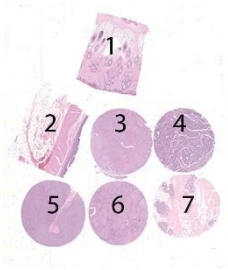
Assessment Run 45 2015

Transcription factor SOX-10 (SOX10)



The slide to be stained for SOX10 comprised:

1. Skin, 2. Colon, 3. Schwannoma, 4. Colon adenocarcinoma, 5-6. Malignant melanoma, 7. Breast hyperplasia

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing SOX10 staining as optimal included:

- A moderate to strong, nuclear staining reaction of virtually all melanocytes in the skin and Schwann cells in the colon.
- An at least moderate nuclear staining reaction of the majority of myoepithelial cells lining sweat glands in the skin and ductal glands in the breast hyperplasia.
- A strong nuclear staining reaction of virtually all neoplastic cells in the Schwannoma and the malignant melanoma tissue core no. 5.
- An at least weak to moderate nuclear staining reaction of the majority of neoplastic cells in the malignant melanoma tissue core no. 6.
- No nuclear staining reaction of other cells.

A weak cytoplasmic staining reaction in cells with a strong nuclear staining reaction was accepted.

Participation

Number of laboratories registered for SOX10, run 45	96
Number of laboratories returning slides	86 (90%)

Results

86 laboratories participated in this assessment. 39 (45%) achieved a sufficient mark.
Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful performance of polyclonal SOX10 antibodies
- Too low concentration of the primary Ab
- Insufficient HIER - too short efficient HIER time

Performance history

This was the first NordiQC assessment of SOX10. A relatively low pass rate of 45% was observed.

Table 2: **Proportion of sufficient results for SOX10 in the first NordiQC run performed**

	Run 45 2015
Participants, n=	86
Sufficient results	45%

Conclusion

The mmAb clones **BC34**, **BS7**, **SOX10/1074** and the rmAb clones **EP268** and **SP267** were the most successful antibodies and could all be used to obtain an optimal staining result for SOX10. Irrespective of the clone applied, efficient HIER and use of a sensitive and specific 3-step polymer/multimer based detection system gave the highest proportion of optimal results. The concentration of the primary antibody must be carefully calibrated.
All polyclonal antibodies applied in this assessment gave less successful results.
Skin and appendix are at present the most recommendable positive and negative tissue controls for SOX10.
Virtually all melanocytes, Schwann cells in appendix and myoepithelial cells in sweat glands of skin must show a moderate to strong nuclear staining reaction. In cells with a strong nuclear staining reaction, a weak cytoplasmic staining due to epitope diffusion must be expected. No nuclear staining reaction in other cells should be seen.

Table 1: **Abs and assessment marks for SOX10, run 45**

Concentrated antibodies:	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BC34**	23 1	Biocare Abcam	8	6	7	3	60%	63%
mAb clone **BS7**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **SOX10/1074**	1	Immunologic	1	0	0	0	-	-
rmAb clone **EP268**	1 4 4	Immunologic Epitomics Cell Marque	7	1	1	0	89%	100%
pAb **383A-XX**	23	Cell Marque	0	5	13	5	21%	-
pAb **44-387**	1	Menarini	0	0	1	0	-	-
pAb **ab108408**	1	Abcam	0	0	0	1	-	-
pAb **ILP3833-C1**	2	Immunologic	0	0	2	0	-	-
pAb **NBP-1-68983**	1	Novus biological	0	0	1	0	-	-
pAb **Z2242**	1	Zeta	0	0	1	0	-	-
pAb **PA0813**	1	Leica	0	0	1	0	-	-
pAb **RBK057**	2	Zytomed	0	0	1	1	-	-
gpAb **sc17432**	3	Santa Cruz	0	0	2	1	-	-
Unknown	2		0	1	1	0	-	-
Ready-To-Use antibodies:								
mAb clone **BC34 API3099**	5	Biocare	3	1	1	0	80%	-
rmAb clone **EP268 383R-18**	3	Cell Marque	2	1	0	0	-	-
rmAb clone **EP268 MAD-000656QD**	1	Master Diagnostica	0	1	0	0	-	-
rmAb clone **SP267 760-4968**	1	Ventana	1	0	0	0	-	-
pAb **AB5727**	1	Milipore	0	0	0	1	-	-
pAb **383A-78**	1	Cell Marque	0	0	1	0	-	-
pAb **PA0813**	1	Leica	0	0	1	0	-	-
gpAb **GAB-0722**	1	Maixin	0	0	1	0	-	-
Total	86		23	16	35	12	-	
Proportion			27 %	18 %	41 %	14 %	45 %	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Following protocol parameters were central to obtain an optimal staining:

Concentrated Abs:
mmAb clone **BC34**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (1/3), Cell Conditioning 1 (CC1, Ventana)(5/9) or Diva low pH (Biocare)(1/1) as retrieval buffer. The mmAb was typically diluted in the range of 1:25– 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 12 of 19 (63%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mmAb clone **BS7**: One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9. The mmAb was diluted 1:200 using a 2-step polymer based detection system. Peroxidase blocking was performed after incubation with primary Ab.

mmAb clone **SOX10/1074**: One protocol with an optimal result was based on HIER using Bond Epitope Retrieval Solution (BERS2, Leica). The mmAb was diluted 1:50 using a 3-step polymer based detection system.

rmAb clone **EP268**: The protocols with optimal results were all based on HIER using either Tris-EDTA/EGTA pH 9 (3/3)*, Target Retrieval Solution pH 9 (3-in-1) (TRS, Dako) (1/1) or CC1 (Ventana)(3/4) as retrieval buffer. The rmAb was typically diluted in the range of 1:30– 1:100 depending on the total

sensitivity of the protocol employed. Using these protocol settings 8 of 8 (100%) laboratories produced a sufficient staining result.

Table 3: **Proportion of optimal results for SOX10 for the most commonly used antibody as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**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 **BC34**	0/2**	0/1	5/9 (56%)	-	0/2	-

* 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 antibodies:
mmAb clone **BC34**, product no. **API3099,** Biocare, IntelliPATH: One protocol with an optimal result was based HIER in Citrate pH 6 using a pressure cooker, 30 min. incubation of the primary Ab and a 3-step polymer based detection system (MACH4).

rmAb clone **SP267**, product no. **760-4968**, Ventana, BenchMark Ultra: One protocol with an optimal result was based on HIER in Cell Conditioning 1 for 32 min., 32 min. incubation of the primary Ab and a 3-step multimer based detection system (OptiView).

Comments
In this first NordiQC assessment of SOX10, the prevalent features of an insufficient staining result were either a generally too weak staining reaction of cells expected to be demonstrated and/or a poor signal-to-noise ratio compromising the interpretation.
Too weak or false negative staining reaction was seen in 62% of the insufficient results (29 of 47). The majority of the laboratories were able to demonstrate SOX10 in cells with high-level antigen expression as Schwann cells and normal melanocytes, whereas demonstration of SOX10 in cells with low antigen expression as myoepithelial cells and neoplastic cells in especially the melanoma tissue core no 6 could only be obtained by an optimally calibrated protocol. In the remaining 38% of the insufficient results, both a too weak specific staining reaction and an excessive background staining compromising the interpretation were seen. This staining pattern was virtually only seen for the pAbs used for SOX10.

The mmAb clone BC34 (mainly Biocare) and the pAb 383A (Cell Marque) were the most widely used concentrated antibodies for demonstration of SOX10 within a laboratory developed (LD) assay. As seen in table 1, mmAb clone BC34 gave a superior performance compared to pAb 383A as no results were assessed optimal and a lower pass rate was observed for the pAb.
mAb clone BC34 gave an overall pass rate of 60% (14 of 24) of which 33% were optimal (see table 1). Both HIER in alkaline and non-alkaline buffers could be used to obtain optimal results. A prerequisite for obtaining optimal results after HIER in non-alkaline buffers was the use of a sensitive 3-step polymer system, whereas optimal results could be achieved with both 2 and 3-step polymer/multimer systems when using alkaline HIER buffers. For mmAb clone BC34, sufficient results could be obtained on all main IHC system, whereas optimal results only were obtained on the BenchMark system, Ventana, as shown in table 3.
Within a LD assay, the rmAb clone EP368 was most successful providing an overall pass rate of 89% (8 of 9 protocols) of which 7 (88%) were optimal. HIER in alkaline buffer and careful calibration of the titre of the primary Ab were fundamental settings for optimal results. A weak to moderate cytoplasmic staining reaction of neurons was seen and this was fully accepted as the interpretation of the specific nuclear staining reaction was not impaired. The pAb 383A and virtually all other pAbs used within LD assays provided an less successful performance. Grouped together, a pass rate of 14% (5 of 36) for laboratories using a protocol based on a pAb for SOX10 was seen, none of which was evaluated as optimal. The staining results based on pAbs were typically characterized by a too weak specific staining reaction combined with an aberrant and excessive background staining compromising the interpretation. The poor signal-to-noise ratio by the pAbs complicated the ability to amplify the specific signals as this would also increase the aberrant and undesired staining reactions. Similar protocols settings were applied by the laboratories using pAb and the more successful monoclonal antibodies for SOX10.

Controls
Skin and appendix are recommended as positive and negative tissue controls for SOX10. In skin, moderate to strong nuclear staining reaction in virtually all melanocytes must be seen. The vast majority of myoepithelial cells lining sweat glands must show an at least moderate nuclear staining reaction. In appendix, virtually all Schwann cells must display an as strong as possible nuclear staining reaction in combination with no or only minimal cytoplasmic staining reaction of epithelial cells and smooth muscle

cells. At present, no tissue with consistent low level expression of SOX10 reliable for positive tissue control to monitor technical sensitivity has been identified. Due to this issue both skin and appendix are needed as tissue controls for SOX10.

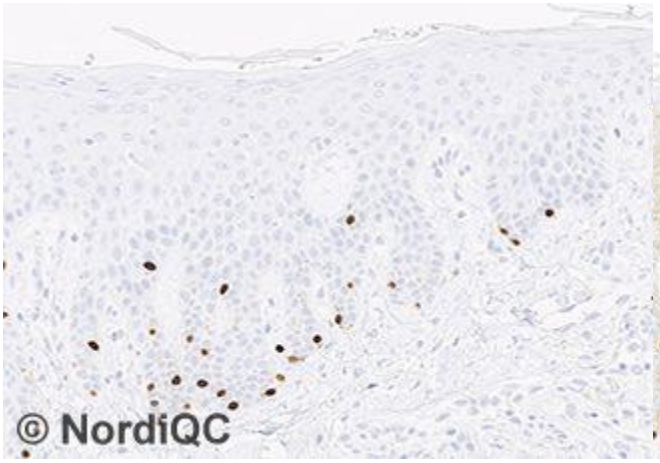


Fig. 1a
Optimal SOX10 staining of the skin using the mmAb clone BC34 diluted 1:40, HIER in CC1 pH 8.5 for 48 min., a 3-step multimer based detection kit and performed on BenchMark Ultra, Ventana.
Virtually all melanocytes show a strong, distinct nuclear staining reaction. No background reaction is seen.
Also compare with Figs. 2a – 5a, same protocol.

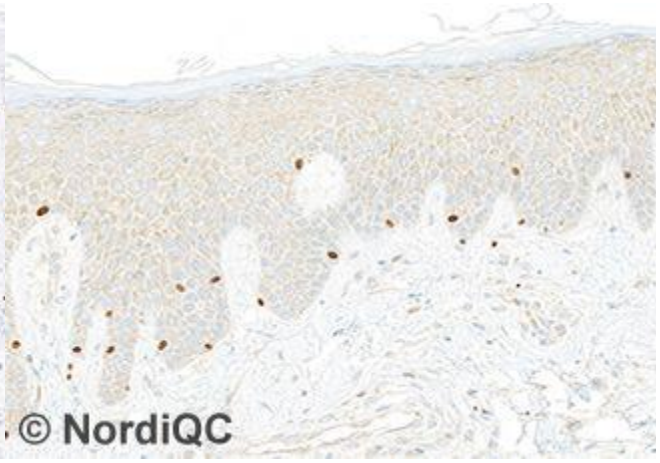


Fig. 1b
SOX10 staining of the skin using an insufficient protocol based on a pAb (383A, Cell Marque) diluted 1:25, HIER in CC1 pH 8.5 for 32 min., a 3-step multimer based detection kit and performed on BenchMark Ultra, Ventana.
The melanocytes are identified, but also an aberrant cytoplasmic staining in the squamous epithelial cells is seen. Compare with Fig. 1a. – same field.
Also compare with Figs. 2b - 5b – same protocol

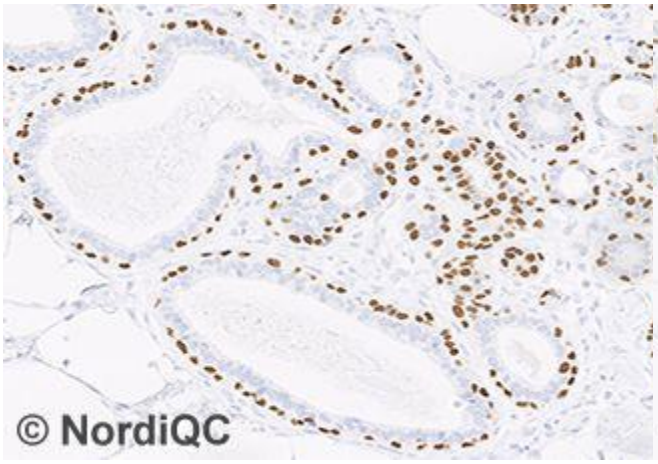


Fig. 2a
Optimal SOX10 staining of the breast hyperplasia using same protocol as in Fig. 1a. All the myoepithelial cells show a moderate to strong nuclear staining reaction. No background reaction is seen.

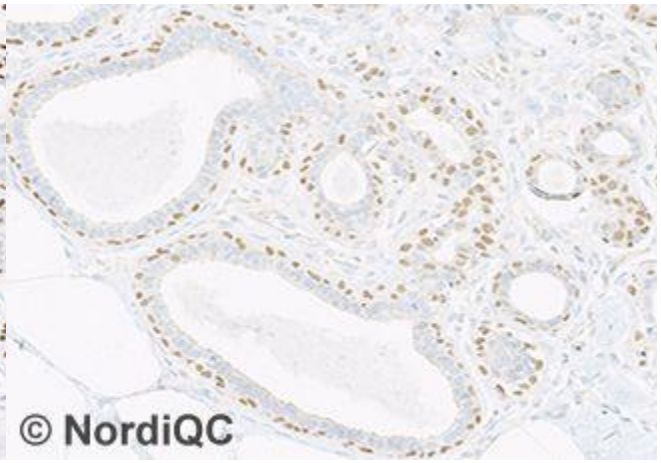


Fig. 2b
SOX10 staining of the breast hyperplasia using same protocol as in Fig. 1b. – same field as in Fig. 2b. The myoepithelial cells are demonstrated, but the intensity is reduced compared to the level expected.
Also compare with Figs. 3b – 5b, same protocol.

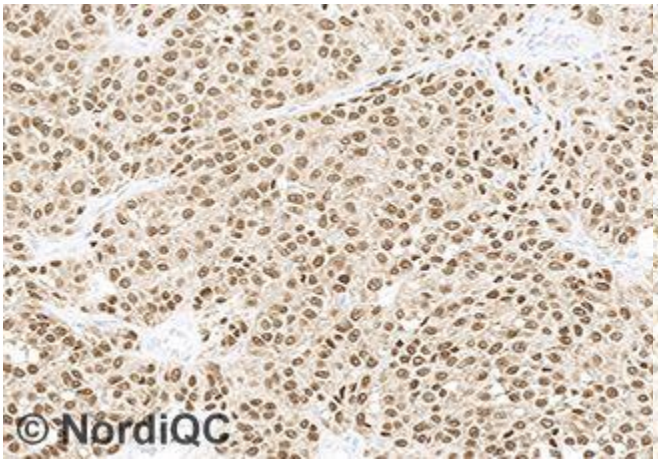


Fig. 3a
Optimal SOX10 staining of the malignant melanoma tissue core no. 5 using same protocol as in Figs. 1a and 2a. Virtually all neoplastic cells show a moderate nuclear staining reaction. A weak cytoplasmic staining reaction is seen, but no staining of the background or in cells not expressing nuclear SOX10.

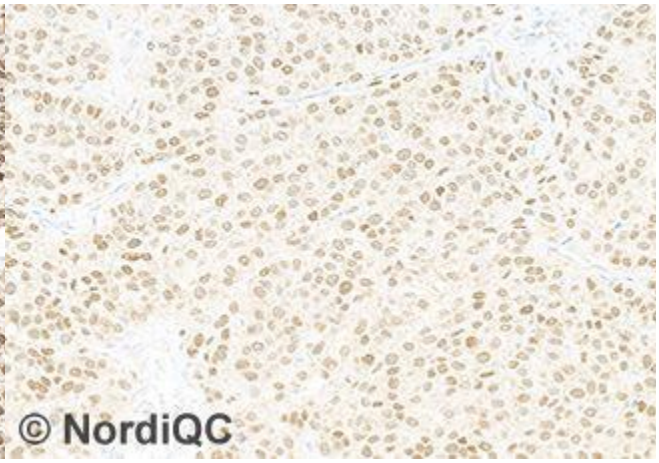


Fig. 3b
SOX10 staining of the malignant melanoma tissue core no. 5 using the same protocol as in Figs. 1b and 2b. The neoplastic cells display a weak nuclear staining reaction. Compare with Fig. 3a. – same field.
Also compare with the melanoma, tissue core 6, Fig. 4b

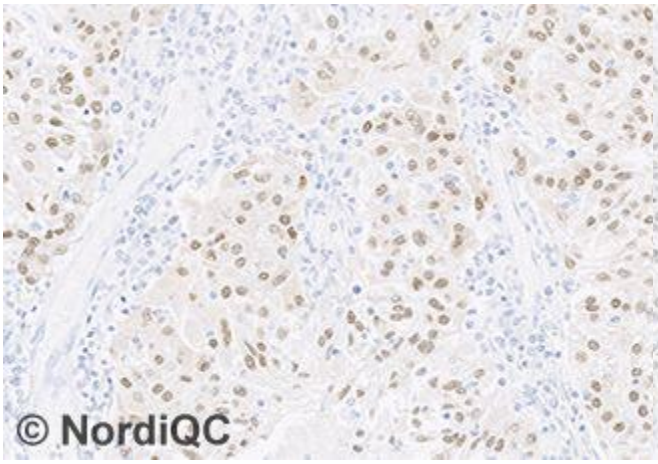


Fig. 4a
Optimal SOX10 staining of the malignant melanoma tissue core no. 6 using same protocol as in Figs. 1a - 3a. The majority of the neoplastic cells show a weak to moderate nuclear staining reaction. No background reaction is seen.

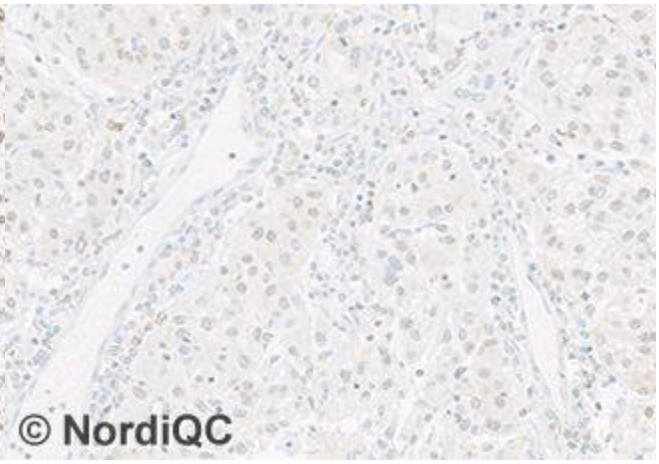


Fig. 4b
Insufficient SOX10 staining of the malignant melanoma tissue core no. 6 using same protocol as in Figs. 1b - 3b. - same field as in Fig. 4a. Only scattered neoplastic cells show a weak and equivocal staining reaction.

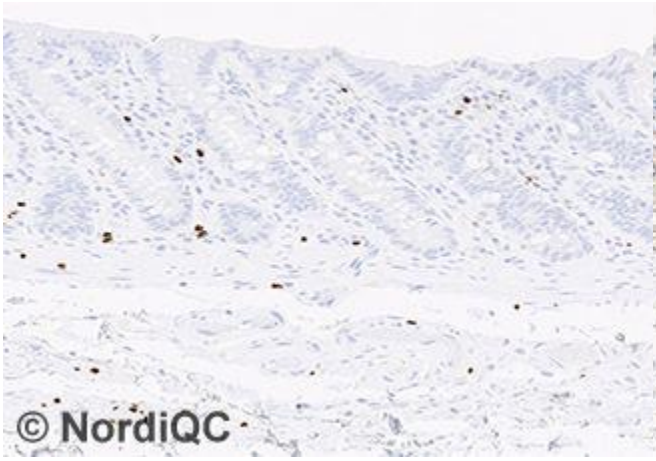


Fig. 5a
Optimal SOX10 staining of the colon using same protocol as in Figs. 1a - 4a. Schwann cells show a strong nuclear staining reaction. No background reaction is seen.

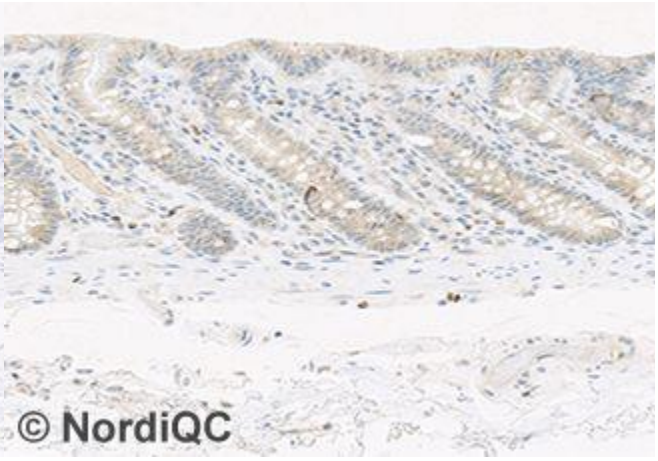


Fig. 5b
Insufficient SOX10 staining of the colon using same protocol as in Figs. 1b – 4b. Only a weak specific nuclear staining reaction in Schwann cells is seen and simultaneously a cytoplasmic staining in epithelial cells is seen.
This staining pattern as shown in Figs. 1b – 5b was typical observed for all pAbs for SOX10. A low affinity for the specific nuclear SOX10 antigen combined with a crossreaction to a cytoplasmic protein, prevented a high signal-to-noise ratio.

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