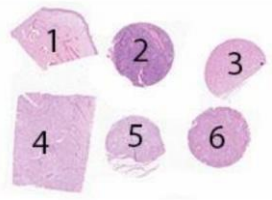
Assessment Run 70 2024
GATA3

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for GATA3, typically identifying urothelial and breast carcinomas in the diagnostic work-up of cancer of unknown primary (CUP) origin. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for GATA3 (see below).

Material
The slide to be stained for GATA3 comprised:



1. Uterine cervix 2. Tonsil 3. Kidney, 4. Breast carcinoma (TNBC), 5. Urothelial carcinoma, 6. Non Small Cell Lung Carcinoma (NSCLC)

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a GATA3 staining as optimal included:

- A weak to moderate, distinct nuclear staining reaction in the majority of squamous epithelial cells situated in the basal and intermediate compartment of the surface epithelium in the uterine cervix.
- An at least moderate, distinct nuclear staining reaction of virtually all podocytes (renal glomeruli) and of epithelial cells in the collecting ducts of the kidney.
- An at least moderate, distinct nuclear staining reaction of virtually all T helper cells in all specimens, in particular Th2 cells in T-zones of the tonsil and dispersed T-cells in the NSCLC.
- A weak to strong, distinct nuclear staining reaction of the majority of neoplastic cells in the breast ductal carcinoma.
- An at least moderate, distinct nuclear staining reaction of the vast majority of neoplastic cells in the urothelial carcinoma.
- No staining reaction of the neoplastic cells in the NSCLC and of the squamous epithelial cells in the tonsil.

A weak cytoplasmic background was accepted e.g in the epithelial cells in the tubuli of the kidney as long as the interpretation was not compromised.

KEY POINTS FOR GATA3 IMMUNO ASSAYS
- The widely used mAb clone **L50-823** is recommendable both as concentrated and RTU.
- 3-step detection systems are mandatory for optimal performance
- Uterine cervix and tonsil are recommendable positive and negative tissue controls

Participation

Number of laboratories registered for GATA3, run 70	422
Number of laboratories returning slides	390 (92%)

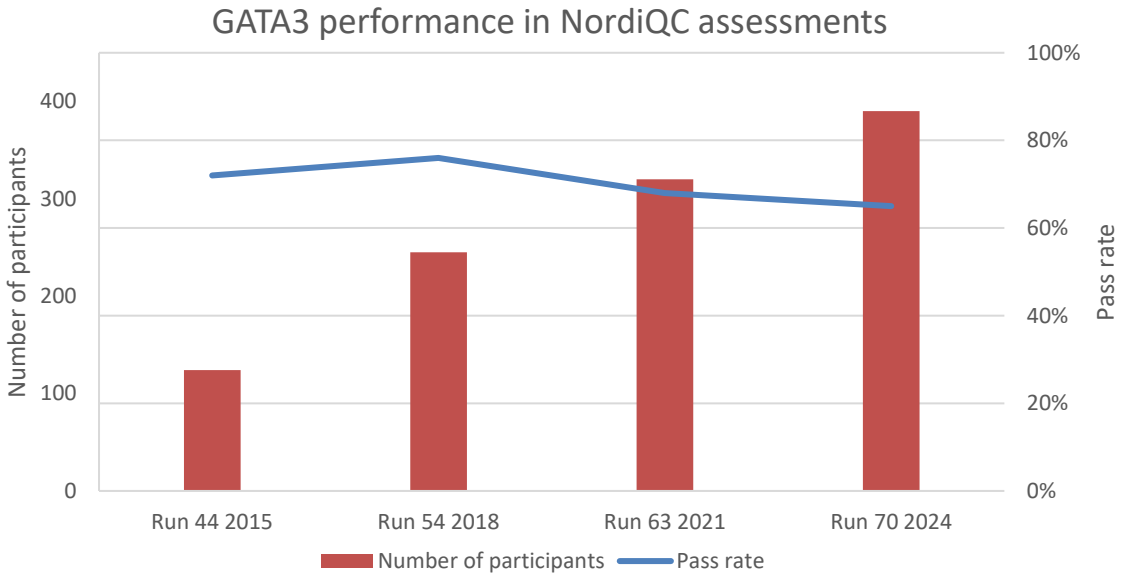
Results
At the date of assessment, 92% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

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

The most frequent causes of insufficient staining were:
- Less sensitive detection systems used in combination with other low sensitivity protocol parameters.
- Too low concentration of the primary antibody or too short incubation time.
- Inefficient Heat Induced Epitope Retrieval (HIER) – too short time or use or acidic buffer.

Performance history
This was the fourth NordiQC assessment of GATA3. A pass rate of 65% was observed, which was slightly lower compared to the previous run 63, 2021.

Graph 1. **Proportion of sufficient results for GATA3 in the four NordiQC runs performed**



Controls
Uterine cervix and tonsil are recommended as positive and negative tissue controls for GATA3. In uterine cervix a weak to moderate staining reaction in the majority of the basal and intermediate squamous epithelial cells must be seen whereas the superficial epithelial cells and stroma cells must be negative. In the tonsil the vast majority of T helper cells (Th2) in the T-zones must show an at least moderate but distinct nuclear staining reaction. No staining of B-cells should be seen.

Conclusion
The mAb clone **L50-823** and the rmAb clones **EP368** and **QR018** could all be used for an optimal demonstration of GATA3. The vast majority of participants used the mAb clone L50-823 either within a laboratory developed (LD) assay or as a Ready-to-use (RTU) format. Used within a LD assay, optimal results could be obtained on all four main IHC systems (Dako Autostainer, Dako Omnis, Ventana BenchMark and Leica Bond). Efficient HIER, preferable in an alkaline buffer, careful calibration of the primary antibody and use of a 3-layer detection system were the most important prerequisites for optimal staining results.
The RTU system 760-4897 (Ventana) also based on the mAb clone **L50-823** provided a high proportion of sufficient and optimal results, especially if OptiView (760-700) was used as detection system.

Table 1a. **Overall results for GATA3, run 70**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	135	35	47	40	13	60%	26%
Ready-To-Use antibodies	255	120	52	72	11	68%	47%
Total	390	155	99	112	24		
Proportion		40%	25%	29%	6%	65%	

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

Table 1b. **Concentrated antibodies and assessment marks for GATA3, run 70**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **L50-823**	87 21 3 1 4 2 2 1 6	Cell Marque BioCare Bio SB BD Pharmingen Zytomed Systems Gennova Immunologic Diagnostic Bio DBS	30	46	39	12	60%	24%
mAb clone **HG3-31**	1	Santa Cruz	-	-	-	1	-	-
rmAb clone **EP368**	4 1	Cell Marque Quartett	4	1	-	-	100%	80%
rmAb clone **ZR358**	1	Thermo Fisher Scientific	-	-	1	-	-	-
rmAb clone **QR018**	1	Quartett	1	-	-	-	-	-
Total	135		35	47	40	13		
Proportion			26%	35%	30%	9%	60%	

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 GATA3, run 70**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **L50-823 760-4897**[3]	30	Ventana/Roche **UltraView**, 760-500	1	3	26	-	13%	3%
mAb clone **L50-823 760-4897**[3]	44	Ventana/Roche **OptiView**, 760-700	34	7	2	1	93%	77%
mAb clone **L50-823 760-4897**[4]	33	Ventana/Roche **UltraView**	8	6	18	1	42%	24%
mAb clone **L50-823 760-4897**[4]	58	Ventana/Roche **OptiView**	43	9	5	1	90%	74%
mAb clone **L50-823 760-4897**	4	Ventana/Roche Other platform	-	1	3	-	-	-
mAb clone **L50-823 390M-17,18,10**	57	Cell Marque	24	16	13	4	70%	42%
mAb clone **L50-823 PM 405AA**	11	BioCare Medical	5	4	2	-	82%	46%
mAb clone **L50-823 MAD-000632QD**	6	Master Diagnostica Vitro SA	1	4	-	1	83%	17%
mAb clone **L50-823 HAM199**	1	Path N Situ	-	-	1	-	-	-
rmAb clone **QR018, 8357-C010**	1	Sakura	1	-	-	-	-	-
mAb clone **L50-823, BMS054**	5	Zytomed systems	-	-	2	3	0%	0%
mAb clone **DA060, RMB1A070**	1	Dartmon	1	-	-	-	-	-
rmAb clone **2555B6B8 PA077**	1	Abcarta	-	1	-	-	-	-
rmAb clone **EP368, RMA-1067**	2	Fuzhou Maixin	1	1	-	-	-	-
rmAb clone **EP368, I12012E-05**	1	BioLynx Biotechnology	1	-	-	-	-	-
Total	255		120	52	72	11		
Proportion			47%	20%	28%	4%	68%	

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).
5) Product used on another platform than developed for

Detailed analysis of GATA3, Run 70
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **L50-823**: Protocols with optimal results were all based on HIER in an alkaline buffer using Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (10/26)*, Cell Conditioning 1 (CC1, Ventana/Roche) (14/41), Target Retrieval Solution (TRS) pH 9 (Dako/Agilent) (5/37) and TRS High (3-in-1) (Dako/Agilent) pH 9 (1/11) as retrieval buffer. The mAb was typically diluted in the range of 1:50-400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 65 of 103 (63%) laboratories produced a sufficient result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP368**: Protocols with optimal results were based on HIER using TRS pH 9 (3/3) and TRIS-EDTA/EGTA pH 9 (1/1) as retrieval buffer. The rmAb was diluted in the range of 1:100-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 5 of 5 (100%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for GATA3 for the most commonly used antibodies as concentrate on the four main IHC systems***

Concentrated antibody	**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 **L50-823**	1/11** (9%)	0/2	5/37 (14%)	0/3	14/41 (34%)	-	10/26 (39%)	0/4
rmAb clone **EP368**	0/1	-	3/3	-	-	-	-	-

* 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, XT, Ultra, Ultra plus
3) Bond III

Ready-To-Use antibodies and corresponding systems
mAb clone **L50-823**, product no. **760-4897**, Ventana, BenchMark XT, ULTRA, ULTRA Plus:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 24-64 min.), 16-48 min. incubation of the primary Ab and UltraView with or without amplification (760-500/760-080) or OptiView with or without amplification (760-700/760-099) as detection system. Using these protocol settings, 80 of 142 (56%) laboratories produced a sufficient staining result.

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 according 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 GATA3 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/Plus/XT mAb **L50-823 760-4897 UltraView**	13% (4/30)	3% (1/30)	42% (14/33)	24% (8/33)
VMS Ultra/Plus/XT mAb **L50-823 760-4897 OptiView**	93% (41/44)	77% (34/44)	90% (52/58)	74% (43/58)

* 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, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments
In this fourth NordiQC assessment for GATA3, the prevalent feature of an insufficient staining result was a too weak or false negative staining reaction of the cells expected to be demonstrated. This pattern was seen in 89% of the insufficient results (121 of 136 laboratories). Too weak staining result was characterized by a reduced staining reaction both in regard to the intensity and proportion of cells expected to be demonstrated. The remaining insufficient results were caused by poor-signal too noise ratio or excessive counterstaining compromising interpretation (Figs. 5a and 5b). Virtually all laboratories were able to demonstrate GATA3 in high-level antigen expressing cells, such as neoplastic cells of the urothelial carcinoma, the epithelial cells of the collecting ducts and podocytes in glomeruli of the kidney. However, demonstration of GATA3 in low-level antigen expressing cells as normal T helper cells (Th2) (all specimens), the neoplastic cells of the breast carcinoma or squamous epithelial cells situated in the basal and intermediate layer of the surface epithelium of the uterine cervix was more challenging and required optimally calibrated protocols.

135 laboratories used concentrated antibodies within LD-assays, providing a pass-rate of 60% (82/135) of which 26% (35/135) were assessed as optimal. Optimal results could be obtained using the mAb clone L50-823 or the rmAb clones EP368 and QR018. However, the mAb clone L50-823 was by far the most applied antibody for demonstration of GATA3 and used by 94% (127/135) of the laboratories using a concentrated format. As shown in Table 2, this antibody clone gave optimal results on all main IHC platforms (see Table 2).

The performance of the mAb **L50-823** was as in previous runs influenced by the company/distributor of the primary Ab among the concentrated formats. In this assessment, 17% (21 of 127) and 69% (87 of 127) of the laboratories purchased the Ab from Biocare or Cell Marque, respectively. Using similar protocol settings, and applying the mAb L50-823 from Biocare, the overall pass rate was only 38% (8 of 21) of which 14% (3 of 21) were assessed as optimal, whereas the proportion of sufficient results were 67% (57 of 87) of which 28% (24 of 87) were giving an optimal mark if laboratories used the primary Ab from Cell Marque. The discrepancy in performance observed between the two products of the mAb clone L50-853 is difficult to elucidate upon and can be related to different parameters. The inferior performance could be related to a lower anti-GATA3 immunoglobulin fraction in the Biocare product compared to the product from Cell Marque or other manufacturing differences impeding the antibody affinity. In this aspect, the average dilution factor for a sufficient result was 1:100 and 1:180 for the Biocare and Cell Marque L50-823 product, respectively.

In previous runs it was observed that the Cell Marque **L50-823** product provided an optimal result with all commercially available antibody diluents, whereas the Biocare L50-823 product required the use of a low pH diluent as Van Gogh or Renoir Red (Biocare). Only 3 laboratories used a low pH diluent with the concentrate from Biocare all 3 receiving a sufficient result.

As mentioned in previous reports, parameters as efficient HIER in an alkaline buffer in combination with a careful calibration of the primary Ab was critical for optimal performance of mAb clone **L50-823**. In addition, the choice of detection system also impacted the overall performance of the assays. Using optimal protocol settings as described above, a pass rate of 0% (0/4) was observed for protocols based on 2-step multimer/polymer detection systems (e.g., UltraView, Ventana/Roche or EnVision Flex, Dako/Agilent). In comparison, the pass rate was 63% (65 of 104) of which 26% (27 of 104) were optimal if protocols were based on a 3-step multimer/polymer detection system (e.g., UltraView + Amplification, OptiView, Ventana/Roche or EnVision Flex+, Dako/Agilent).

In previous runs it was observed that using OptiView was superior to using UltraView with amplification, but in this assessment that pass rate of the two different 3-layer methods was similar with a pass rate of 75% for both settings.

Table 4 summarizes the proportion of sufficient and optimal marks for the mAb clone L50-823 using either 2- or 3-layer detection systems regardless of the protocol settings applied e.g., HIER time and/or incubation time in the primary Ab (≥10 protocols assessed) and primary Ab format (conc. or RTU).

Table 4. **Summarization of the proportion of sufficient and optimal marks using either 2- or 3-layer detection systems**.**

		2-layer detection system		3-layer detection system	
Antibodies	**n**	**Sufficient**	**Optimal**	**Sufficient**	**Optimal**
mAb conc **L50-823** Cell Marque	87	0% (0/4)	0% (0/4)	69% (57/83)	29% (24/83)
mAb conc **L50-823** Biocare	21	-	-	38% (8/21)	14% (3/21)
mAb clone RTU **L50-823 760-4897*** Ventana/Roche	169	14% (7/51)	2% (1/51)	89% (105/118)	72% (85/118)
mAb clone RTU **L50-823 390M-17,18,10** Cell Marque	57	46% (6/13)	15% (2/13)	77% (34/44)	55% (24/44)
mAb clone RTU **L50-823 PM 405AA** Biocare	11	-	-	82% (9/11)	45% (5/11)

*Only protocols performed on the intended IHC stainer device are included.

Although the number of participants using the rmAb clone **EP368** was low, the antibody provided a relative high proportion of optimal results (80%, 4 of 5) primarily on the platforms from Dako/Agilent. One optimal protocol was obtained on the Gene Tech GeneStainer. As for the mAb clone L50-823, assays based on EP368 require protocol settings providing appropriate level of analytical sensitivity and specificity, which for the rmAb clone EP368 implied use of HIER in an alkaline buffer, a typical dilution range of 1:100-200 and use of a 3-step polymer detection system.

RTU formats were used by 65% (255 of 390) of the laboratories providing a pass rate of 68%, 47% being optimal. The only "true" RTU system with more than 5 protocols assessed was the product **760-4897** from Ventana/Roche based on the mAb clone **L50-823** and obtained among all other assays, both LD-assays and RTU formats, the highest pass rate in the assessment (see Table 1c).

According to the instructions giving by the vendor (Ventana/Roche), both UltraView and OptiView can be used as detection systems. Applying vendor recommended protocol settings based on OptiView, HIER in CC1 for 32 min. and incubation in the primary Ab for 32 min., the pass-rate was 93%, 77% being optimal. However, and using the vendor recommendations based on UltraView, HIER in CC1 for 64 min. and incubation in the primary ab for 32 min., the proportion of sufficient results declined significantly to 13% (4 of 30) and only one optimal result was achieved. As shown in Table 1c and 3, 55% (91/165) of the laboratories applied laboratory modified protocol settings to the Ventana/Roche RTU system typically adjusting HIER time, incubation time in the primary Ab and/or choice of detection system, giving nearly identical results compared to vendor recommended protocol settings. Four laboratories used this clone on a non-intended platform with mixed results. In general, the choice of detection system was very important as mentioned above and for the RTU system 760-4897, the proportion of optimal results was considerably higher using a 3-layer multimer detection system instead of a 2-step multimer detection system, 72% and 2%, respectively, regardless of other protocol settings applied e.g., HIER time in CC1 and/or incubation time in the primary Ab.

The Ready-to-Use products from Cell Marque (mAb clone **L50-823**, product no. **390M-17, -18, -10**) and Biocare (mAb clone **L50-823**, product no. **PM 405AA**) had very similar pass rates. The challenges regarding choice of titre, diluent and as such inferior performance for the concentrated format from Biocare was not seen for the corresponding RTU product with optimal protocol settings. Both RTU products could produce optimal results on the four main IHC platforms. However, these two RTU formats are developed and validated by "third-party" IHC provider and not within a total Ready-To-Use system and thus, laboratories are obligated to optimize protocols and validate assay performance in relation to the

applied in-house platform(s) and more importantly, to relevant clinical samples displaying a broad spectrum of antigen densities for GATA3 and to critical staining indicators as described for the controls.

This was the fourth assessment of GATA3 in NordiQC (see Graph 1). The pass rate declined slightly in this run compared to the latest run 63, 2021. The number of participants increased with 22% and most of these new users applied a RTU format. The reduced pass rate in this run was in particular related to the LD assays based on the concentrated format of mAb clone L50-823 and extended use of RTU formats in combination with 2-layer detection systems providing a too low level of analytical sensitivity.

In this assessment the included breast carcinoma was a triple negative breast tumor harboring lower expression level of GATA3 compared to the breast carcinoma (high expressing level of GATA3) used in run 54. Combined with the tonsil and uterine cervix these three tissue samples were critical indicators of the analytical sensitivity of the protocol applied (Figs. 1a-3a). Kidney is less useful as an indicator of an optimally calibrated protocol as it might not unravel lack of analytical sensitivity due to the high level of GATA3 seen in both podocytes and epithelial cells of the collecting ducts (Figs. 4a and b).

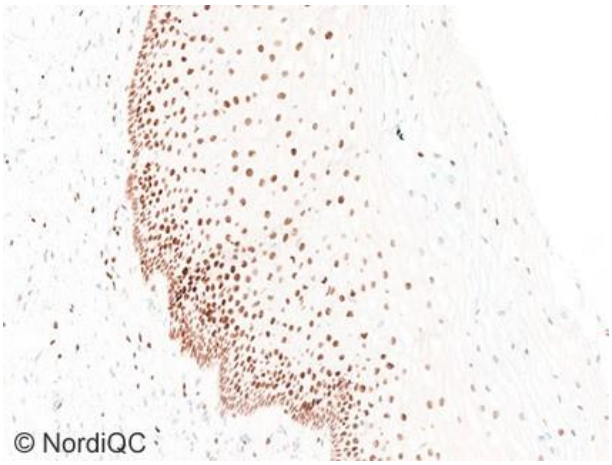


Fig. 1a (x100)
Optimal GATA3 staining reaction of the uterine cervix using the RTU system 760-4897 (Ventana/Roche), based on the mAb clone L50-823, applying vendor recommended protocol settings and OptiView as detection system. The squamous epithelial cells in the basal and intermediate layer of the surface epithelium display a weak to moderate, but distinct nuclear staining reaction, whereas the nuclei of superficial layers and stroma cells are negative. Same protocol settings as in Figs. 2a-4a.

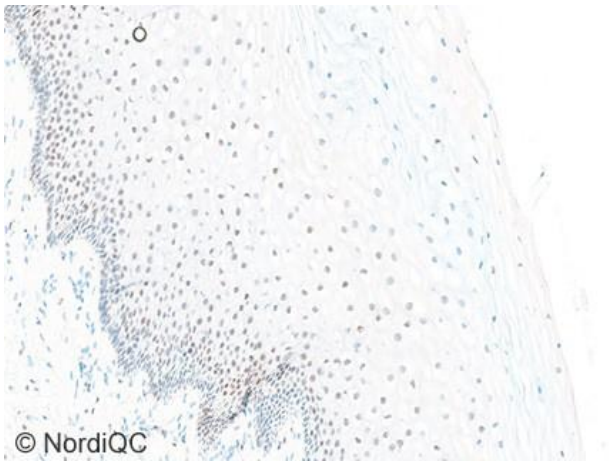


Fig. 1b (x100)
Insufficient GATA3 staining reaction of the uterine cervix using the same RTU system as in Fig. 1a, but with the vendor recommended protocol settings based on UltraView as the detection system. The proportion and intensity of cells expected to be demonstrated is significantly reduced, displaying only faint or false negative staining reaction. Same protocol settings as in Figs. 2b-4b. Same field as Fig 1a.

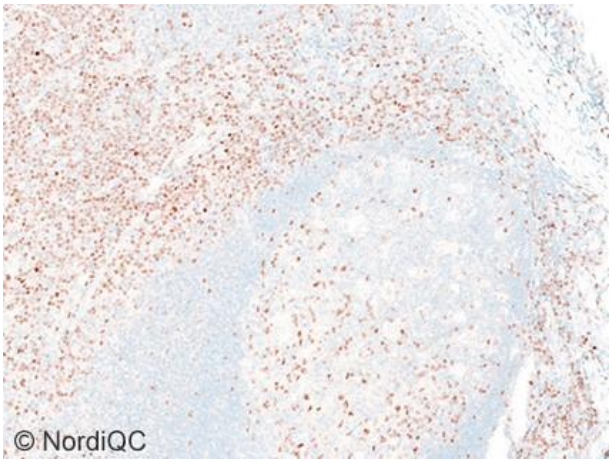


Fig. 2a (x100)
Optimal GATA3 staining reaction of the tonsil using the same protocol as in Figs. 1a-4a. The vast majority of T helper cells (Th2) display a moderate but distinct nuclear staining reaction, whereas the B-cells are negative.

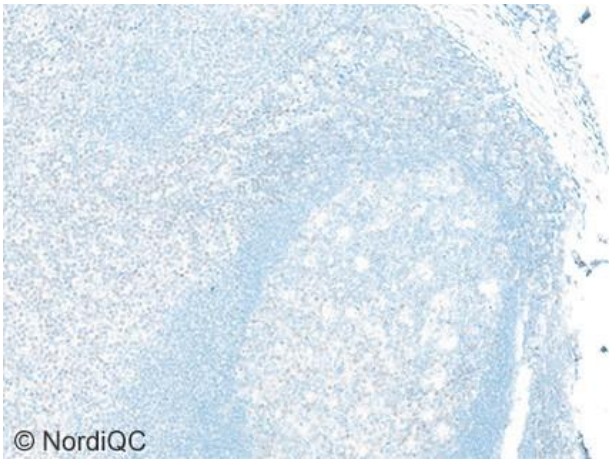


Fig. 2b (x100)
Insufficient GATA3 staining reaction of the tonsil using the same protocol as in Figs. 1b-4b. The vast majority of T helper cells (Th2) are false negative and only a fraction of germinal centre T-cells are weakly demonstrated – compare with Fig. 2a, same field.

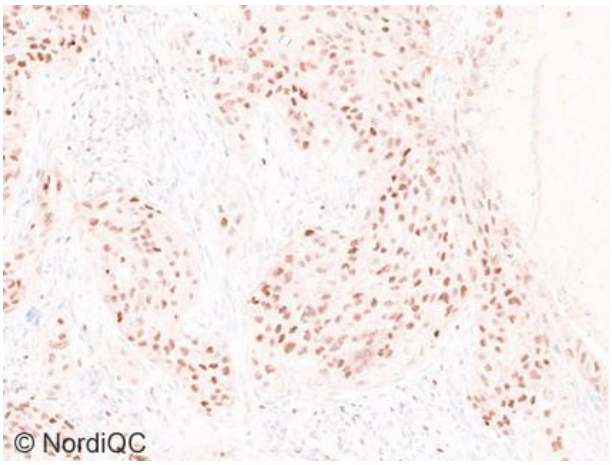


Fig. 3a (x100)
Optimal GATA3 staining reaction of the breast carcinoma using same protocol settings as in Figs. 1a-4a. A weak to strong nuclear staining reaction of virtually all neoplastic cells are seen.

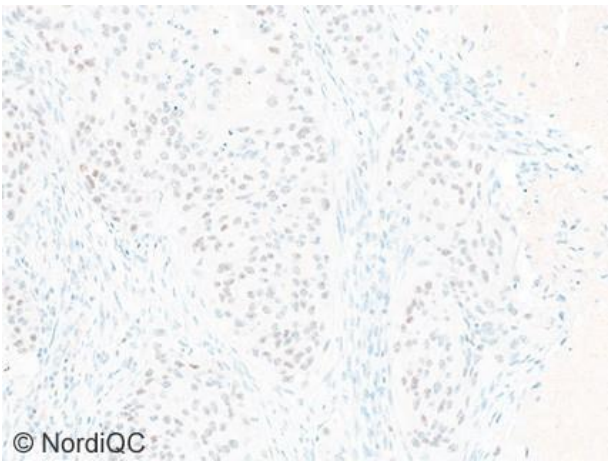


Fig. 3b (x100)
Insufficient GATA3 staining reaction of the breast carcinoma using the same protocol settings as in Figs. 1b-4b. Many of the neoplastic cells are false negative – compare with Fig. 3a

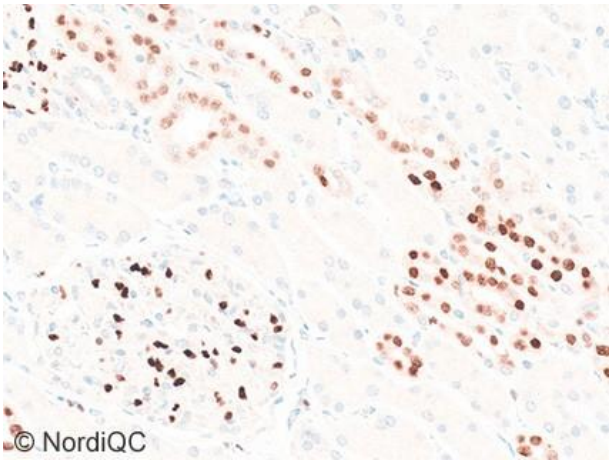


Fig. 4a (x200)
Optimal GATA3 staining reaction of the kidney using the same protocol as in Figs. 1a-4a. All epithelial cells of the collecting ducts and podocytes in glomeruli show a moderate to strong and distinct nuclear staining reaction.

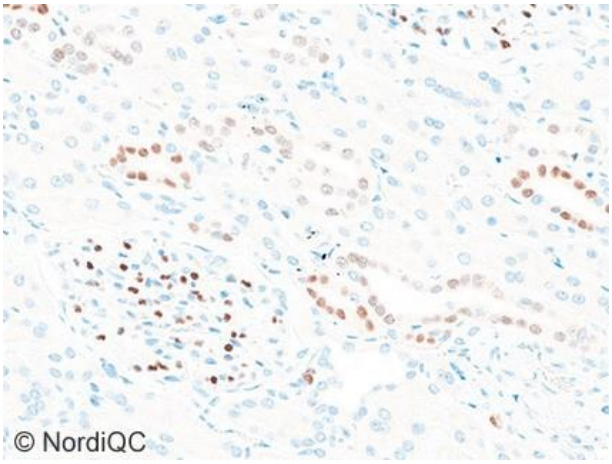


Fig. 4b (x200)
Insufficient GATA3 staining reaction of the kidney using the same protocol as in Figs. 1b-4b. The staining intensity is significantly reduced displaying only a weak nuclear staining reaction of e.g., the collecting ducts - compare with Fig. 4a.

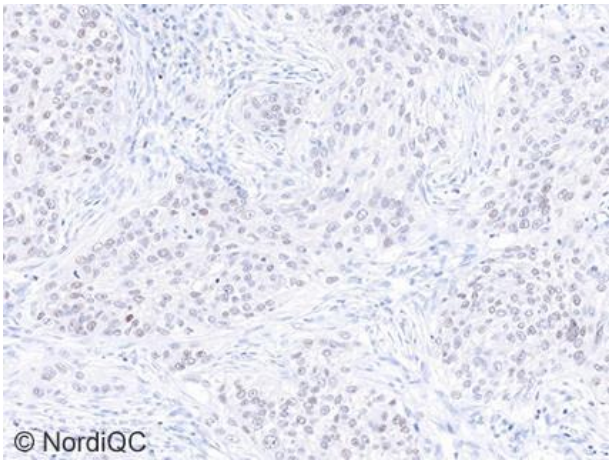


Fig. 5a (x100)
Insufficient staining reaction of the breast carcinoma using the concentrate from Cell Marque, based on the mAb clone L50-823, on the Ventana Ultra (Ventana/Roche) and OptiView as the detection system. Interpretation is difficult due to too weak specific staining reaction in combination with an excessive counterstaining, risking misdiagnosis in the diagnostic work of CUP – compare with Fig. 3a.

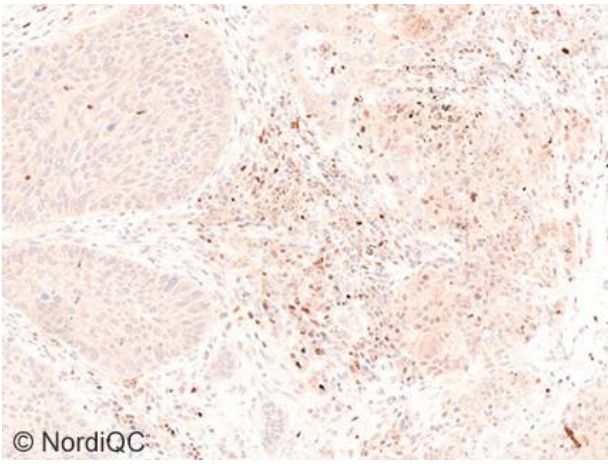


Fig. 5b (x100)
Insufficient GATA3 staining reaction of the NSCLC using the mAb clone L50-823 within a LD-assay and on the Dako Omnis platform (Dako/Agilent). EnVision Flex+ was used as the detection system. An aberrant granulated cytoplasmic reaction of the neoplastic cells is displayed and mainly caused by too high concentration of the primary antibody. This aberrant staining pattern was seen in all tissue cores.

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