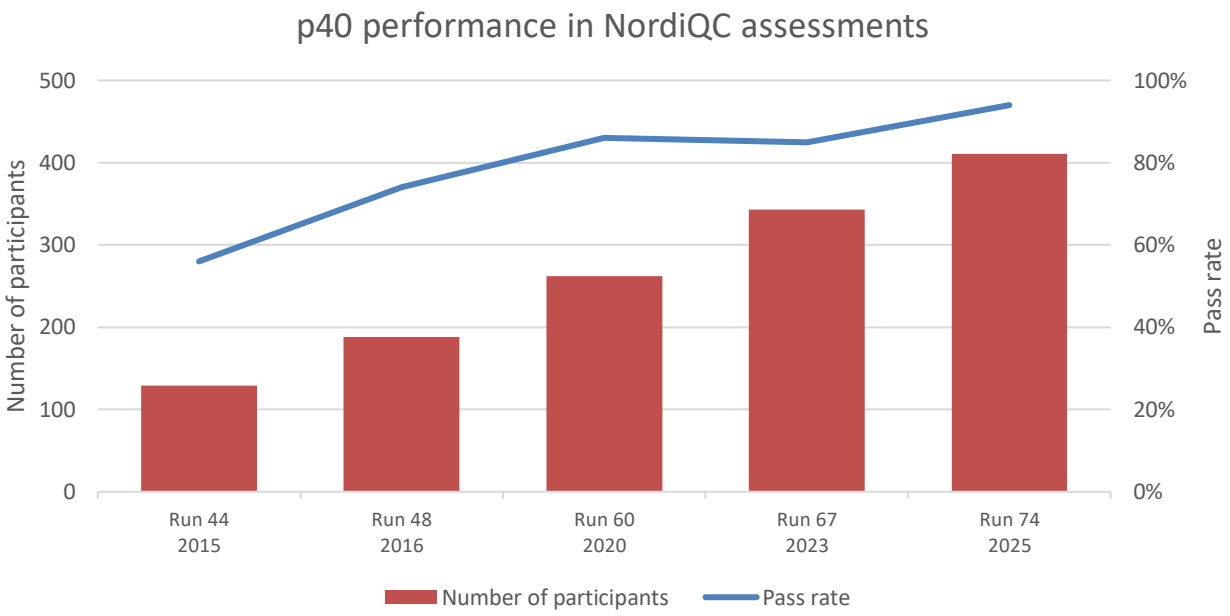
All slides returned after the assessment were assessed and received advice if the result being insufficient, but the data were not included in this report.

Results
412 laboratories returned slides for this assessment. 1 participant stained on a wrong slide and was excluded from the analysis. Of the remaining 411 laboratories, 94% achieved a sufficient mark (optimal or good), see Table 1a (see 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 were:
- Inefficient HIER
- Too short incubation time of primary Ab
- Less successful primary Ab
- Use of less sensitive detection systems

Performance history
This was the fifth NordiQC assessment of p40. An increased pass rate was observed in this run 74 compared to the previous runs (see Graph 1).

Graph 1. **Proportion of sufficient results for p40 in the five NordiQC runs**



Controls
Placenta is recommended as primary critical positive tissue control for p40, where an at least weak to moderate, distinct nuclear staining reaction of cytotrophoblasts must be seen. The cytotrophoblasts should be visible even at a low magnification (5x objective).
Supportive to placenta, tonsil can be used as positive and negative tissue control to guide analytical specificity. Virtually all squamous epithelial cells must show a moderate to strong, distinct nuclear staining reaction. No nuclear or cytoplasmic staining reaction should be seen in other cell types.

Conclusion
Optimal staining results for p40 could be obtained with the mAb clone **BC28** and rmAb clones **DAK-p40**, **ZR8** and **SP225.** mAb clone BC28 was the most commonly used p40 antibody, giving an overall pass rate of 94%, 57% optimal. The RTUs from Leica Biosystem based on mAb clone BC28 and from Dako/Agilent based on rmAb clone DAK-p40 were the most successful antibodies, giving pass rates of 100% and high proportion of optimal results especially when using the vendor recommended protocol settings. The recently launched RTU from Ventana/Roche based on rmAb clone SP225 provided a 100% pass rate when following the recommend protocol settings for OptiView detection system.
The concentrated format of mAb clone **BC28** provided optimal staining results on the main platforms from Dako/Agilent, Leica Biosystems and Ventana/Roche. Irrespective of the clone applied, efficient Heat Induced Epitope retrieval (HIER) in an alkaline buffer and use of a sensitive and specific 3-step polymer / multimer based detection system gave the highest proportion of optimal results. The concentration of the primary antibody must be carefully calibrated. As seen in previous assessment runs, polyclonal Abs were less successful and should be avoided.

Table 1a. **Overall results for p40, run 74**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	97	68	23	3	3	94%	70%
Ready-To-Use antibodies	314	178	118	16	2	94%	54%
Total	411	246	141	19	5		
Proportion		60%	34%	5%	1%	94%	

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

Table 1b. **Concentrated antibodies and assessment marks for p40, run 74**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **BC28**	4	abcam	2	2	0	0	-	-
	69	Biocare Medical	56	12	1	0	99%	81%
	7	Zytomed	6	1	0	0	100%	86%
mAb clone **rTP40/3690**	1	abcam	0	0	0	1	-	-
mAb clone **IHC058**	1	GenomeMe	0	1	0	0	-	-
rmAb clone **ZR8**	2	BioSB	0	2	0	0	-	-
	1	Cell Marque	0	1	0	0	-	-
	1	Immunologic	1	0	0	0	-	-
	3	Zeta Corporation	1	1	1	0	-	-
rmAb clone **QR020**	2	Quartett	0	2	0	0	-	-
Ab clone **10C8-E1**	1	Wondfo	1	0	0	0	-	-
Ab clone **HGL-p40**	1	Bio-Highgrade	1	0	0	0	-	-
pAb **ACI3030**	1	Biocare Medical	0	1	0	0	-	-
pAb **RP163**	3	Diagnostic Biosystems	0	0	1	2	-	-
Total	97		68	23	3	3		
Proportion			70%	24%	3%	3%	94%	

1) Proportion of sufficient stains (optimal or good) (≥5 asessed protocols).
2) Proportion of Optimal Results (≥5 asessed protocols).

Table 1c. **Ready-To-Use antibodies and assessment marks for p40, run 74**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **BC28 API/AVI/VLTM 3066**	12	Biocare Medical	9	3	0	0	100%	75%
mAb clone **BC28 790-4950 (VRPS)[3]**	28	Ventana/Roche	1	23	4	0	86%	4%
mAb clone **BC28 790-4950 (LMPS)[4]**	135	Ventana/Roche	60	62	11	2	90%	44%
mAb clone **BC28 MSG097/BMS050**	3	Zytomed	3	0	0	0	-	-
mAb clone **BC28 NOA-BMS050**	1	Zytovision	0	1	0	0	-	-
mAb clone **BC28 PA0163 (VRPS)[3]**	18	Leica Biosystems	17	1	0	0	100%	94%
mAb clone **BC28 PA0163 (LMPS)[4]**	13	Leica Biosystems	10	3	0	0	100%	77%
mAb clone **BC28 8341-C010**	3	Sakura Finetek	3	0	0	0	-	-
mAb clone **C3B4 CPM-0133**	1	Celnovte	1	0	0	0	-	-
mAb clone **IHC052 IHC052**	1	GenomeMe	0	1	0	0	-	-
Ab clone **513M2A7 PA560**	1	abcarta	1	0	0	0	-	-
Ab clone **GM008 GT2531**	1	Gene Tech	0	1	0	0	-	-
Ab clone **BP6033 I10172E**	1	Biolynx Biotechnology	1	0	0	0	-	-
rmAb clone **SP225 790-7219 (VRPS)[3]**	12	Ventana/Roche	2	9	1	0	92%	17%
rmAb clone **SP225 790-7219 (LMPS)[4]**	8	Ventana/Roche	3	5	0	0	100%	38%
rmAb clone **BY004 BFM-0131**	1	Bioin Biotechnology	1	0	0	0	-	-
rmAb clone **DAK-p40 GA784 (VRPS)[3]**	51	Dako/Agilent	50	1	0	0	100%	98%
rmAb clone **DAK-p40 GA784 (LMPS)[4]**	18	Dako/Agilent	13	5	0	0	100%	72%
rmAb clone **MXR010 RMA-1006**	2	Fuzhou Maixin	2	0	0	0	-	-
rmAb clone **ZR8 MAD-000686QD**	1	Master Diagnostica	0	1	0	0	-	-
rmAb clone **ZR8 LS-C312131**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **TP40/3980R ANA43**	1	BioGenex	0	1	0	0	-	-
pAb **API 3030**	1	Biocare Medical	0	1	0	0	-	-
Total	314		178	118	16	2		
Proportion			57%	37%	5%	1%	94%	

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 p40, Run 74
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **BC28**: Protocols with optimal results were all based on HIER using Cell Conditioning 1 (CC1, Ventana/Roche) (29/37)*, Target Retrieval Solution (TRS) High pH (Dako/Agilent) (22/25), TRS Low pH (Dako/Agilent) (1/1) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (13/15). 61 of 64 optimal protocols applied a 3-layer detection system. The mAb was typically diluted in the range of 1:25-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 77 of 78 (99%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **ZR8**: Protocols with optimal results were based on HIER using BERS2 (Leica Biosystems) (1/3) or CC1 (Ventana/Roche) (1/3). The rmAb was diluted in the range of 1:50-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings, 6 of 6 (100%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for the two most commonly applied p40 antibodies as concentrate on the 4 main IHC systems***

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 **BC28**	3/4**	-	18/21 (86%)	1/1	29/37 (78%)	-	13/15 (87%)	0/2
rmAb clone **ZR8**	0/1	-	-	-	1/3	-	1/3	-

* 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).
1) Autostainer Classical, Link 48.
2) BenchMark GX, Ultra, Ultra plus
3) Bond III, Prime

Ready-To-Use antibodies and corresponding systems
mAb clone **BC28**, product no. **790-4950**, Ventana/Roche, BenchMark GX / XT / ULTRA / ULTRA PLUS:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min.), 16-64 min. incubation of the primary Ab and UltraView (760-500) with amplification (760-080), OptiView (760-700) or OptiView (760-700) with OptiView Amplification Kit (760-099 / 860-099) as detection system. Using these protocol settings 132 of 144 (92%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **BC28**, product no. **PA0163**, Leica Biosystems, Bond MAX / III / Prime:
Protocols with optimal results were typically based on HIER using BERS2 (efficient heating time 20-30 min.), 15-20 min. incubation of the primary Ab and Bond Refine (DS9800) as detection system. Using these protocol settings 31 of 31 (100%) laboratories produced a sufficient staining result.

rmAb clone **DAK-p40**, product no. **GA784**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH 9 (efficient heating time 20-30 min. at 97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (GV800 + GV809) as the detection system. Using these protocol settings, 63 of 63 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP225**, product no. **790-7219**, Ventana/Roche, BenchMark ULTRA / ULTRA PLUS:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 64 min.), 32 min. incubation of the primary Ab and OptiView (760-700) or OptiView (760-700) with OptiView Amplification Kit (760-099 / 860-099) as detection system. Using these protocol settings 13 of 13 (100%) laboratories produced a sufficient staining result.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 assessed protocols). 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 (in Table 1 LMPS also includes off label use on deviant IHC stainers).

Table 3. **Proportion of sufficient and optimal results for p40 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS BenchMark mAb **BC28** **790-4950 UltraView**	15/17 (88%)	0/17 (0%)	31/39 (79%)	4/39 (10%)
VMS BenchMark mAb **BC28** **790-4950 OptiView**	9/11 (81%)	1/11 (9%)	87/92 (95%)	52/92 (57%)
Leica BOND mAb **BC28** **PA0163**	18/18 (100%)	17/18 (94%)	13/13 (100%)	10/13 (77%)
Dako Omnis rmAb **DAK-p40** **GA784**	51/51 (100%)	50/51 (98%)	13/13 (100%)	12/13 (92%)
VMS BenchMark rmAb **SP225** **790-7219 UltraView**	2/3	0/3	3/3	0/3
VMS BenchMark rmAb **SP225** **790-7219 OptiView**	9/9 (100%)	2/9 (22%)	4/4	2/4

* Protocol settings recommended by vendor – retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer were included.

Comments

In concordance with the previous NordiQC assessments for p40, the prevalent feature of an insufficient result was a too weak or completely false negative staining reaction of the cells expected to be demonstrated. This pattern was seen in 58% of the insufficient results (14 of 24 laboratories). The remaining insufficient results were typically characterized by a general poor signal-to-noise ratio (29%) or false positive nuclear staining reactions (13%).
Too weak staining result was typically characterized by a reduced staining reaction regarding both the intensity and proportion of cells expected to be demonstrated. This was in particular observed in the cytotrophoblasts of placenta and a significantly reduced intensity and/or proportion of positive neoplastic cells of the lung squamous cell carcinoma, tissue core no. 4. Virtually all laboratories successfully demonstrated p40 in the majority of neoplastic cells of the lung squamous cell carcinoma, tissue core no. 3, with high expression level of p40.

24% (97 of 411) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for p40 with a total pass rate of 94% (91 of 97), 70% optimal.
The mAb clone **BC28** was the most widely used antibody and had the highest proportion of sufficient and optimal results obtained for protocols based on concentrated formats, as seen in Table 1b. Optimal results could be obtained on the 4 most widely used IHC platforms, as shown in Table 2. Efficient HIER in an alkaline buffer in combination with a 3-step polymer/multimer based detection system provided the highest proportion of optimal results. In particular, the choice of detection system influenced the performance for the mAb clone BC28 within LD assays for p40. 85% of protocols (61 of 72) based on a 3-layer detection system provided an optimal result being in clear contrast and superior to the use of 2-layer detection systems only giving an optimal result in 33% (3 of 9).

Sufficient and optimal results could also be achieved with the rmAb clone ZR8, but the data from previous assessments suggests that this antibody can be difficult to optimize. In this assessment 6 of 7 (86%) laboratories achieved sufficient results, but only 29% (n=2) were assessed as optimal.

Two different polyclonal Abs (pAb) were used as concentrates within LD assays (4 protocols in total). Despite protocol settings, as retrieval conditions, detection systems and IHC stainer platforms were identical to the mAb clone BC28 and rmAb clone ZR8, only one sufficient result was provided. The insufficient results were typically characterized by a false negative staining reaction, a poor signal-to-noise ratio and/or false positive staining reaction. This observation was concordant to data generated in runs 48, 60 and 67.

Ready-To-Use (RTU) antibodies were used by 76% (314 of 411) of the laboratories. The most frequently used RTU systems for p40 were the Ventana/Roche 790-4950 system for BenchMark and the GA784 Dako/Agilent for Omnis, based on mAb clone BC28 and rmAb clone DAK-p40, respectively.

The Ventana/Roche RTU system **790-4950** based on mAb clone BC28 was used by 159 participants on the intended BenchMark IHC platform. Following the vendor protocol recommendations, a pass rate of 86% was obtained, only 4% optimal. 82% of the participants applied a laboratory modified protocols, giving an overall pass rate of 90% (118 of 131) despite chosen detection system. The most common and successful modification was a prolonged incubation time of the primary Ab to 24-40 min. (recommended 16 min.). The majority of laboratories using OptiView as detection system also successfully prolonged the HIER time to 48-64 min. (recommended 32 min.). These "positive" modifications of the official RTU protocol, resulted in an increase of optimal results, as 43% of the laboratories achieved optimal results (n=56) compared to 4% of the laboratories using the official RTU protocol (1 of 28).

The newly launched **790-7219** based on rmAb clone SP225 from Ventana/Roche obtained a 92% pass rate when following the vendor protocol recommendations, and 100% when modifying the protocol (see Table 3). The assay has recommended protocols both for UltraView and OptiView. In this assessment, no optimal results were seen when using UltraView as detection system.

The Dako/Agilent RTU system **GA784** based on rmAb clone DAK-p40 was used by 64 participants and obtained a 100% pass rate both when following the vendor protocol recommendations, and when modifying the protocol, with similar proportion of optimal results (see Table 3). The modifications were related to minor adjustments in incubation time in HIER and/or primary Ab.
Five laboratories used the Omnis RTU successfully on Autostainer, using similar protocol settings as recommended for Omnis: HIER in TRS high pH for 20 min., 20 min. incubation of the primary Ab and EnVision Flex+ as detection system.
However, in this context, it must be emphasized that modifications of vendor recommended protocol settings for RTU systems including migration of the RTU Abs to another platform than the intended, require a meticulous validation process for the end-users. As seen in this assessment, modifications can be very successful but may also generate sub-optimal or aberrant results and therefore must be carefully monitored.

The Leica Biosystems RTU system **PA0163** based on mAb clone BC28 was used by 31 participants, and all produced a sufficient result, 87% optimal. Laboratories applying the vendor recommended protocol settings, using HIER in BERS2 for 20 min., 15 min. incubation of the primary Ab and Refine as detection system obtained 94% optimal results (see Table 3).

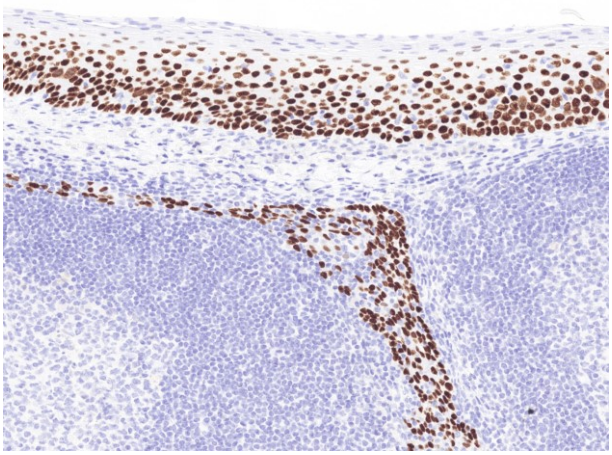


Fig. 1a
Optimal p40 staining reaction of the tonsil using the Dako/Agilent RTU format GA784 for Omnis based on rmAb clone DAK-p40 following the recommended protocol. A moderate to strong nuclear staining reaction is seen in virtually all the squamous epithelial cells. No background staining is seen.
Same protocol used in Figs. 2a - 5a.

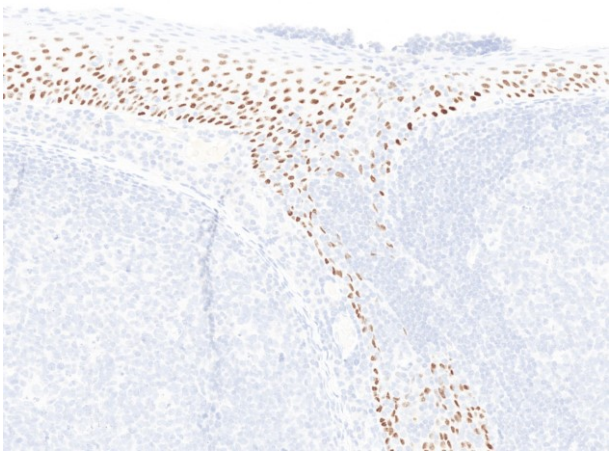


Fig. 1b
Insufficient p40 staining reaction of the tonsil using the Ventana/Roche RTU format 790-4950 based on mAb clone BC28 with the vendor recommended protocol settings for OptiView as detection system.
Compare with Fig. 1a (same field). The staining intensity is reduced.
Also compare with Figs. 2b - 4b, same protocol.

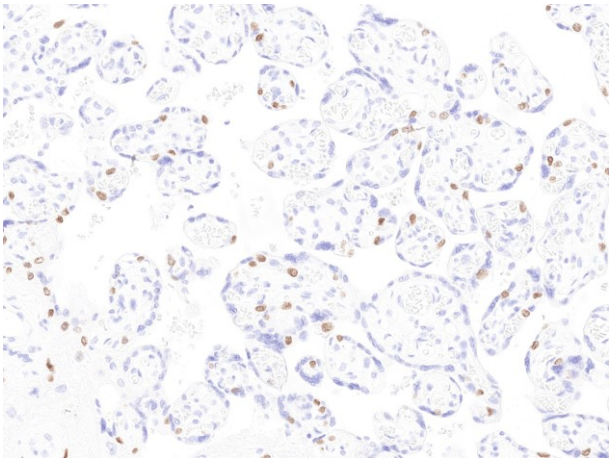


Fig. 2a
Optimal p40 staining reaction of the placenta using same protocol as in Fig. 1a. Scattered cytothrophoblastic cells show a weak to moderate, distinct nuclear staining reaction.
The cells can easily be identified at low magnification (5x).

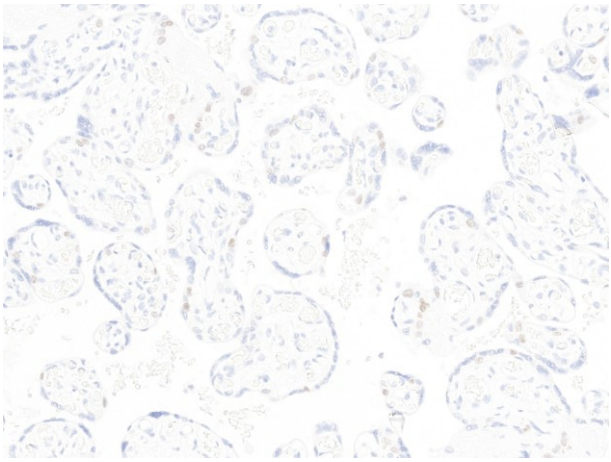


Fig. 2b
Insufficient p40 staining reaction of the placenta using same protocol as in Fig. 1b. Virtually no nuclear staining reaction of cytothrophoblastic cells is seen. Compare with Fig. 2a (same field).
Also compare with Figs. 3b and 4b, same protocol.

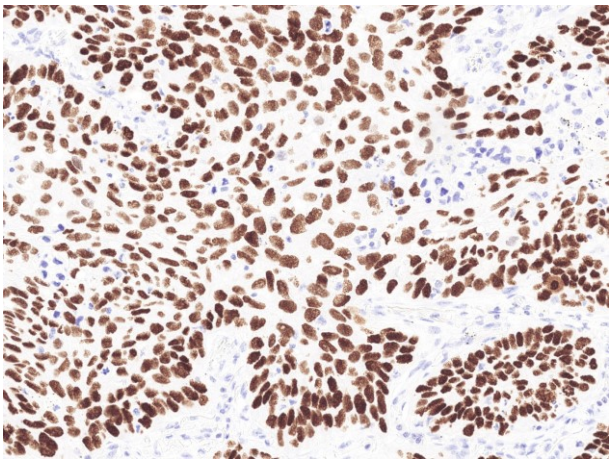


Fig. 3a
Optimal p40 staining reaction of the lung squamous cell carcinoma, tissue core no. 3, using same protocol as in Figs. 1a and 2a. Virtually all neoplastic cells show a moderate to strong nuclear staining reaction.
No background staining is seen.

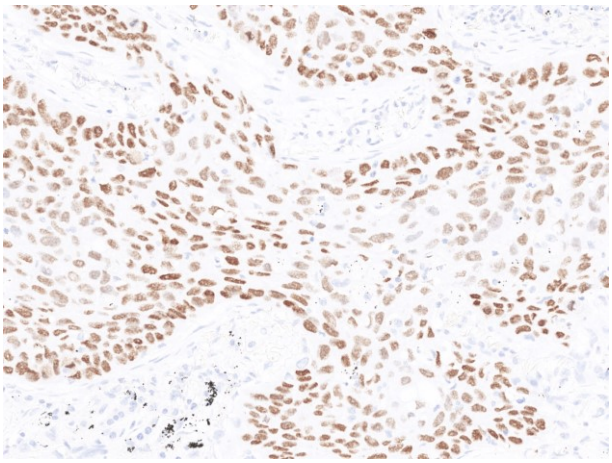


Fig. 3b
p40 staining reaction of the lung squamous cell carcinoma, tissue core no. 3, using same protocol as in Figs. 1b and 2b. The neoplastic cells are demonstrated, though the intensity is reduced compared to the level expected and shown in Fig. 3a (same field). However also compare with Fig. 4b, same protocol.

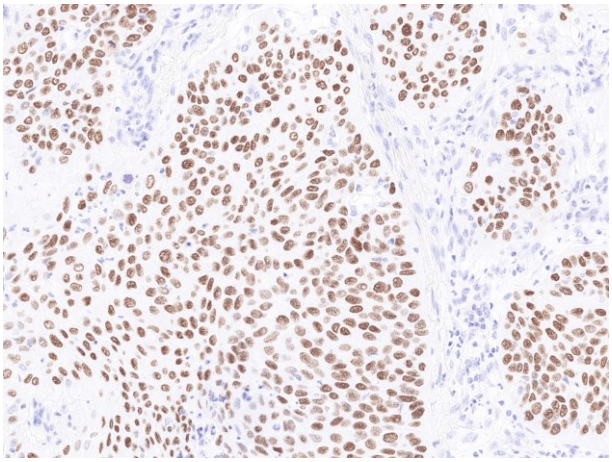


Fig. 4a
Optimal p40 staining reaction of the lung squamous cell carcinoma, tissue core no. 4, using same protocol as in Figs. 1a - 3a.
The majority of neoplastic cells show a weak to moderate nuclear staining reaction.

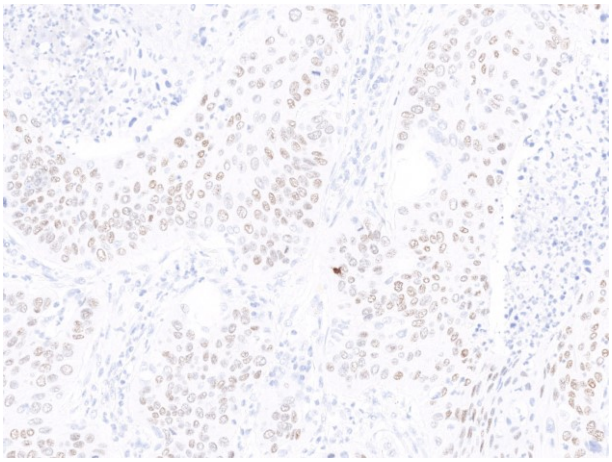


Fig. 4b
Insufficient p40 staining reaction of the lung squamous cell carcinoma, tissue core no. 4, using same protocol as in Figs. 1b – 3b.
The neoplastic cells only show a faint nuclear staining reaction. Compare with Fig. 4a (same field).

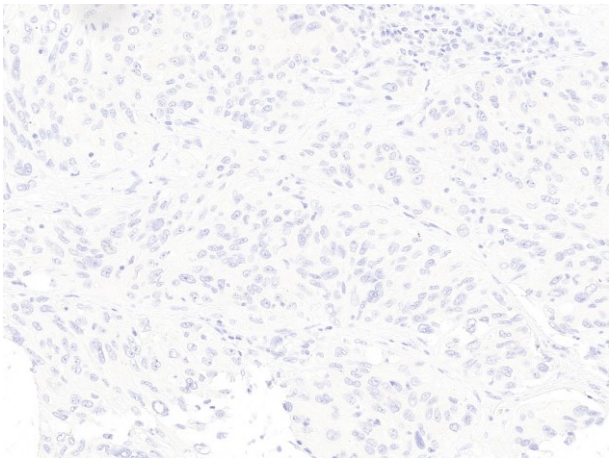


Fig. 5a
Optimal p40 staining reaction of the lung adenocarcinoma, using same protocol as in Figs. 1a-4a.
No staining is observed.

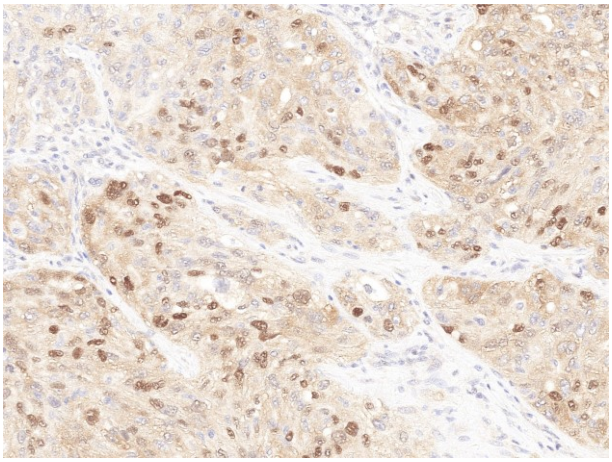


Fig. 5b
Insufficient p40 staining reaction of the lung adenocarcinoma, using mAb clone rTP40/3690, with HIER in an alkaline buffer and a 2-step detection system.
Dispersed neoplastic cells show a weak to moderate nuclear staining reaction. In addition, a cytoplasmic staining is seen. Compare with Fig. 5a (same field).

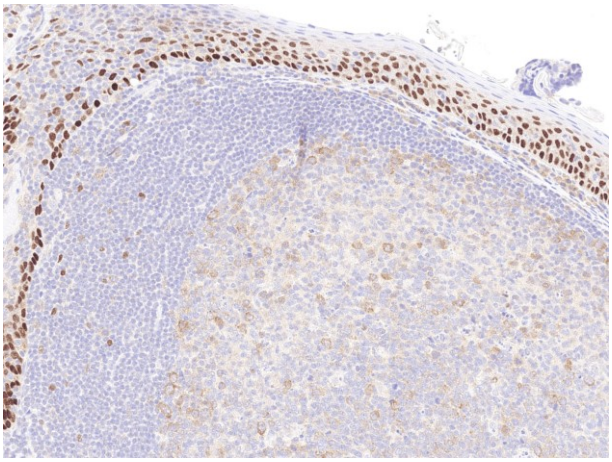


Fig. 6a
Insufficient p40 staining reaction of tonsil using same protocol as in Fig. 5b. The squamous epithelium shows a moderate to strong nuclear staining reaction as expected, but lymphocytes in germinal centres are false positive.

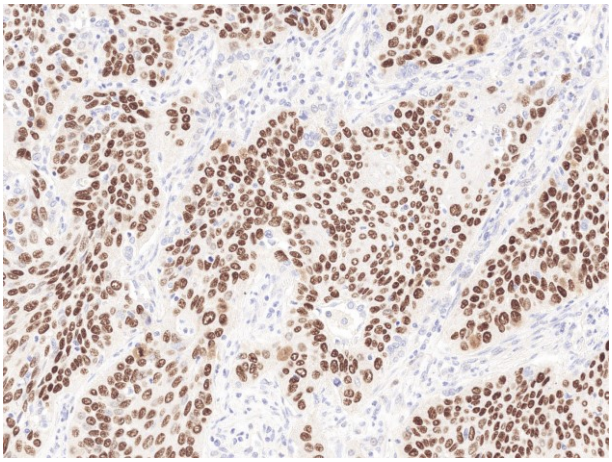


Fig. 6b
p40 staining reaction of the lung squamous cell carcinoma, tissue core no. 4, using same protocol as in Fig. 5b and 6a.
The majority of neoplastic cells show an at least weak to moderate nuclear staining reaction as expected.

HLK/LE/SN 19.06.2025

Version	Description of change and reason	Date	Authorized by
2	Table 3 updated as an error found in data	16.07.2025	LE/HLK