

GATA3

Material

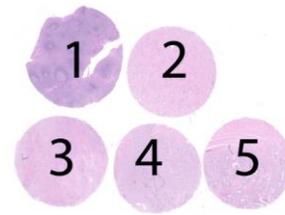
The slide to be stained for GATA3 comprised:

1. Tonsil
2. Kidney
3. Urothelial carcinoma
4. Breast ductal carcinoma
5. Colon adenocarcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing GATA3 staining as optimal included:

- An at least moderate, distinct nuclear staining reaction of virtually all epithelial cells in collecting ducts and podocytes in glomeruli in the kidney
- An at least weak nuclear staining reaction of the majority of T-cells in the T-zones in the tonsil and dispersed T-cells in all tissues
- A moderate to strong nuclear staining reaction of virtually all neoplastic cells in the breast ductal carcinoma
- An at least weak to moderate nuclear staining reaction of the majority of neoplastic cells in the urothelial carcinoma
- No staining reaction of neoplastic cells of the colon adenocarcinoma



Participation

Number of laboratories registered for GATA3, run 44	129
Number of laboratories returning slides	124 (96%)

Results

124 laboratories participated in this assessment. Out of these, 89 (72%) laboratories achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:

- Less successful primary antibody
- Use of less sensitive detection systems
- Too low concentration of the primary antibody
- Insufficient HIER (too short efficient heating time)

Performance history

This was the first NordiQC assessment of GATA3. A pass rate of 72% was observed.

Table 2. **Proportion of sufficient results for GATA3 in the first NordiQC run performed**

	Run 44 2015
Participants, n=	124
Sufficient results	72%

Conclusion

The mAb clone **L50-823** was found to be a very robust and recommendable antibody clone for demonstration of GATA3. In concentrated format within a laboratory developed assay, optimal results could be obtained on all three main IHC platforms (Dako, Leica and Ventana) and in general a high pass rate was observed. Efficient HIER, preferable in an alkaline buffer, and careful calibration of the primary antibody were the most important prerequisites for optimal staining results.

Corresponding RTU formats of the mAb clone **L50-823** and especially the Ventana/Cell Marque system provided a high pass rate and proportion of optimal results.

Normal kidney and tonsil are recommendable as positive and negative tissue controls for GATA3. Virtually all epithelial cells in collecting ducts and podocytes in glomeruli in the kidney must show a moderate to strong nuclear staining reaction, while an at least weak but distinct nuclear staining reaction of T-cells must be seen. No staining reaction of B-cells should be seen.

Table 1. **Antibodies and assessment marks for GATA3, run 44**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone L50-823	27 48 3 1	Biocare Cell Marque Immunologic Zeta	23	31	22	3	68%	69%
mAb clone HG3-31	6	Santa Cruz	0	0	0	6	0%	-
Polyclonal	1	Acris	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone L50-823 760-4897	20	Ventana/Cell Marque	13	7	0	0	100%	100%
mAb clone L50-823 MAD000632-QD	3	Master Diagnostica	1	1	1	0	-	-
mAb clone L50-823 390M-18	9	Cell Marque	2	7	0	0	100%	100%
mAb clone L50-823 PM405AA	3	BioCare	1	2	0	0	-	-
mAb clone L50-823 MAB-0695	1	Maixin	0	1	0	0	-	-
mAb clone L50-823 ZM-0498	1	Zeta	0	0	1	0	-	-
mAb clone HG3-31	1	Santa Cruz	0	0	0	1	-	-
Total	124		40	49	24	11	-	
Proportion			30%	41%	20%	9%	72%	

1) Proportion of sufficient stains (optimal or good)

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

Detailed analysis of GATA3, Run 44

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **L50-823**: The protocols with an optimal result were all based on heat induced epitope retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (3/19), TRS pH 6.1 (Dako) (1/6) Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/7) or Cell Conditioning 1 (BenchMark, Ventana)(17/41) as retrieval buffer. The mAb was typically diluted in the range of 1:70–1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings 48 of 70 (69 %) laboratories produced a sufficient staining (optimal or good).

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

Table 3. **Proportion of optimal results for GATA3 for the most commonly used antibody as concentrate on the 3 main IHC systems***

Concentrated antibodies	Dako		Ventana		Leica	
	Autostainer Link / Classic		BenchMark XT / Ultra		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 L50-823	3/16** (19%)	1/6 (17%)	18/36 (50%)	-	1/7 (14%)	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)

Ready-To-Use antibodies and corresponding systems

mAb clone **L50-823**, product no. **760-4897**, Ventana, BenchMark XT, ULTRA:

Protocols with optimal results were typically based on HIER using Cell Conditioning 1 (efficient heating time 24-64 min.), 16-32 min. incubation of the primary Ab and iView (760-091), UltraView (760-500) or OptiView (760-700) as detection system. **Using these protocol settings 20 of 20 (100%) laboratories produced a sufficient staining result.**

Comments

In this first NordiQC assessment for GATA3, 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 94% of the insufficient results (33 of 35 laboratories). A 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 a poor signal-to-noise ratio compromising the interpretation.

Virtually all laboratories were able to demonstrate GATA3 in high-level antigen expressing cells, such as neoplastic cells of the breast ductal carcinoma and epithelial cells of the renal collecting ducts. However, demonstration of GATA3 in low-level antigen expressing cells as normal T-cells in the tonsil and neoplastic cells of urothelial carcinoma was more challenging and required optimally calibrated protocols.

Optimal staining results could only be obtained with the mAb clone L50-823. Used as concentrated format within a laboratory developed (LD) assay, optimal results could be provided on all three main IHC platforms from Dako, Leica and Ventana (see table 3). For optimal performance of mAb clone L50-823, efficient HIER in an alkaline buffer in combination with a careful calibration of the primary Ab seem to be the most critical parameters. Both 2- and 3-step polymer and multimer based detection systems could be used to obtain an optimal result.

The mAb clone HG3-31, Santa Cruz showed a less successful performance. The Ab was used by 6 laboratories as a concentrate and despite similar protocol settings, e.g. HIER, detection systems etc., as for the mAb clone L50-823 were applied, all protocols produced insufficient staining results (borderline or poor) as shown in table 1. The main feature of these 6 insufficient protocols was too weak or false negative staining reaction.

Corresponding Ready-To-Use (RTU) formats for mAb clone L50-823 provided higher pass rates and proportion of optimal results compared to LD assays.

Especially the Ventana RTU system based on the mAb clone L50-823 was successful as a pass rate of 100% was seen. Optimal results could both be obtained when using the official protocol recommendations given by Ventana and by laboratory modified protocol settings, typically adjusting HIER time, incubation time of the primary Ab and/or choice of detection system.

Controls

Kidney and tonsil is recommended as positive and negative tissue controls for GATA3. In kidney, moderate to strong nuclear staining reaction in virtually all epithelial cells lining the collecting ducts and podocytes in glomeruli must be seen. In tonsil the vast majority of T-cells in the T-zones must show an at least weak but distinct nuclear staining reaction. No staining of B-cells should be seen (dispersed intra-germinal T-cells will be identified).

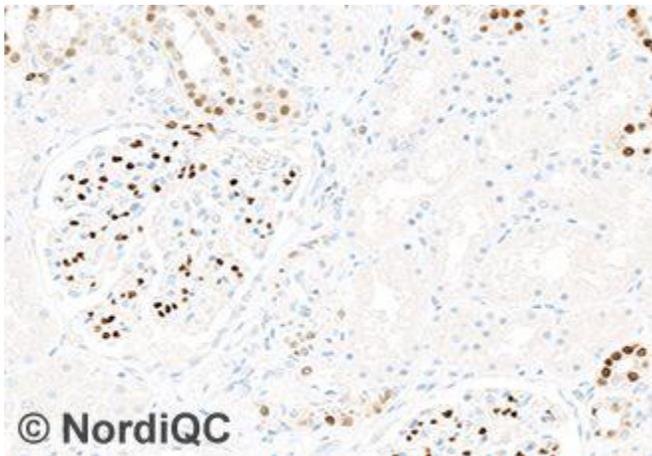


Fig. 1a (x200)

Optimal GATA3 staining of the kidney using the mAb clone L50-823 as a concentrate, optimally calibrated with HIER in an alkaline buffer (CC1 pH 8.5, Ventana) and a 3-step multimer based detection system (UltraView, Ventana). A moderate to strong nuclear staining reaction is seen in the majority of epithelial cells lining the collecting ducts and in podocytes in glomeruli. Same protocol used in Figs. 1a - 4a.

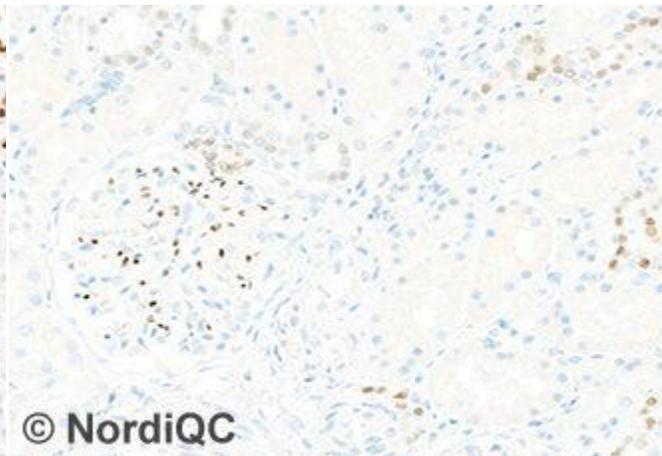
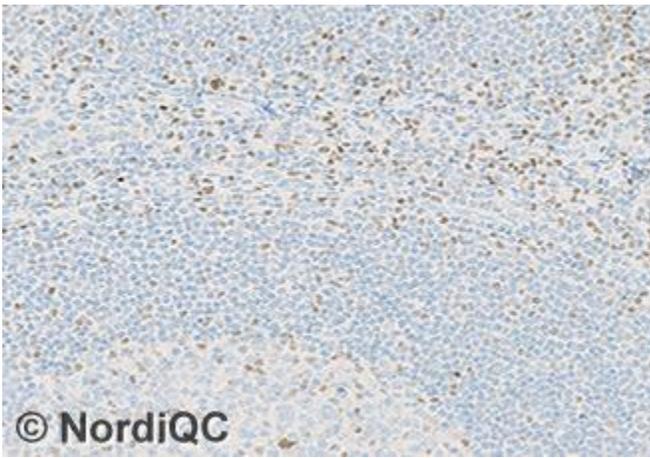


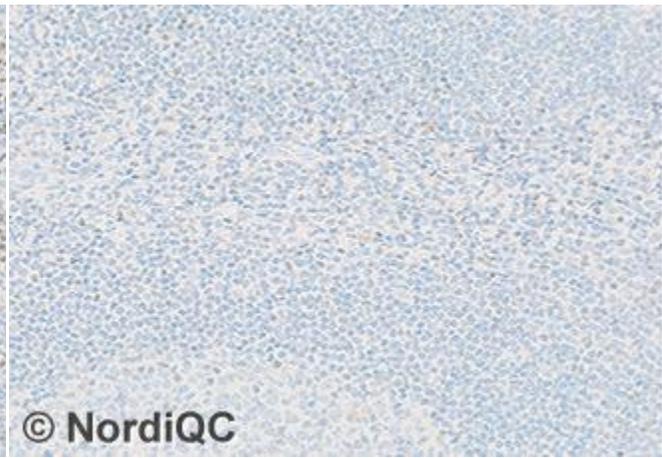
Fig. 1b (x200)

Insufficient GATA3 staining of the kidney using the mAb clone L50-823. The protocol provided an overall reduced sensitivity primarily caused by a too low concentration of the primary Ab. The intensity and proportion of cells demonstrated is reduced compared to the level expected - same field as in Fig. 1a. Also compare with Figs. 2b - 4b, same protocol.



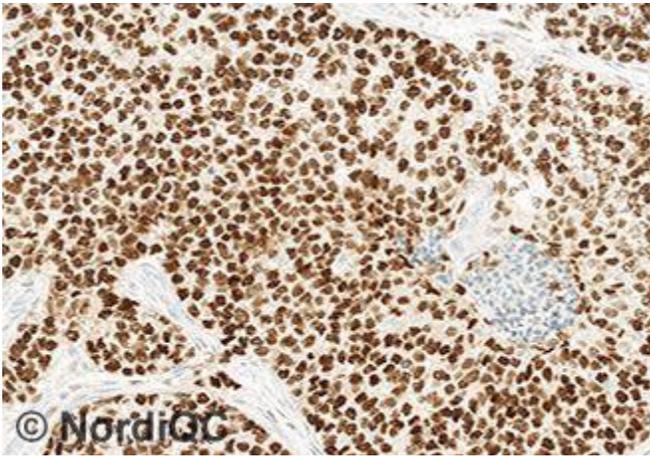
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Fig. 2a (x200)
Optimal GATA3 staining of the tonsil using same protocol as in Fig. 1a. T-cells primarily located in the T-zones but also within the germinal center show a weak to moderate, distinct nuclear staining reaction. No background staining is seen.



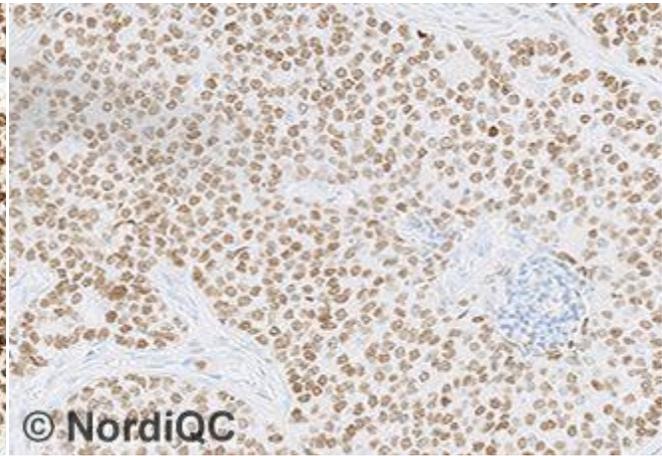
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Fig. 2b (x200)
Insufficient GATA3 staining of the tonsil using same protocol as in Fig. 1b – same field as in Fig. 2a. Virtually no staining reaction of T-cells is seen. Also compare with Figs. 3b and 4b, same protocol.



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Fig. 3a (x200)
Optimal GATA3 staining of the breast ductal carcinoma using same protocol as in Figs. 1a & 2a. Virtually all neoplastic cells show a moderate to strong nuclear staining reaction. No background staining is seen.



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Fig. 3b (x200)
Staining of the breast ductal carcinoma using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a. The vast majority of neoplastic cells are demonstrated, whereas the intensity is reduced, compared to the level expected and seen in Fig. 3a. However compare with Figs. 4a and 4b.

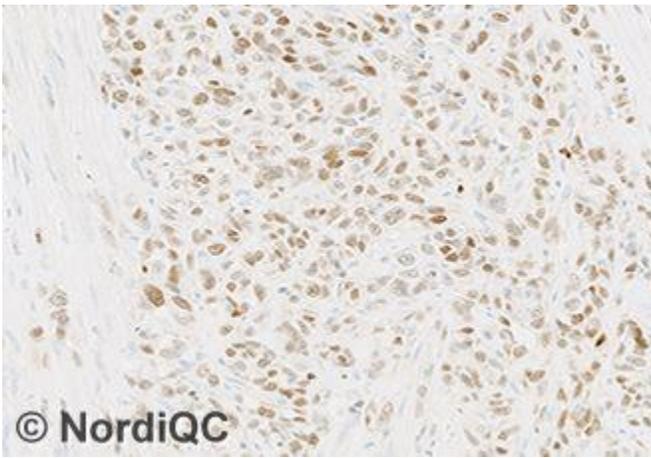


Fig. 4a (x200)
Optimal GATA3 staining of the urothelial carcinoma using same protocol as in Figs. 1a - 3a. The majority of neoplastic cells show a distinct, weak to moderate nuclear staining reaction.

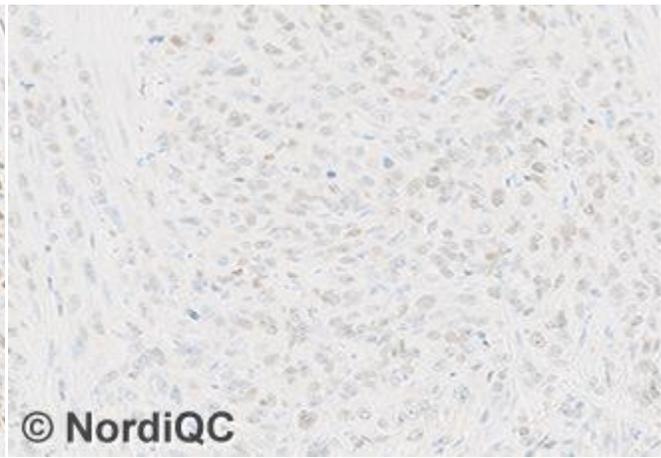


Fig. 4b (x200)
Insufficient GATA3 staining of the urothelial carcinoma using same protocol as in Figs. 1b - 3b - same field as in Fig. 4a. Only a weak and equivocal nuclear staining reaction in dispersed neoplastic cells is observed.

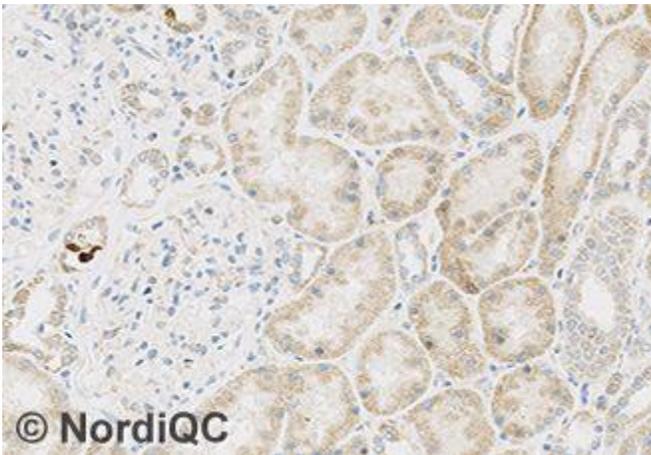


Fig. 5a (x200)
Insufficient GATA3 staining of the kidney using a pAb with HIER in an alkaline buffer (TRS pH 9, Dako) and a 2-step polymer based detection system (FLEX, Dako). A poor signal-to-noise ratio is seen due to an aberrant cytoplasmic staining reaction in combination with only a faint specific nuclear staining. Also compare with Fig. 5b, same protocol.

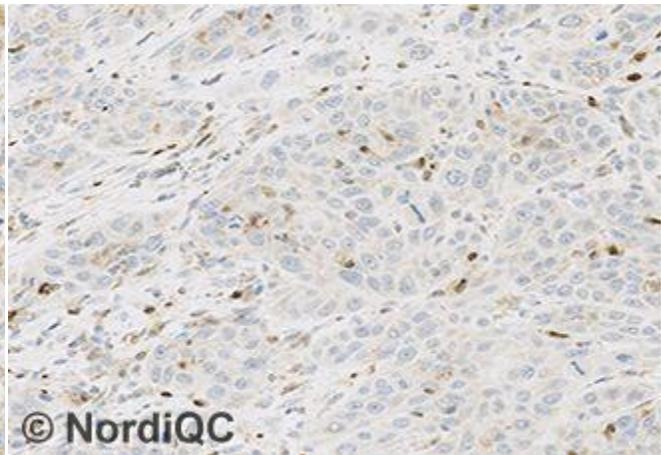


Fig. 4b (x200)
Insufficient GATA3 staining of the urothelial carcinoma using same protocol as in Fig. 5a based on a pAb. A nuclear staining reaction in stromal cells is seen, while the neoplastic cells are false negative.

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