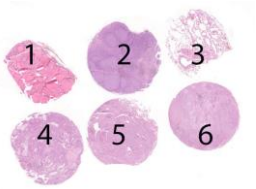
Assessment Run 68 2023
Thyroid transcription factor-1 (TTF1)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of the IHC assays for TTF1 performed by the NordiQC participants, identifying lung as origin for adenocarcinomas of unknown origin and differentiation between lung adenocarcinoma and lung squamous cell carcinoma. Relevant clinical tissues, both normal and neoplastic, were selected to include a wide spectrum of TTF1 antigen densities (see below).

Material
The slide to be stained for comprised:



1. Thyroid gland, 2. Tonsil, 3. Normal lung, 4-5. Lung adenocarcinoma, 6. Lung squamous cell carcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a TTF1 staining as optimal included:

- A strong, distinct nuclear staining reaction of all type II pneumocytes and basal cells of the terminal bronchioles in the lung.
- An at least weak to moderate, distinct nuclear staining reaction of the vast majority of luminal epithelial cells of the terminal bronchioles in the normal lung.
- A strong, distinct nuclear staining reaction of all follicular epithelial cells in the thyroid gland.
- A moderate to strong nuclear staining reaction of the majority of neoplastic cells in the lung adenocarcinoma, tissue core no. 4, and at least weak to moderate, distinct nuclear staining reaction of the majority of neoplastic cells in the lung adenocarcinoma, tissue core no. 5.
- No staining reaction of neoplastic cells in the lung squamous cell carcinoma (a strong staining reaction is expected in remnants of normal pneumocytes).
- No staining reaction in the tonsil*.

*A weak nuclear staining reaction of scattered lymphocytes (<1%) mainly in germinal centres was accepted[1].

[1] Van Bockstal M, Camboni A, De Vlieghere E, et al. Some diffuse large B cell lymphomas (DLBCLs) present with clone-dependent TTF-1 positivity. Histopathology. 2018;72(7):1228–1230.

Participation

Number of laboratories registered for TTF1, run 68	433
Number of laboratories returning slides	401 (93%)

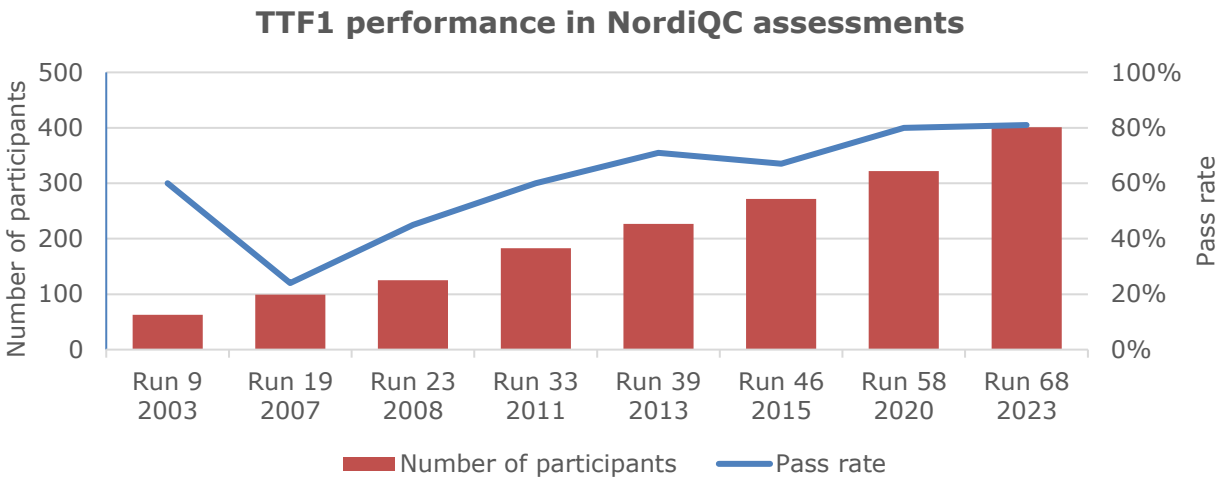
All slides returned after the assessment were assessed and received advice if the result being insufficient, but the data were not included in this report.

Results
401 laboratories participated in this assessment. 81% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies used and assessment marks (see page 3).

The most frequent causes of insufficient staining reactions were:
- Less successful primary Ab (the mAb clone 8G7G3/1).
- Too low concentration of the primary Ab.

Performance history
This was the eighth NordiQC assessment of TTF1. A pass rate of 81% was seen, which is the highest level obtained in all NordiQC assessments of TTF1 (see Graph 1).

Graph 1: **Proportion of sufficient results for TTF1 in the NordiQC runs performed**



Conclusion

In this run, and in concordance with previous NordiQC assessments for TTF1, the mAb clone SPT24 and the rmAb clone SP141 were most successful for the demonstration of TTF1 providing pass rates of 97% and 96%, respectively. In contrast, mAb clone 8G7G3/1 was less successful and provided a significantly lower pass rate of 6%.

The overall pass rate for TTF1 run 68 was 81%. Compared to previous assessments, increased use of the successful clones at the expense of 8G7G3/1 and extended use of sensitive 3-step detections systems, seemed to have a positive impact on the pass rate both in this and the previous assessment.

Controls

Normal lung is recommendable as positive tissue control for TTF1. The columnar epithelial cells of the terminal bronchioles serve as a "low expressor" (LE) positive tissue control and must show an at least weak to moderate and distinct nuclear staining reaction. The type II pneumocytes and the basal cells of the terminal bronchioles all serve as "high expressors" (HE), in which a strong nuclear staining reaction must be seen. The nuclear staining in the HE should be as strong as possible without significant cytoplasmic reaction. Thyroid epithelial cells and lung pneumocytes are less reliable as positive tissue control for TTF1 as these cells only express very high levels of TTF1, making it difficult to evaluate the level of analytical sensitivity of the protocol used. Tonsil can serve as negative tissue control, in which no staining should be seen. However scattered lymphocytes (<1%), mainly in germinal centres, may show a weak nuclear staining reaction, which is accepted. The recommendations of the tissue controls for IHC mentioned above are concordant to the guidelines published by the International Ad Hoc Expert Committee[2].

[2]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 1. **Antibodies and assessment marks for TTF1, run 68**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **8G7G3/1**	3 14 1 1 1	Cell Marque Dako/Agilent Quartett Santa Cruz Zytomed	0	2	10	8	10%	-
mAb clone **SPT24**	6 1 1 3 3 96 8 1	Biocare Medical Biogenex Epredia Gennova Immunologic Leica Biosystems Monosan Unknown	102	14	2	1	97%	86%
rmAb clone **EP229**	3	Cell Marque	0	3	0	0	-	-
rmAb clone **IHC141**	1	GenomeMe	1	0	0	0	-	-
rmAb clone **QR046**	1	Quartett	1	0	0	0	-	-
rmAb clone **ZR176**	1	Zeta Corporation	0	1	0	0	-	-
Ready-To-Use Antibodies								
mAb clone **8G7G3/1 790-4398 (VRPS)[3]**	3	Ventana/Roche	0	0	2	1	-	-
mAb clone **8G7G3/1 790-4398 (LMPS)[4]**	15	Ventana/Roche	0	0	9	6	0%	0%
mAb clone **8G7G3/1 IR056 (VRPS)[3]**	6	Dako/Agilent	0	0	6	0	0%	0%
mAb clone **8G7G3/1 IR056 (LMPS)[4]**	22	Dako/Agilent	0	1	8	13	5%	0%
mAb clone **847G1A6 PA352**	1	Abcarta	1	0	0	0	-	-
mAb clone **8G7G3/1 BFM-0063**	1	Bioin Biotechnology	1	0	0	0	-	-
mAb clone **C7H7 FTM0022**	1	Celnovte	1	0	0	0	-	-
mAb clone **MX011 MAB-0677**	2	Fuzhou Maixin	2	0	0	0	-	-
mAb clone **NX2.1/690, AMA25**	1	Biogenex	0	0	1	0	-	-
rmAb **EP229 8224-C010**	2	Sakura Finetek	2	0	0	0	-	-
rmAb clone **SP141 790-4756 (VRPS)[3]**	51	Ventana/Roche	47	2	2	0	96%	92%
rmAb clone **SP141 790-4756 (LMPS)[4]**	97	Ventana/Roche	85	8	2	2	96%	88%
mAb clone **SPT24 PA0364 (VRPS)[3]**	13	Leica/Novocastra	10	3	0	0	100%	77%
mAb clone **SPT24 PA0364 (LMPS)[4]**	26	Leica/Novocastra	22	3	0	1	96%	85%
rmAb clone **BP6079 BX50074**	2	Biolynx Biotechnology	2	0	0	0	-	-
mAb clone **SPT24 MAD-000486QD**	5	Master Diagnostica SL	3	1	1	0	80%	60%
mAb clone **SPT24 API 3126**	4	BioCare Medical	2	2	0	0	-	-
Unknown	4		4	0	0	0	-	-
Total	401		286	40	43	32		
Proportion			71%	10%	11%	8%	81%	

1) Proportion of sufficient stains (optimal or good). For Laboratory Developed (LD) assays (≥5 asessed protocols)
2) Proportion of Optimal Results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 asessed protocols).

Detailed analysis of TTF1, Run 68
The following protocol parameters were central to obtain optimal staining:

Concentrated Antibodies
mAb clone **SPT24**: Protocols with optimal results were all based on Heat Induced Epitope Retrieval (HIER) using either Cell Conditioning 1 (CC1; Ventana/Roche) (43/45)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (46/54), Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (8/9), Bond Epitope Retrieval Solution 1 (BERS1; Leica Biosystems) (2/4) or unknown (3/3) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings, 110 of 113 (97%) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this HIER buffer)

Table 2. **Proportion of optimal results for TTF1 for the mAb clone SPT24 as concentrate on the main IHC systems***

Concentrated antibodies	**Dako Autostainer**		**Dako Omnis**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max / PRIME**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **SPT24**	6/6** (100%)	0/2	40/48 (83%)	0/1	43/45 (96%)	-	8/9 (89%)	2/4

* 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).

Ready-To-Use (RTU) antibodies and corresponding systems
rmAb clone **SP141** product no. **790-4756**, Ventana/Roche, BenchMark GX, XT and Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min.), 8-48 min. incubation of the primary Ab and UltraView (760-500), UltraView + Amplification (760-500+760-080) or OptiView (760-700) as detection system. Using these protocol settings, 140 of 146 (96 %) laboratories produced a sufficient staining result.
1 laboratory used product no. 790-4756 on another platform. This was not included in the description above.

mAb clone **SPT24** product.no. **PA0364**, Leica Biosystems, Bond III and Max:
Protocols with optimal results were typically based on HIER using BERS1 or BERS2 (efficient heating time 15-30 min.), 15-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 30 of 30 (100 %) laboratories produced a sufficient staining result.
9 laboratories used product no. PA0364 on other platforms. These were not included in the description above.

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 Laboratory Modified Protocol Settings (LMPS) also includes off label use on deviant IHC stainers).

Table 3. **Comparison of pass rates for vendor recommended and laboratory modified RTU protocols**

RTU systems	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT mAb 8G7G3/1 **790-4398**	0/3	0/3	0/15 (0%)	0/15 (0%)
Dako AS Link 48+ mAb 8G7G3/1 **IR056**	0/6 (0%)	0/6 (0%)	0/7 (0%)	0/7 (0%)
VMS Ultra/XT rmAb SP141 **790-4756**	49/51 (96%)	47/51 (92%)	92/96 (96%)	85/96 (89%)
Leica BOND III/Max mAb SPT24 **PA0364**	13/13 (100%)	10/13 (77%)	17/17 (100%)	15/17 (88%)

* 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 this assessment and in concordance with previous assessments for TTF1, the prevalent feature of an insufficient result was a general too weak or false negative staining of cells/structures expected to be demonstrated. Too weak or false negative staining reaction was seen in 87% of the insufficient results (65 of 75). 11% (8 of 75) of the insufficient results were caused by poor signal-to-noise ratio.

Virtually all the participating laboratories were able to demonstrate TTF1 in structures with a high-level antigen expression (thyroid epithelial cells and type II pneumocytes of the lung). Cells with low levels of antigen expression, as columnar epithelial cells of terminal bronchioles of the lung and in particular neoplastic cells of the lung adenocarcinoma, tissue core no. 5, were more challenging and could only be demonstrated when using a correctly calibrated protocol.

36% (145 of 401) of the participants used Abs as concentrated format within laboratory developed (LD) assays for TTF1, with an overall pass rate of 86% (124 of 145), 72% optimal. 14% (20 of 145) of the LD assays was based on the less successful mAb clone 8G7G3/1, with a pass rate of 10% (2 of 20), none optimal (see Table 1). 82% (119 of 145) of LD assays was based on mAb clone SPT24, with a significant higher pass rate of 97% (116 of 119), 70% optimal.

mAb clone SPT24 was the most widely used antibody for demonstration of TTF1. Used as a concentrate within a LD assay, it provided an optimal staining result on all the three main IHC platforms from Dako/Agilent, Leica Biosystems and Ventana/Roche (see Table 2). It was observed that especially use of HIER at high pH in combination with a 3-step detection system was found successful.

64% (256 of 401) of the participants used Abs in RTU formats, with an overall pass rate of 79% (202 of 256), 71% optimal. 18% (47 of 256) of the RTU systems was based on mAb clone 8G7G3/1 with a pass rate of 4% (2 of 47), one optimal. When using an RTU based on another clone, a significant higher pass rate of 96% (200 of 209) was obtained, 87% optimal.

The Ventana/Roche RTU format, 790-4756 based on rmAb clone SP141 obtained a high pass rate both when applying vendor recommended protocol settings (96%) and when modifying the protocol settings (96%), see Table 3. The most common modifications seen were shorter HIER time and prolonged incubation time of primary Ab.

mAb clone SPT24 PA0364, RTU format from Leica Biosystems, obtained a pass rate of 100% both when applying the protocol settings as recommended by Leica Biosystems or when modifying the protocol settings on the Bond platform, see Table 3.

Most important for optimal and consistent result for TTF1 was the choice of the primary Ab: The mAb clone SPT24 and the rmAb clone SP141 had significantly higher pass rate compared to the mAb clone 8G7G3/1. In this run, pass rates of 97% and 96% were seen for mAb clone SPT24 and rmAb clone SP141, respectively. In comparison, a pass rate of 6% was seen when the mAb clone 8G7G3/1 was used. This pattern was also observed in the previous NordiQC assessments for TTF1.
In the last four TTF1 assessments, one of 258 submitted protocols based on the mAb clone 8G7G3/1 produced an optimal result despite protocol settings were similar to those used for the mAb clone SPT24 or rmAb clone SP141. The mAb clone 8G7G3/1 has thus shown to have a significantly lower affinity/analytical sensitivity for TTF1 compared to the mAb clone SPT24 and the rmAb clone SP141. In this and all previous NordiQC assessments for TTF1, the reduced affinity/analytical sensitivity have induced false negative results in both lung adenocarcinomas and lung carcinoids with the risk of misclassification of carcinoma of unknown primary origin. Cumulated data and pass rates for the last four runs (Run 39, 46, 58 and 68) are shown in Table 4.

Table 4. **The overall pass rate in the last four runs for the mAb clones SPT24, 8G7G3/1 and the rmAb clone SP141**

	SPT24 All protocol settings		**SP141 All protocol settings**		**8G7G3/1 All protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal	Sufficient	Optimal
Participants	92% (568/619)	69% (429/619)	97% (305/316)	80% (252/316)	10% (25/258)	0% (1/258)

Althouth the data so far is limited, new Abs for TTF1 are being introduced as e.g. the rmAb clone EP229 and seem to provide promising results on par to clones SPT24 and SP141 on the material used for the NordiQC TTF1 assessment – see Table 1. However more data is needed to confirm the diagnostic sensitivity and specificity for the new Abs.

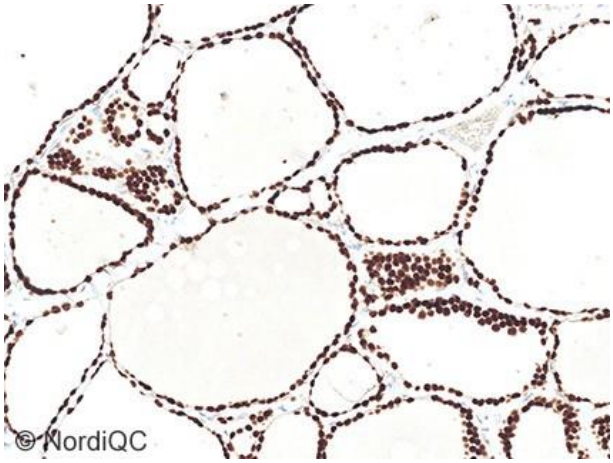


Fig. 1a
Optimal staining of TTF1 in thyroid using the rmAb clone SP141 as an RTU (790-4756, Ventana/Roche) using the vendor recommended protocol settings, being HIER in CC1 for 64 min., 12 min. incubation of the primary Ab and performed on the BenchMark Ultra, using the OptiView detection system.
A strong nuclear staining reaction is seen in virtually all follicular epithelial cells.
Also compare with Figs. 2a - 5a – same protocol.

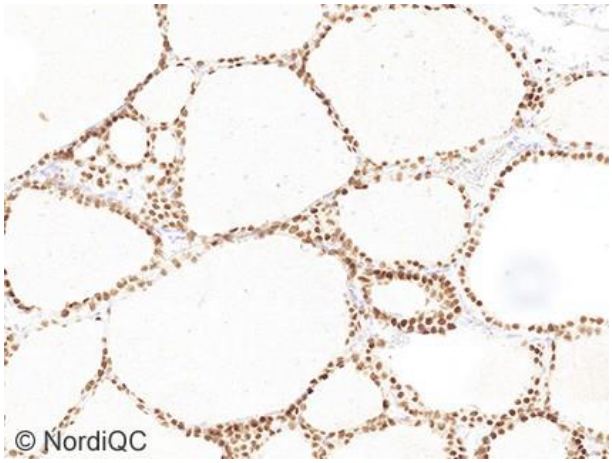


Fig. 1b
TTF1 staining of the thyroid using the mAb clone 8G7G3 as an RTU (IR056, Dako/Agilent) performed on the Omnis platform, using HIER in TRS High pH, 20 min. incubation of the primary Ab and EnVision Flex+ as detection system.
Only a moderate nuclear staining reaction is seen in the majority of follicular epithelial cells – same field as in Fig. 1a. Also compare with Figs. 2b - 5b – same protocol. Overall an insufficient result was provided.

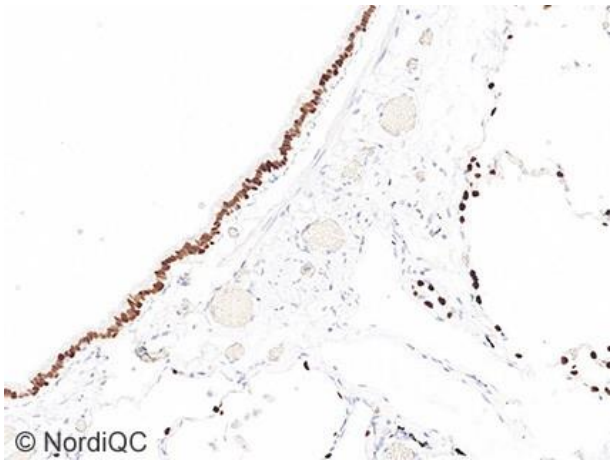


Fig. 2a
Optimal staining of TTF1 in the normal lung using same protocol as in Fig. 1a.
The type II pneumocytes and dispersed basal epithelial cells lining the terminal bronchioles show a strong distinct nuclear staining reaction, whereas the columnar epithelial cells show a weak to moderate nuclear staining reaction.

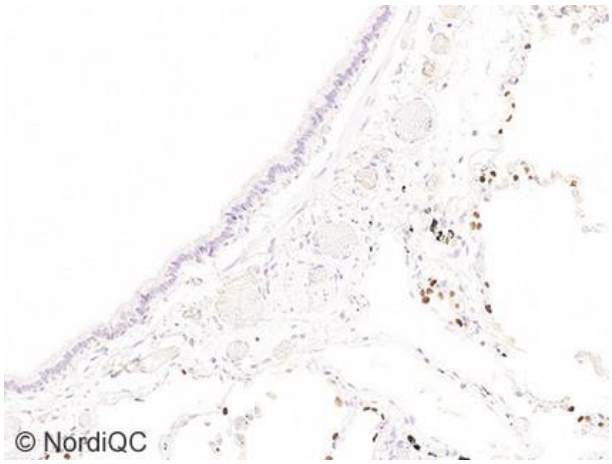


Fig. 2b
Insufficient staining of TTF1 in the normal lung using same protocol as in Fig. 1b.
Only type II pneumocytes are demonstrated, while no staining reaction is seen in the columnar epithelial cells - same field as in Fig. 2a.

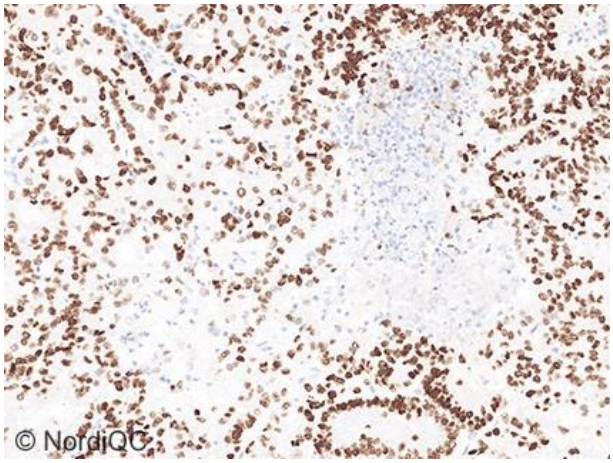


Fig. 3a
Optimal staining of TTF1 in the lung adenocarcinoma, tissue core no. 4 (high expression of TTF1), using same protocol as in Figs. 1a - 2a.
Virtually all the neoplastic cells show a moderate to strong and distinct nuclear staining reaction.

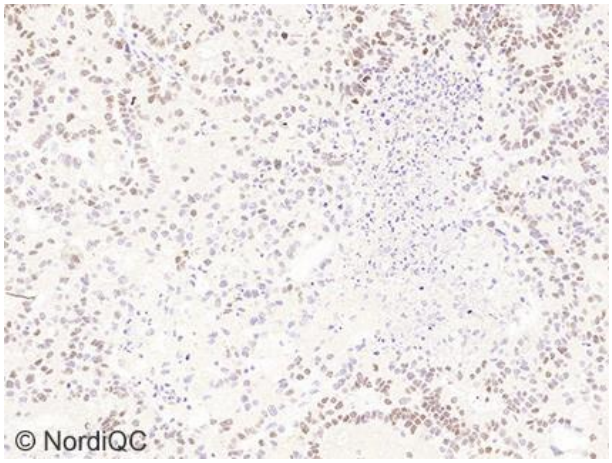


Fig. 3b
Insufficient staining of TTF1 in the lung adenocarcinoma, tissue core no. 4, using same insufficient protocol as in Figs. 1b - 2b.
Despite being a tumour with high expression level of TTF1, both the proportion of positive neoplastic cells and staining intensity is significant reduced compared to the level expected and obtained by optimal protocol settings as seen in Fig. 3a – same field.

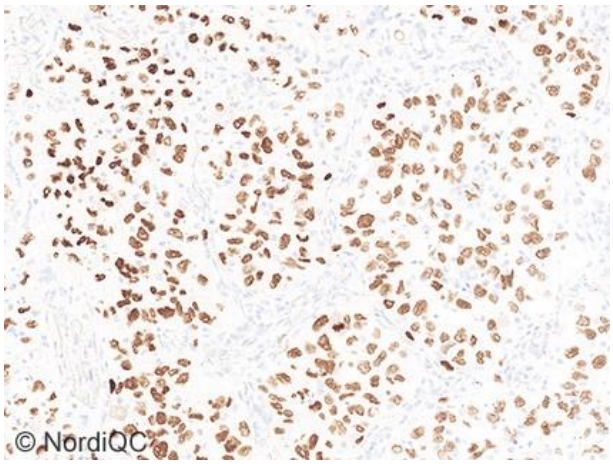


Fig. 4a
Optimal staining of TTF1 in the lung adenocarcinoma, tissue core no. 5, with low level TTF1 expression, using same protocol as in Figs. 1a - 3a.
Virtually all the neoplastic cells show a weak to moderate nuclear staining reaction.

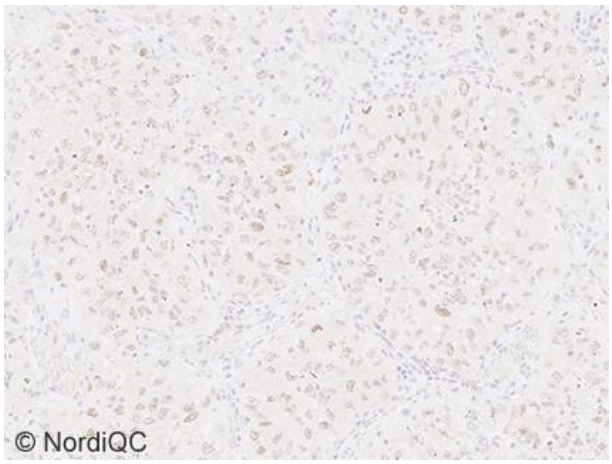


Fig. 4b
Insufficient staining of TTF1 in the lung adenocarcinoma, tissue core no. 5, with low level TTF1 expression, using same protocol as in Figs. 1b - 3b.
Virtually all neoplastic cells are only faintly positive compared to the intensity expected and simultaneously a weak diffuse cytoplasmic staining reaction is seen compromising the read-out – same field as in Fig. 4a.
Also compare with Fig. 5b.

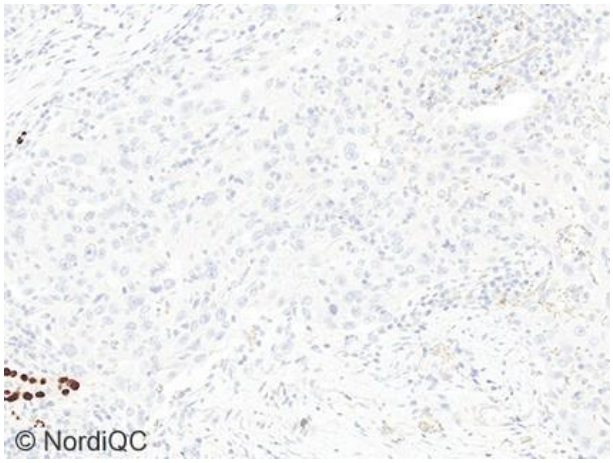


Fig. 5a
Optimal staining of TTF1 in the lung squamous cell carcinoma using same protocol as in Figs. 1a - 4a. No staining reaction is seen in the neoplastic cells. Compare with Fig. 5b.

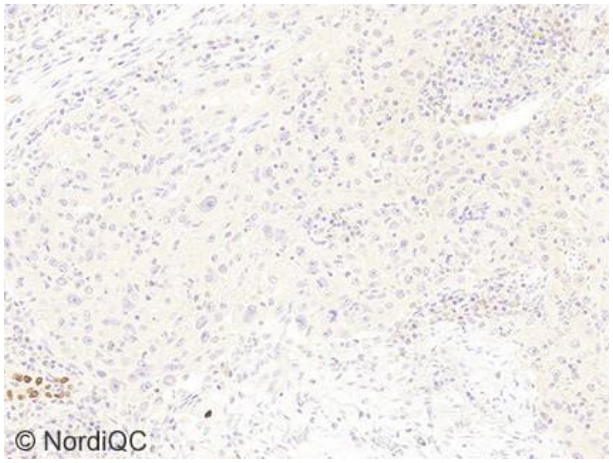


Fig. 5b
Insufficient staining of TTF1 in the lung squamous cell carcinoma using same protocol as in Figs. 1b - 4b giving a poor signal-to-noise ratio, interfering the interpretation - same field as in Fig. 5a.

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