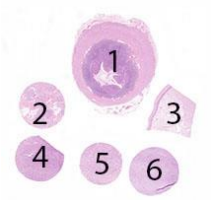
Assessment Run 72 2024

Trichorhinophalangeal syndrome 1 (TRPS1)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for TRPS1, typically used to identify breast carcinomas including triple negative breast carcinomas in the diagnostic work-up of carcinomas of unknown primary origin.
Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for TRPS1 (see below).

Material
The slide to be stained for TRPS1 comprised:



1. Appendix, 2. Breast hyperplasia, 3. Uterine cervix, 4. Non-small cell lung carcinoma (NSCLC) 5-6. Triple negative breast carcinoma (TNBC).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing TRPS1 staining as optimal included:

- A strong and distinct nuclear staining reaction of virtually all luminal epithelial cells in the breast hyperplasia.
- An at least weak to moderate distinct nuclear staining reaction in most suprabasal and intermediate squamous epithelial cells in the uterine cervix.
- An at least weak, but distinct nuclear staining reaction of the vast majority of neoplastic cells in the TNBC tissue core 5.
- An at least moderate to strong distinct nuclear staining reaction of the vast majority of neoplastic cells in the TNBC tissue core 6.
- No staining reaction of the appendiceal columnar epithelial cells and neoplastic cells in the NSCLC.
- A weak, nuclear staining reaction in scattered lymphocytes and ganglion cells in appendix was accepted.

KEY POINTS FOR TRPS1 IMMUNOASSAYS
- The rmAb clone **ZR382** is recommendable both as a concentrated Ab and a RTU.
- The widely used rmAb clone **EP392** and **pAb PA5-84874 from Invitrogen** seem less reproducible overall and more challenging to calibrate properly.
- IHC assays for TRPS1 must be calibrated carefully to provide desired diagnostic sensitivity and specificity as TRPS1 protein is expressed in many cells/neoplasias.

Participation

Number of laboratories registered for TRPS1, run 72	101
Number of laboratories returning slides	89 (88%)

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

Results
89 laboratories participated in this assessment. 65 (73%) achieved a sufficient mark (optimal or good), see Table 1a (see page 2). Tables 1b and 1c summarizes the antibodies (Abs) used and assessment marks (see page 2 and 3).

The most frequent cause of insufficient staining reactions were:
- Less successful primary Ab
- Inadequate calibration of primary Ab and/or overall too low analytical sensitivity/specificity of the IHC assay

Performance history
This was the first NordiQC assessment of TRPS1 and the overall pass rate was 73%.

Controls
Luminal epithelial cells in breast hyperplasia should show a strong nuclear staining reaction. In uterine cervix, most suprabasal squamous epithelial cells and dispersed stromal cells should display an at least weak to moderate nuclear staining reaction. No staining should be seen in epithelial cells in appendix. A weak to moderate, distinct nuclear staining reaction might be seen in ganglion cells and scattered lymphocytes in appendix. Smooth muscle cells should be negative or "as weak as possible".

Conclusion
The rmAb clone **ZR382** was the most successful Ab for the demonstration of TRPS1. As concentrated (conc.) format within a laboratory developed assay, optimal results were obtained on all main, fully automated stainer platforms. Efficient HIER in an alkaline buffer and carefully calibrated primary Ab together with a sensitive detection system were the most important prerequisites for a sufficient staining. The rmAb clone EP392 and pAbs gave overall an inferior and less reproducible performance.

Table 1a. **Overall results for TRPS1, run 72**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	59	22	20	12	5	71%	37%
Ready-To-Use antibodies	30	13	10	2	5	76%	43%
Total	89	35	30	14	10		
Proportion		39%	34%	16%	11%	73%	

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

Table 1b. **Concentrated antibodies and assessment marks for TRPS1, run 72**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **TRPS1/8131R**	1	ImmunoLogic	-	1	-	-	-	-
rmAb clone **BP6231**	1	Biolynx Biotechnology	1	-	-	-	-	-
rmAb clone **EP392**	16	Bio SB	7	4	3	2	69%	44%
	3	Epitomics	-	-	2	1	-	-
rmAb clone **EPR16171**	3	Abcam	2	-	-	1	-	-
	1	Diagnostic BioSystems	-	-	1	-	-	-
rmAb clone **MSVA-512R**	1	MS Validated Antibodies	-	1	-	-	-	-
rmAb clone **ZR382**	17	Zeta Corporation	12	4	1	-	94%	71%
Polyclonal	1	Atlas Antibodies	-	1	-	-	-	-
	1	Gennova	-	-	-	1	-	-
	14	Invitrogen, PA5-84874	-	9	5	-	64%	0%
Total	59		22	20	12	5		
Proportion			37%	34%	20%	9%	71%	

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

Table 1c. **Ready-To-Use antibodies and assessment marks for TRPS1, run 72**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **EP392 BSB-3790-x**	7	Bio SB	1	1	1	4	29%	14%
rmAb clone **EPR16171 GT245402**	1	Gene Tech	1	-	-	-	-	-
rmAb clone **MXR063 RMA-1141**	1	Fuzhou Maixin	-	1	-	-	-	-
rmAb clone **ZR382 8359-C010**	1	Sakura Finetek	1	-	-	-	-	-
rmAb clone **ZR382 Z2673RP**	18	Zeta Corporation	9	8	-	1	94%	50%
Ab clone **506I3C7 PA588**	1	abcarta	-	-	1	-	-	-
Ab clone **BY193 BFM-0560**	1	Bioin Biotechnology	1	-	-	-	-	-
Total	30		13	10	2	5		
Proportion			43%	33%	7%	17%	76%	

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

Detailed analysis of TRPS1, Run 72

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

rmAb clone **EP392**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS, Dako/Agilent) High pH (6/9)*, TRS Low pH (Dako/Agilent) (1/1) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (1/7) as retrieval buffer. The rmAb was typically diluted in the range of 1:50-1:500. Using these protocol settings, 11 of 17 (65%) laboratories produced a sufficient staining result (optimal or good).
*(number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **ZR382**: Protocols with optimal results were based on HIER using TRS High pH (Dako/Agilent) (1/1), Cell Conditioning 1 (CC1, Ventana/Roche) (10/13) or BERS2 (Leica Biosystems) (1/2) as retrieval buffer. The mAb was diluted in the range of 1:50-1:1.000. Using these protocol settings, 15 of 16 (94%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for TRPS1 for the most commonly used antibody concentrates on the four 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
rmAb clone **EP392**	2/3**	-	4/6 (67%)	-	0/2	-	1/7 (14%)	-
rmAb clone **ZR382**	-	-	1/1	-	10/13 (77%)	-	1/2	0/1

* 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 Link 48.
2) BenchMark XT, Ultra, Ultra plus
3) Bond III

Ready-To-Use antibodies and corresponding systems

No Ready-To-Use Abs with a corresponding system (≥5 assessed protocols) were giving optimal results in this assessment.

Comments

In this assessment, the prevalent feature of an insufficient result was a too weak or completely false negative staining reaction of cells and structures expected to be positive. This pattern was observed in 63% of the insufficient results (15 of 24). Virtually all laboratories were able to demonstrate TRPS1 in high-level antigen expressing structures such as neoplastic cells of the TNBC, tissue core no 6, and luminal epithelial cells in the breast hyperplasia. Demonstration of TRPS1 in low-level expressing structures as neoplastic cells of the TNBC, tissue core no 5, was more challenging and required a carefully calibrated protocol. 29% (7 of 24) of the insufficient results was caused by a poor signal-to-noise ratio or an excessive background staining reaction. 8% (2 of 24) of the insufficient results were caused by a false

positive staining reaction in cells and structures expected to be negative, e.g. smooth muscle cells and neoplastic cells in the NSCLC included in the TMA.

66% (59 of 89) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for TRPS1. The most successful **rmAb clone ZR382** was used by 17 participants, giving a pass rate of 93%, 71% optimal (see Table 1b). Optimal results could be obtained on the main fully automated platforms from Dako/Agilent, Ventana/Roche and Leica Biosystems (see Table 2). The main prerequisites for sufficient staining were use of HIER in an alkaline buffer and careful calibration of the titre of the primary Ab.

The **rmAb clone EP392** was the most widely used antibody for demonstration of TRPS1 as a concentrate, and gave an overall pass rate of 69%, 44% optimal. The main prerequisites for sufficient staining were careful calibration of the titre of the primary Ab and use of a 3-step detection system. However, it was observed that despite similar protocol settings were applied by the participants, both sufficient and insufficient results were obtained, and the insufficient results being caused by either false negative or false positive staining reactions. No cause for this irreproducibility has been found in the submitted data.

16 laboratories used a polyclonal Ab, giving an overall pass rate of 64%, but no optimal. Of particular notice, it was observed that the frequently cited Ab, pAb PA5-84874 Invitrogen being used by 14 participants gave an overall inferior performance caused by a poor-signal-to-noise ratio compromising the read-out for TRPS1. The protocol settings for the pAbs were similar to the rmAbs above with HIER in an alkaline buffer and a 3-step detection system. 31% (5 of 16) gave an aberrant primarily cytoplasmic staining reaction in nerves and smooth muscle cells in appendix. No single cause for this staining pattern has been found in the submitted data. In this context, different lot numbers in combination with different protocol settings might have impacted the inferior performance of the pAbs especially the PA5-84874. Internal NordiQC studies have revealed lot-to-lot variation of this Ab and only by careful calibration of the Ab and applying an Ab diluent with background reducing capacity as "Background sniper" (Biocare Medical) a successful result was obtainable[1].

The RTU format of the **rmAb clone ZR382** (**Z2673RP**) from Zeta Corporation gave a high proportion of sufficient and optimal results as shown in Table 1c. Optimal and sufficient results were seen on the main platforms from Dako/Agilent, Ventana/Roche and Leica Biosystems, with similar protocol settings as for the concentrated format.

Overall, for both concentrated and RTU Abs, the rmAb clone ZR382 gave a superior performance with a pass rate of 94% (34 of 36), 61% optimal (n=22) compared to the rmAb clone EP392 with a 50% pass rate (13 of 26), 31% optimal (n=8).

An aberrant, typically granular, cytoplasmic staining reaction was seen in 13% (12 of 89) of the laboratories using various clones/Abs. The aberrant staining pattern was typically seen for rmAb clone EP392 (n=5) and for polyclonal Abs (n=4). In appendix, ganglion cells and axon in the muscle layer showed an extensive granular cytoplasmic staining reaction, and in few cases, a cytoplasmic staining reaction was observed in epithelial cells. Comparable protocol settings were applied to optimal protocols, and no single cause for this staining pattern has been found in the submitted data as it was seen with various products and protocols.

This was the first NordiQC assessment of TRPS1 as an IHC assay to identify breast carcinomas including triple negative breast carcinomas in the diagnostic work-up of carcinomas of unknown primary origin. A satisfactory pass rate of 73% was obtained, but the assessment and newer publications also revealed that the IHC assays for TRPS1 must be carefully calibrated, as TRPS1 is expressed in many cells and corresponding neoplasias, to give the expected diagnostic sensitivity and specificity for breast carcinomas. In this assessment we included a NSCLC verified to be TRPS1 negative by the NordiQC reference standard method based on clone MSVA-512R, MS Validated antibodies, but recent publications do indicate that TRPS1 is expressed in many non-breast neoplasias as e.g. gynaecological and lung carcinomas, despite typically at a reduced expression level[2,3]. This multi-origin expression profile must be considered when optimizing and validating IHC assays for TRPS1.

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[1]Truumees B. et al. TRPS1 in Breast Cancer: A comparative study of five different IHC assays. Abstract: 36th European Congress of Pathology – Abstracts. Virchows Arch 485 (Suppl 1), 1–546 (2024)

[2]Lennartz M. et al. TRPS1 is a Highly Sensitive Marker for Breast Cancer: A Tissue Microarray Study Evaluating More Than 19,000 Tumors From 152 Different Tumor Entities. Am J Surg Pathol. 2024 Jun 1;48(6):637-651

[3] Rammal R. et al. Utility of TRPS1 immunohistochemistry in confirming breast carcinoma: Emphasis on staining in triple-negative breast cancers and gynecologic tumors. Am J Clin Pathol. 2023 Oct 3;160(4):425-434.

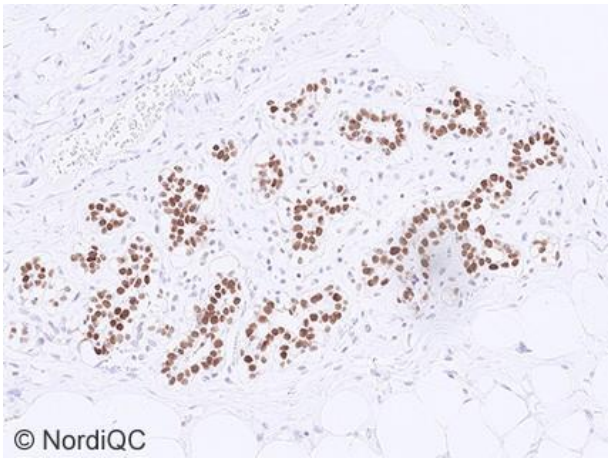

Fig. 1a
Optimal TRPS1 staining reaction of the breast hyperplasia using the rmAb clone ZR382 in a concentrated format (1:100), using HIER at high pH for 64 min., 32 min. incubation of the primary Ab, OptiView as detection system and performed on BenchMark Ultra.
The epithelial cells show an intense, distinct nuclear staining reaction.
Also compare with Figs. 2a - 7a – same protocol.

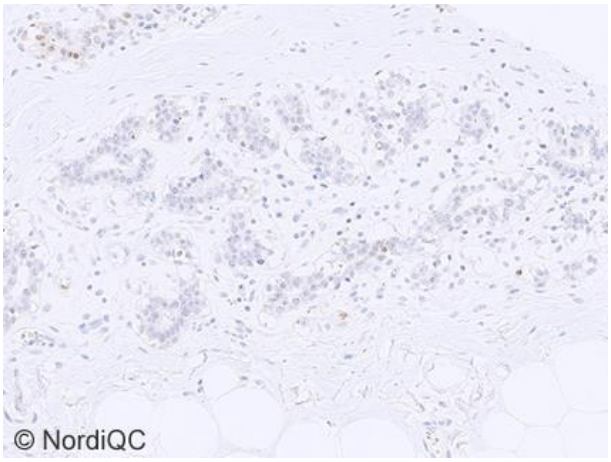

Fig. 1b
Insufficient TRPS1 staining reaction of the breast hyperplasia using a protocol not calibrated appropriately, being false negative in the vast majority of epithelial cells.
The protocol was based on the rmAb clone EP392 as a RTU format using HIER at high pH for 92 min., 100 min. incubation of the primary Ab, OptiView with Amplification as detection system and performed on BenchMark Ultra.
Also compare with Figs. 2b - 5b – same protocol.

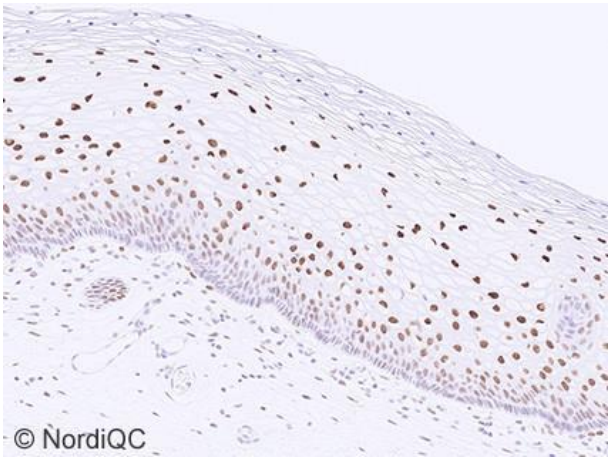

Fig. 2a
Optimal TRPS1 staining reaction of the uterine cervix using same protocol as in Fig. 1a. The suprabasal and intermediate squamous epithelial cells show a weak to moderate, distinct nuclear staining reaction. Dispersed stroma cells are demonstrated.
Also compare with Figs. 3a - 7a – same protocol.

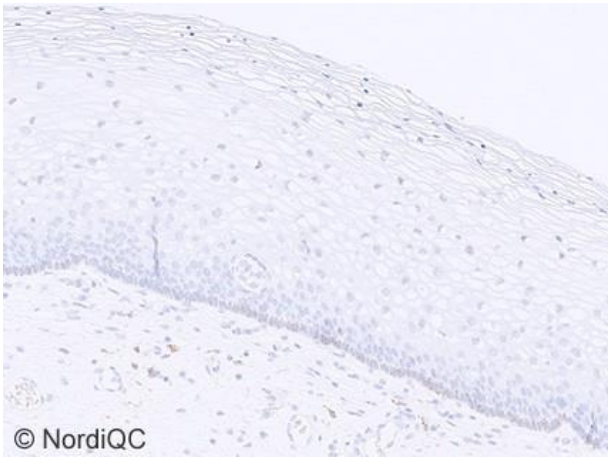

Fig. 2b
Insufficient TRPS1 staining reaction of the uterine cervix using same protocol as in Fig. 1b – same field as in Fig. 2a.
The squamous epithelial cells are false negative.
Also compare with Figs. 3b - 5b – same protocol.

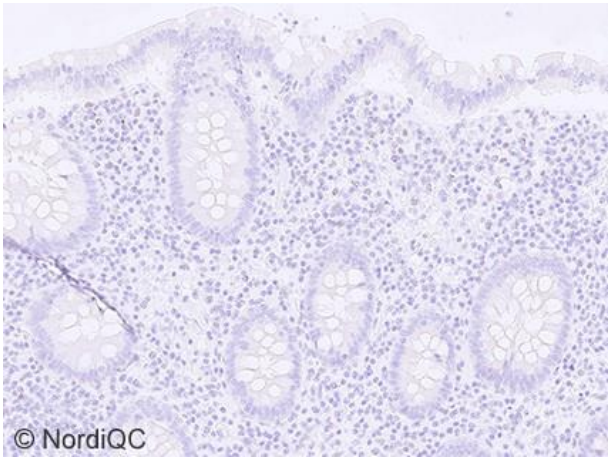

Fig. 3a
Optimal TRPS1 staining reaction of the appendix using same protocol as in Figs. 1a and 2a.
No staining reaction is seen in the epithelial cells. Scattered lymphocytes show a faint nuclear staining reaction.
Also compare with Figs. 4a - 7a – same protocol.

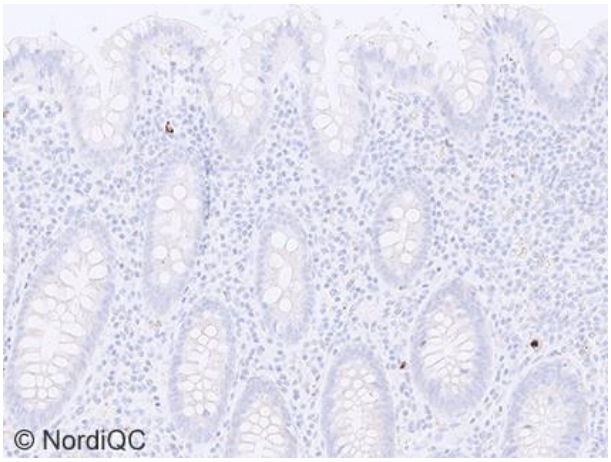

Fig. 3b
TRPS1 staining reaction of the appendix using the same insufficient protocol as in Figs. 1b and 2b – same field as in Fig. 3a. No staining reaction is observed in the epithelial cells, whereas a strong staining reaction is seen in scattered lymphocytes.
Also compare with Figs. 4b and 5b – same protocol.

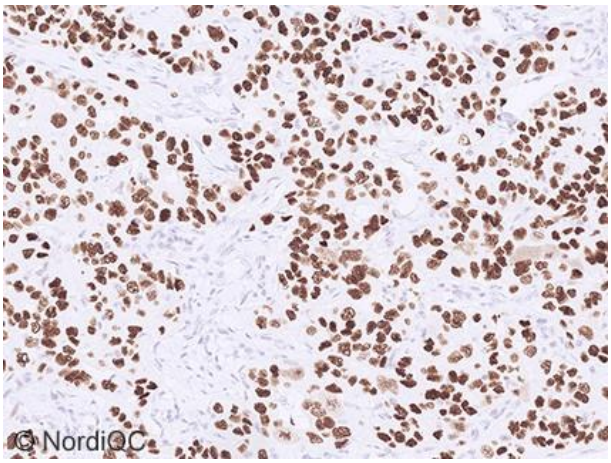

Fig. 4a
Optimal TRPS1 staining reaction of TNBC, tissue core no. 6, using same protocol as in Figs. 1a – 3a.
Virtually all neoplastic cells show a moderate to strong, distinct nuclear staining reaction.
Also compare with Figs. 5a - 7a – same protocol.

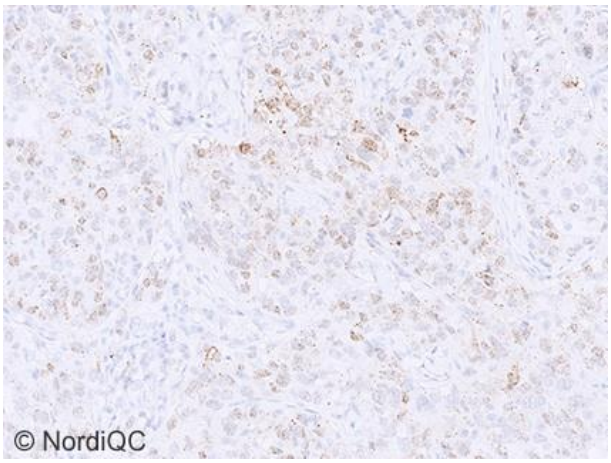

Fig. 4b
Insufficient TRPS1 staining reaction of the TNBC, tissue core no. 6, using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
The vast majority of neoplastic cells are negative, and an aberrant, weak, granular cytoplasmic staining reaction is observed.
Also compare with Fig. 5b – same protocol.

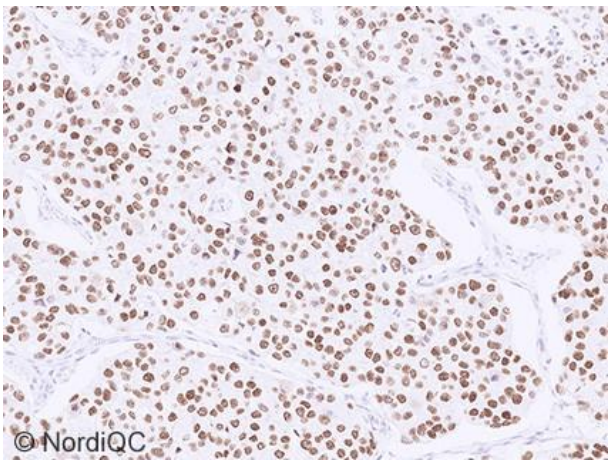

Fig. 5a
Optimal TRPS1 staining reaction of the TNBC, tissue core no. 5, using same protocol as in Figs. 1a – 4a.
Virtually all neoplastic cells show a weak to moderate, distinct nuclear staining reaction.
Also compare with Figs. 6a and 7a – same protocol.

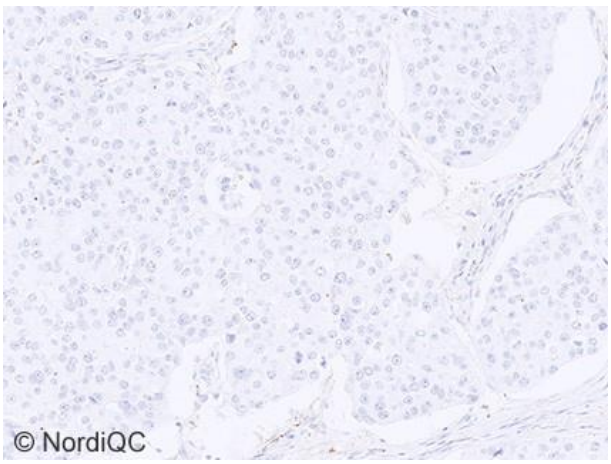

Fig. 5b
Insufficient TRPS1 staining reaction of the TNBC, tissue core no. 5, using same protocol as in Figs. 1b - 4b.
No staining reaction is seen. Compare with optimal result in Fig. 5a.

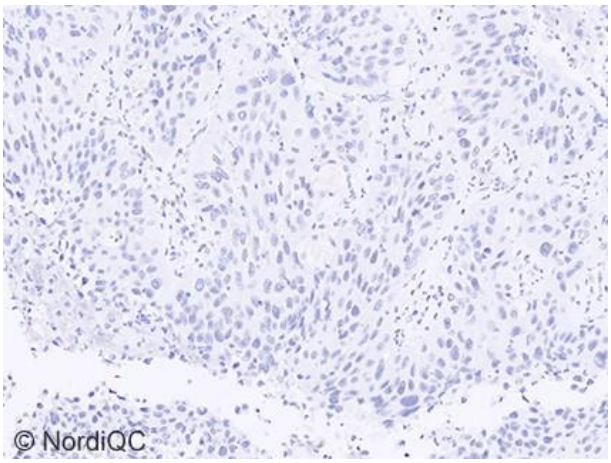

Fig. 6a
Optimal TRPS1 staining reaction of the NSCLC using same protocols as in Figs. 1a – 5a.
No staining reaction is seen.

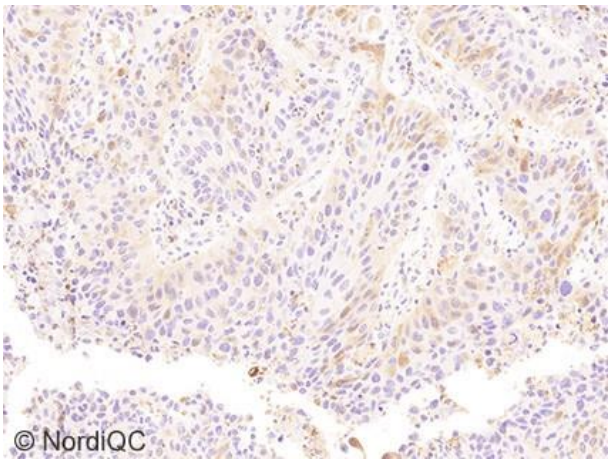

Fig. 6b
Insufficient TRPS1 staining of the NSCLC, using the rmAb clone EP392 as a concentrated format (1:150) using HIER at high pH for 20 min., 20 min. incubation of the primary Ab, on a Bond III.
An aberrant, primarily cytoplasmic staining reaction is observed in the vast majority of neoplastic cells.
Compare with optimal result in Fig. 6a.

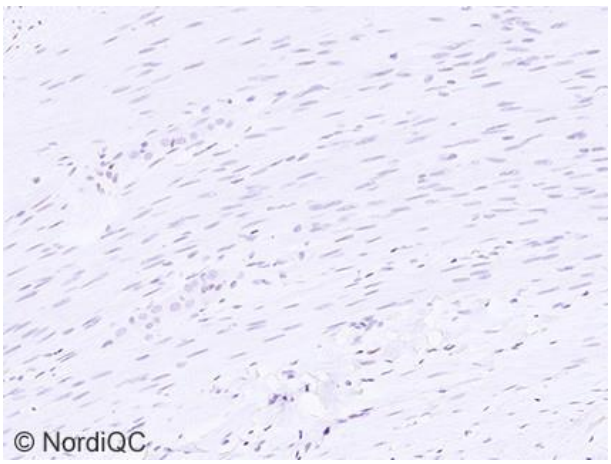

Fig. 7a
Optimal TRPS1 staining reaction of the muscle layer in appendix using same protocols as in Figs. 1a – 6a. A faint nuclear staining reaction can be observed in ganglion cells, while smooth muscle cells are negative.

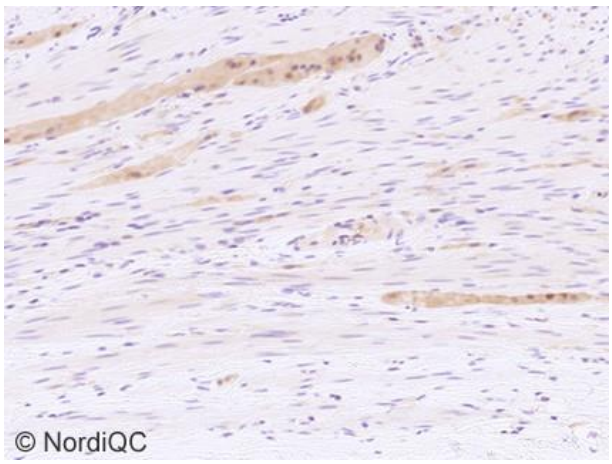

Fig. 7b
Insufficient TRPS1 staining reaction of the muscle layer in appendix, using same protocol as in Fig. 6b.
An aberrant, primarily cytoplasmic staining reaction is observed in muscle cells and ganglion cells. Compare with optimal result in Fig. 7a.

HLK/LE/SN 20.11.2024