

Material

The slide to be stained for TTF1 comprised:

1. Thyroid gland, 2. Liver, 3. Normal lung, 4-6. Lung adenocarcinoma 7. Colon adenocarcinoma.

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, Clara cells and basal cells of the terminal bronchioles in the lung.
- A moderate to strong, distinct nuclear staining reaction of the vast majority of columnar epithelial cells of the terminal bronchioles in the lung.
- A strong, distinct nuclear staining reaction of all the follicular epithelial cells in the thyroid gland.
- A strong nuclear staining reaction of the majority of neoplastic cells in the two lung adenocarcinomas no. 5 & 6 and at least weak to moderate, distinct nuclear staining reaction of the majority of neoplastic cells in the lung adenocarcinoma no. 4.
- No nuclear staining reaction of the colon adenocarcinoma.
- No nuclear staining reaction of the liver. Cytoplasmic staining in the hepatocytes was accepted when using mAb 8G7G3/1.



Participation

Number of laboratories registered for TTF1, run 46	287
Number of laboratories returning slides	272 (95%)

Results

272 laboratories participated in this assessment. 67% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:

- Less successful primary Ab (the mAb clone 8G7G3/1)
- Too low concentration of the primary antibody
- Use of detection systems with low sensitivity

Performance history

This was the sixth NordiQC assessment of TTF1. A minor decrease of the pass rate was seen compared to run 39 in 2013 (see table 2).

Table 2. Proportion of sufficient results for TTF1 in the last 5 NordiQC runs performed

	Run 19 2007	Run 23 2008	Run 33 2011	Run 39 2013	Run 46 2015
Participants, n=	99	125	183	227	272
Sufficient results	24%	45%	60%	71%	67%

Conclusion

In this run, and in concordance with previous NordiQC assessments for TTF1, the mAb clone SPT24 and the rmAb clone SP141 were very robust and sensitive markers for the demonstration of TTF1. Pass rates of 88% and 94% were seen for mAb clone SPT24 and rmAb clone SP141, respectively. Optimal results

were also seen with the recently introduced mAb clone MX011 and rmAb clone EP229. In contrast, mAb clone 8G7G3/1 once again failed to produce optimal mark and the antibody had showed a significantly lower analytical sensitivity compared to the other clones, resulting in an alarming overall pass rate of only 3%.

Lung tissue is recommendable as positive control: Columnar epithelial cells of the terminal bronchi must show moderate to strong, distinct nuclear staining and type II pneumocytes and basal epithelial cells a strong, distinct nuclear staining.

Table 1. **Antibodies and assessment marks for TTF1, run 46**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone 8G7G3/1	15	Dako/Agilent	0	0	14	11	0%	-
	3	Thermo/NeoMarkers						
	6	Cell Marque						
	1	Zeta Corp.						
mAb clone SPT24	120	Leica/Novocastra	76	43	14	3	88%	89%
	9	Monosan						
	5	Immunologic						
	2	BioCare						
rmAb clone EP229	1	Cell Marque	1	0	0	0	-	-
rmAb clone SP141	1	Spring Bioscience	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone 8G7G3/1 790-4398	16	Ventana/Roche	0	1	7	8	7%	-
mAb clone 8G7G3/1 IR056	31	Dako/Agilent	0	1	24	6	3%	-
rmAb clone SP141 790-4756	50	Ventana/Roche	30	17	3	0	94%	94%
mAb clone SPT24 PA0364	8	Leica/Novocastra	7	1	0	0	100%	100%
mAb clone SPT24 MAD-000486QD	2	Master Diagnostica SL	2	0	0	0	-	-
mAb clone SPT24 API 3126	1	BioCare	1	0	0	0	-	-
mAb clone MX011 MAB-0677	1	Maixin	1	0	0	0	-	-
Total	272		118	64	62	28	-	
Proportion			43%	24%	23%	10%	67%	

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

2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of TTF1, Run 46

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 (Ventana) (24/58)*, Target Retrieval Solution pH 9 (3-in-1) (Dako) (14/17), TRS pH 9 (Dako) (30/41), TRS pH 6.1 (Dako) (2/3), Bond Epitope Retrieval Solution 2 (Leica) (11/15), Bond Epitope Retrieval Solution 1 (Leica) (2/2), Diva Decloaker pH 6.2 (Biocare) (1/2), Tris-EDTA/EGTA pH 9 (5/10), or Citrate pH 6 (1/3) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 118 of 132 (89 %) laboratories produced a sufficient staining (optimal or good).

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

rmAb clone **EP229**: One protocol with an optimal result was based on HIER using Cell Conditioning 1 as retrieval buffer (efficient heating time 40 min at 100°C). The rmAb was diluted 1:200 and incubated for 30 min at 37°C using the OptiView (Ventana 760-700) as detection system.

Table 3. **Proportion of optimal results for TTF1 for the mAb clone SPT24 as concentrate on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic / OMNIS		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	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	30/40** (75%)	2/2	24/58 (41%)	-	11/15 (73%)	2/2

* 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

mAb clone **SP141** product no. **790-4756**, Ventana, BenchMark GX, XT and Ultra:

Protocols with optimal results were typically based on HIER using Cell Conditioning 1 (efficient heating time 36-92 min), 8-56 min. incubation of the primary Ab and UltraView (Ventana 760-500) or OptiView (Ventana 760-700) as detection system. Using these protocol settings, 47 of 50 (94 %) laboratories produced a sufficient staining result.

mAb clone **SPT24** product.no. **PA0364**, Leica/Novocastra, Bond III and Max:

Protocols with optimal results were typically based on HIER using Bond Epitope Retrieval Solution 1 or Bond Epitope Retrieval Solution 2 (efficient heating time 15-40 min.), 15-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (Leica DS9800) as detection system. Using these protocol settings, 8 of 8 (100 %) laboratories produced a sufficient staining result.

mAb clone **SPT24** product.no. **MAD-000486QD**, Master Diagnostica SL, LabVision Autostainer:

Protocols with optimal result were based on HIER using Tris-EDTA/EGTA pH 8, (efficient heating time 20 min. at 100°C), 10 min. incubation of the primary Ab and Quanto (Master Diagnostica product no. MAD-021881QK) as detection system. Using this protocol settings, 2 of 2 (100 %) laboratories produced an optimal staining result.

mAb clone **SPT24** product.no. **API3126**, BioCare, IntelliPATH:

One protocol with an optimal result was based on HIER using Diva Decloaker pH 6.2 (Biocare) in a pressure cooker (efficient heating time 15 min. at 110°C), 30 min. incubation of the primary Ab and MACH 4 Universal HRP-Polymer (M4U534) as detection system.

mAb clone **MX011** product.no. **MAB-0677**, Maixin, manual staining:

One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (efficient heating time 20 min. at 100°C), 60 min. incubation of the primary Ab. and KIT-5230 (Maixin) as detection system.

Comments

In this assessment and in concordance with the observations in the 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 99% of the insufficient results (89 of 90)

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 no. 4 were more challenging and could only be demonstrated when using a correctly calibrated protocol.

mAb clone SPT24 was the most widely used antibody for demonstration of TTF1. Used as a concentrate within a laboratory developed (LD) assay, it provided an optimal staining result on all the three main IHC platforms from Dako, Leica and Ventana (see table 3). Despite similar high pass rates on the three platforms, a significantly lower proportion of optimal staining results was seen on the Ventana BenchMark platform (41%) compared to the Dako Autostainer (76%) and Leica Bond platforms (76%). The reason for this is unclear, but might be related to the sensitivity of the detection systems used on the different platforms. On the Ventana BenchMark platform only 34% of the laboratories used a sensitive 3-step multimer system, whereas sensitive 3-step polymer systems were used in 55% and 100% of the laboratories on the Dako Autostainer and Leica Bond platforms, respectively.

Most important for optimal and consistent staining for TTF1 was the choice of the primary Ab: The mAb clone SPT24 and the recently introduced rmAb SP141 had a significant higher pass rate compared to the mAb clone 8G7G3/1. In this run, pass rates of 88% and 94% were seen for mAb clone SPT24 and rmAb SP141, respectively. In comparison, a pass rate of 3% was seen when the mAb clone 8G7G3/1 was used. This pattern was also observed in the previous NordiQC assessments for TTF1. Cumulated data and pass rates for the last 4 runs (Run 23, 33, 39 and 46) are shown in table 4.

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

	SPT24		SP141*		8G7G3/1	
	All protocol settings		All protocol settings		All protocol settings	
	Sufficient	Optimal	Sufficient	Optimal	Sufficient	Optimal
Participants	90% (429/479)	64% (308/479)	94% 59/63	65% 41/63	7% (17/259)	0% (0/259)

* Because rmAb clone SP141 is only recently introduced, data represents Run 39 and 46 only

In the last four TTF1 assessments, none of 259 submitted protocols based on the mAb clone 8G7G3/1 produced an optimal staining despite protocol settings similar to those used for the mAb clone SPT24 or the rmAb clone SP141 as (Fig. 1 – Fig. 4). The mAb clone 8G7G3/1 has thus shown to have a significantly lower affinity for TTF1 compared to the mAb clone SPT24 and the rmAb clone SP141.

In this assessment, the insufficient results with clone SPT24 were all characterized by a generally too weak staining reaction, typically caused by a too low titer of the primary Ab, often in combination with use of a detection system with relative low sensitivity (e.g. 2-step multimer/polymer systems) (Fig. 5 – Fig. 6). 71% of the laboratories (12 out of 17) achieving insufficient results used a 2-step multimer/polymer based detection system in combination with a relative high average SPT24-dilution factor of 1:230. Laboratories achieving sufficient results applied a significantly lower average SPT24-dilution factor of 1:135 and only 51% used a 2-step multimer/polymer based detection system.

Controls

Normal lung is recommendable as positive tissue control for TTF1 (Fig. 2a and Fig. 2b). The columnar epithelial cells of the terminal bronchioles serve as a “low expressor” (LE) positive tissue control, showing a moderate to strong nuclear staining reaction. The type II pneumocytes, the Clara cells and the basal cells of the terminal bronchioles all serve as “high expressors” (HE), in which a strong nuclear staining reaction is seen. The nuclear staining in the “HE” should be as strong as possible without significant cytoplasmic reaction. Thyroid is less reliable as positive tissue control for TTF1, as the thyroid epithelial cells express very high levels of TTF1, making it difficult to evaluate the sensitivity of the protocol used (Fig. 1a and Fig. 1b). Data from previous TTF1 assessments indicate that lung carcinoids – typically expressing low amounts of TTF1 – should also be included as positive control when the initial validation of a TTF1 protocol is being established. Liver can be used as negative tissue control, but a cytoplasmic staining in the hepatocytes must be accepted when using mAb 8G7G3/1.

The recommendations of the above mentioned tissue controls for IHC are concordant to 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.0000000000000163. Review. PubMed PMID: 25474126.



Fig. 1a

Optimal TTF1 staining of the thyroid using the rmAb clone SP141 (Ventana RTU Ab with a conc. of app. 5,7 µg/mL) by an incubation time of 24 min, HIER in an alkaline buffer (CC1 64 min) and performed on the BenchMark Ultra, using the UltraView detection system. A strong nuclear staining reaction is seen in virtually all follicular epithelial cells.

No background staining is seen.

Also compare with Figs. 2a, 3a and 4a – same protocol.

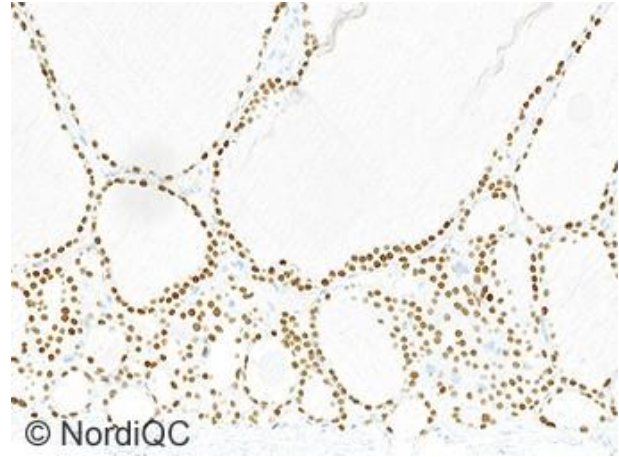


Fig. 1b

TTF1 staining of the thyroid using the mAb clone 8G7G3/1 (Ventana RTU Ab with a conc. of app. 7µg/mL). Similar protocol settings were used as in Fig. 1a:

Incubation time 28 min. - HIER in CC1 for 64 min. and UltraView as detection kit and performed on the BenchMark Ultra, Ventana.

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, 3b and 4b – same protocol.

Overall an insufficient result was provided.

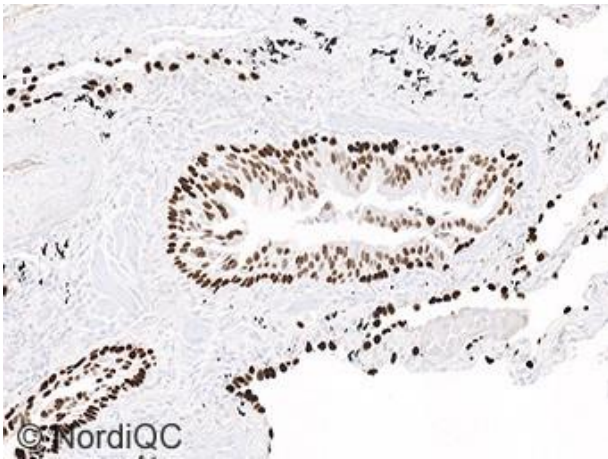


Fig. 2a

Optimal TTF1 staining of the lung using same protocol as in Fig. 1a.

The type II pneumocytes and the basal epithelial cells lining the terminal bronchioles show a strong distinct nuclear staining reaction, whereas the columnar epithelial cells show a moderate nuclear staining reaction.

No background staining is seen.

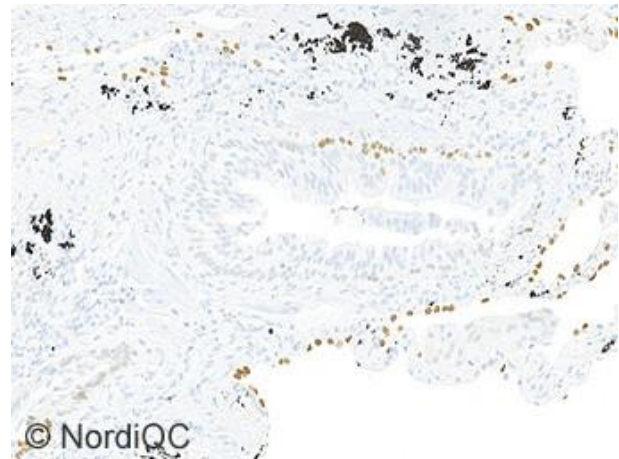


Fig. 2b

Insufficient TTF1 staining of the lung using same protocol as in Fig. 1b.

The majority of type II pneumocytes and the basal epithelial cells lining the terminal bronchioles show only a weak to moderate positive nuclear staining reaction and no reaction is seen in the columnar epithelial cells - same field as in Fig. 2a.

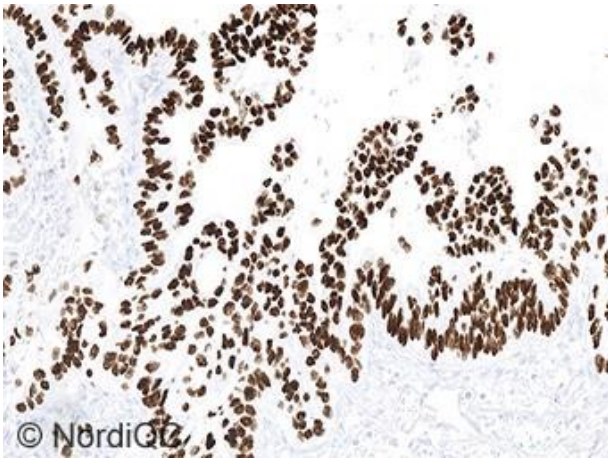


Fig. 3a
 Optimal TTF1 staining of the lung adenocarcinoma no. 7 (high expression of TTF1) using same protocol as in Figs. 1a & 2a.
 Virtually all the neoplastic cells show a strong and distinct nuclear staining reaction. No background staining is seen.

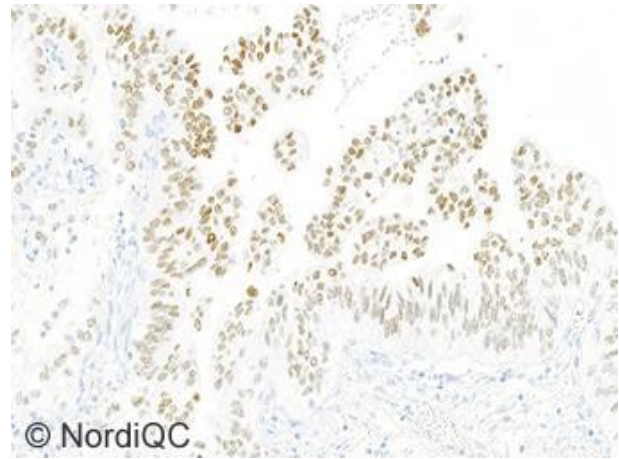


Fig. 3b
 TTF1 staining of the lung adenocarcinoma no. 7 using same insufficient protocol as in Figs. 1b & 2b.
 Despite being a tumour with high expression level of TTF1, the neoplastic cells show only a moderate nuclear staining reaction – same field as in Fig. 3a.

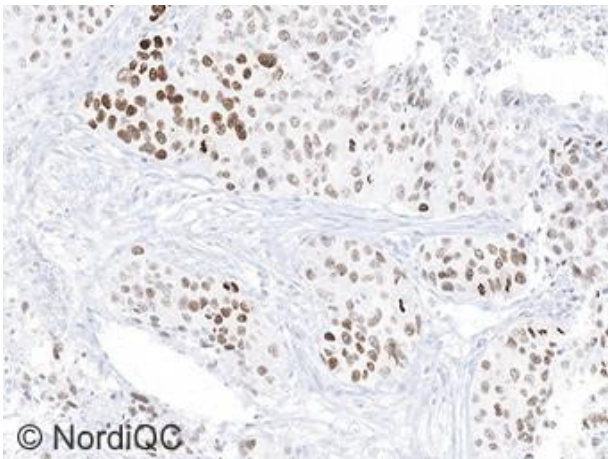


Fig. 4a
 Optimal TTF1 staining of the lung adenocarcinoma no. 4 with low level TTF1 expression using same protocol as in Figs. 1a, 2a & 3a.
 Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction.
 No background staining is seen.

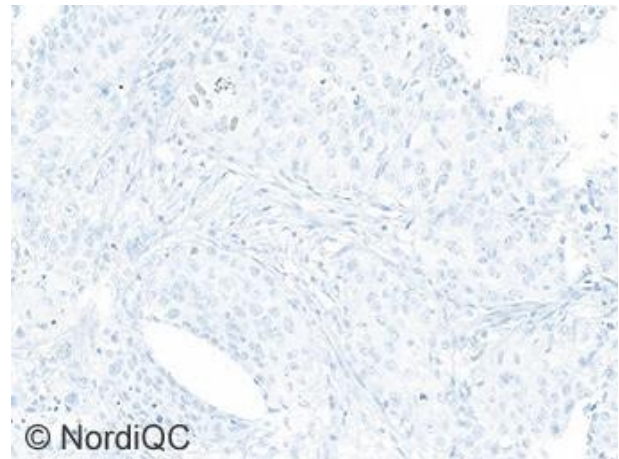


Fig. 4b
 Insufficient TTF1 staining of the lung adenocarcinoma no. 4 with low level TTF1 expression using same protocol as in Figs. 1b, 2b & 3b.
 Virtually all neoplastic cells are negative – same field as in Fig. 4a.

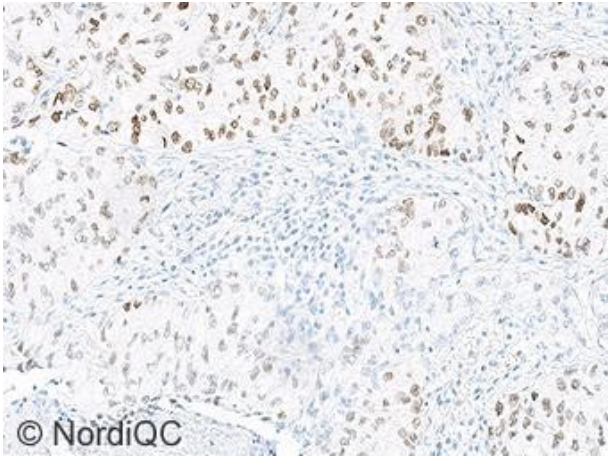


Fig. 5a.
Optimal TTF1 staining of the lung adenocarcinoma no. 4 using mAb clone SPT24 diluted 1:25 with an incubation time of 32 min, HIER in CC1 for 64 min and performed at the BechMark Ultra instrument (Ventana), using the UltraView (2-step multimer) detection system. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. Compare with Fig 5b.

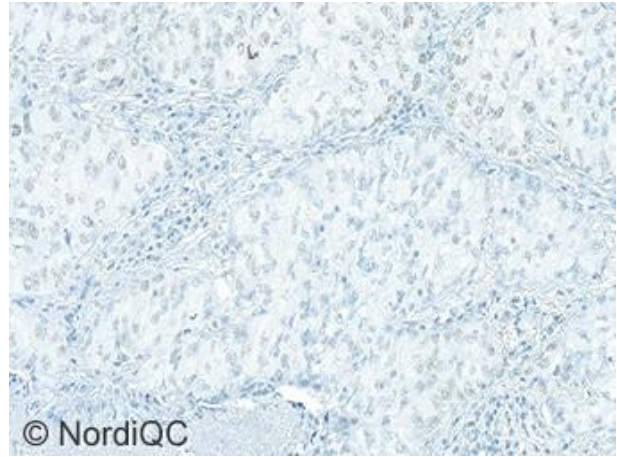


Fig. 5b.
Insufficient TTF1 staining of the lung adenocarcinoma no. 4. Same protocol as in Fig 5a. but with the use of **mAb clone SPT24 diluted 1:200**. The lower concentration of the primary Ab results in a negative staining reaction of virtually all neoplastic cells – same field as in Fig. 5a.

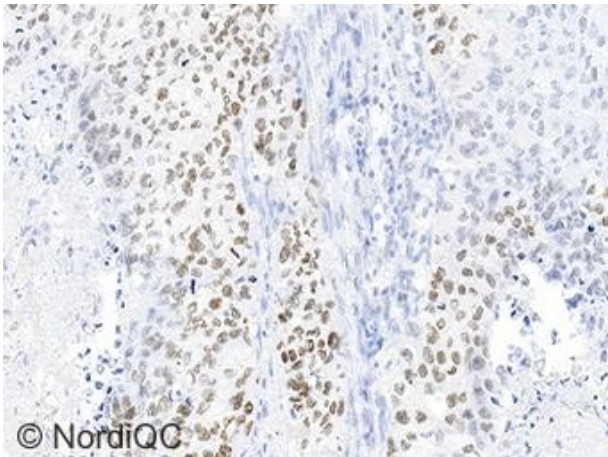


Fig. 6a.
Optimal TTF1 staining of the lung adenocarcinoma no. 4 using mAb clone SPT24 diluted 1:50 with an incubation time of 20 min, HIER in TRS pH 9 for 30 min and performed at the OMNIS instrument (Dako), using the EnVision FLEX (2-step polymer) detection system. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. Compare with Fig. 6b.

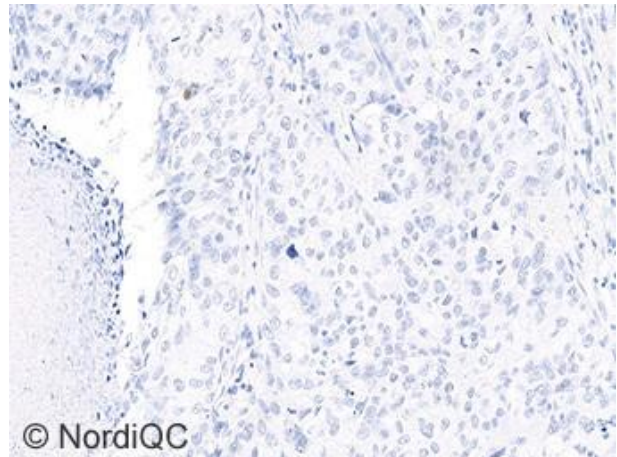


Fig. 6b.
Insufficient TTF1 staining of the lung adenocarcinoma no. 4. Same protocol as in Fig 6a. but with the use of **mAb clone SPT24 diluted 1:600**. The lower concentration of the primary Ab results in a negative staining reaction of virtually all neoplastic cells – same field as in Fig. 6a.

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