Assessment Run 48 2016

Transcription factor SOX-10 (SOX10)

Material
The slide to be stained for SOX10 comprised:

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

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a 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.
- A strong nuclear staining reaction of virtually all neoplastic cells in the Schwannoma and the malignant melanoma, tissue core no. 5.
- An at least moderate nuclear staining reaction of the majority of neoplastic cells in the malignant melanoma, tissue core no. 6.
- No staining reaction in other cellular structures including the neoplastic cells of the colon adenocarcinoma.

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

Participation

Number of laboratories registered for SOX10, run 48	136
Number of laboratories returning slides	121 (89%)

Results
120 laboratories participated in this assessment. One laboratory returned a slide stained for an unknown marker other than SOX10. 81 (68%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Less successful primary antibody – all polyclonals
- Insufficient HIER (too short heating time).
- Less sensitive detection systems
- Unexplained technical issues

Performance history
This was the second NordiQC assessment of SOX10. The overall pass rate was relative low but improved significantly compared to the result obtained in run 45, 2015 (see table 2).

Table 2. **Proportion of sufficient results for SOX10 in the two NordiQC runs performed**

	Run 45 2015	Run 48 2016
Participants, n=	86	120
Sufficient results	45%	68%

Conclusion
The mAb clones BC34 and BS7 and the rmAb clones EP268 and SP267 were the most successful antibodies and could all be used to obtain optimal staining for SOX10. Irrespective of the clone applied, efficient HIER, preferable in an alkaline buffer, precise calibration of the primary Ab concentration and use of a 3-step polymer or multimer based detection system were the main prerequisites for optimal results. Protocols based on the pAbs 383A-76/78 (Cell Marque), ILP3833-C1 (Immunologic), 44-387 (Menarini),

ab108408 (Abcam) and RBK057-05 (ZytoMed) only produced insufficient results. Skin and colon are recommendable positive and negative tissue controls for SOX10. Virtually all melanocytes, Schwann cells in appendix/colon and myoepithelial cells in sweat glands of skin must show moderate to strong nuclear staining reaction. No nuclear staining reaction should be seen in other cells.

Table 1. **Antibodies and assessment marks for SOX10, run 48**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BC34**	36 1 1	Biocare Medical Abcam/Epitomics Klinipath	22	5	7	4	71%	77%
mAb clone **BS7**	5	Nordic Biosite	5	0	0	0	100%	100%
mAb clone **SOX10/1074**	5	Immunologic	0	2	1	2	-	-
mAb clone **DPM15.10**	1	Diagnostic Biosystem	0	1	0	0	-	-
mAb clone **ZM10**	1	Zeta Corporation	0	1	0	0	-	-
rmAb clone **EP268**	23 1 1 1	Cell Marque Epitomics BioSB Diagnostic Biosystems	13	6	3	4	73%	78%
rmAb clone **SP267**	2	Spring Bioscience	0	2	0	0	-	-
pAb **383A-76**	5	Cell Marque	0	0	5	0	0%	-
pAb **ILP3833-C1**	1	Immunologic	0	0	1	0	-	-
pAb **44-387**	1	Menarini	0	0	1	0	-	-
pAb **ab108408**	1	Abcam	0	0	0	1	-	-
pAb **RBK057-05**	1	ZytoMed	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **BC34** **API 3099 AA or H**	2	Biocare Medical	1	0	1	0	-	-
mAb clone **BC34** **API 3099 AA or H**[3]	5	Biocare Medical	2	0	3	0	-	-
rmAb clone **EP268**[4] **383R-10, -17 or -18**	13	Cell Marque	10	1	2	0	85%	91%
rmAb clone **EP268** **MAD-000656QD**	2	Master Diagnostica	0	1	1	0	-	-
rmAb clone **EP268** **RMA-0726**	1	Maixin	1	0	0	0	-	-
rmAb clone **EP268** **PR135**	1	PathSitu/Unknown	0	1	0	0	-	-
rmAb clone **SP267** **760-4968**	5	Ventana/Roche	5	0	0	0	100%	100%
rmAb clone **SP267** **M5671**	2	Spring Bioscience	1	1	0	0	-	-
pAb **383A-78**	2	Cell Marque	0	0	1	1	-	-
Total	120		60	21	26	13	-	
Proportion			50%	18%	22%	10%	68%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only (see below).
3) RTU formats developed for Biocare`s IHC system (IntelliPATH) but used by laboratories off-label on the platforms Ventana Benchmark/Ultra or Leica BOND III.
4) RTU format not developed for a specific IHC system and used by laboratories on different platforms as Ventana Benchmark Ultra/XT, Leica BOND III or Dako Autostainer Link+.

Detailed analysis of SOX10, Run 48

The following protocol parameters were central to obtain optimal staining.

Concentrated antibodies

mAb **BC34**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using an alkaline buffer as Cell Conditioning 1 (BenchMark, Ventana) (16/21) *, TRS High pH (3-1)

(Dako/Agilent) (2/3), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/3), Epitope Retrieval or TRIS-EDTA pH 9 (1/1) as retrieval buffer. Two laboratories obtained an optimal mark performing HIER in acidic buffers using Diva Decloaker pH 6.2 (Biocare Medical) (1/2) or standard citric buffer pH 6 (1/3). The mAb was typically diluted in the range of 1:25 – 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 20 of 26 (86 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb **BS7**: Protocols with optimal results were based on heat induced epitope retrieval (HIER) using an alkaline buffer as TRS High pH (3-1) (Dako/Agilent) (3/3) or Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1). One laboratory obtained an optimal result without performing any pre-treatment at all. The mAb was typically diluted in the range of 1:100 – 1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 of 5 (100 %) laboratories produced an optimal result.

rmAb **EP268**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using an alkaline buffer as Cell Conditioning 1 (BenchMark, Ventana) (9/19) or TRS High pH (3-1) (Dako/Agilent) (4/6). The rmAb was typically diluted in the range of 1:50 – 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 18 of 23 (78 %) laboratories produced a sufficient staining.

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 / 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 **BC34**	1/2 **	0/1	14/16 (88%)	-	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)

Ready-To-Use antibodies and corresponding systems
mAb clone **BC34,** product no. **API 3099 AA or H,** Biocare Medicare, IntelliPATH:
One protocol with an optimal result was based on HIER using Diva Decloaker (Pressure Cooker, efficient heating time 15 min. at 110°C), 30 min. incubation of the primary Ab and MACH4 Universal HRP-polymer (M4U534, Biocare Medical) as detection system.

rmAb clone **SP267,** product no. **760-4968,** Ventana/Roche Benchmark Ultra:
Protocols with optimal results were based on HIER using CC1 (efficient heating time 32-76 min. at 95-100°C), 32-44 min. incubation of primary Ab and UltraView with amplification (Ventana,760-500 + 760-080) or OptiView (Ventana, 760-700) as detection system. Using these protocol settings, 5 of 5 (100%) laboratories produced an optimal result.

Comments
In this second NordiQC assessment of SOX10, the prevalent feature of an insufficient result was either a generally too weak staining reaction of cells expected to be demonstrated and/or poor signal-to-noise ratio compromising the interpretation. Too weak or false negative staining reaction was seen in 85% of the insufficient results (33 of 39). The majority of the laboratories were able to stain SOX10 in Schwann cells of the colon, neoplastic cells of the Schwannoma and the melanoma tissue core.no. 5, whereas demonstration of SOX10 in neoplastic cells of the melanoma tissue core no. 6, normal melanocytes and myoepithelial cells of the skin was more challenging and required a carefully calibrated protocol. In 15 % (6 of 39) of the insufficient results, a general poor signal-to-noise ratio and/or false positive staining reaction was seen, mostly related to poor performance of pAbs.

The mAb clone BC34 and the rmAb clone EP268 were the most widely used antibodies for the demonstration of SOX10. Used as a concentrate by a laboratory developed (LD) assay, mAb clone BC34 gave an overall pass rate of 71% (27 of 38) of which 58% were optimal (see table 1). As shown in table 3, optimal results could be obtained on all three main IHC platforms from Agilent/Dako, Leica/Novocastra and Roche/Ventana. Efficient HIER, preferable in alkaline buffer, careful calibration of the primary Ab concentration and use of a sensitive detection system were the most central parameters for optimal results. For protocols based on the concentrate of mAb clone BC34, HIER time seems to be an important parameter influencing the analytical sensitivity of the assay. If HIER in alkaline buffer (at 95-100°C) was applied in combination with a standard 2- or 3-step multimer/polymer detection system, the efficient Median HIER Time (MHT) for optimal results was 49 min. (range 20-64 min.), whereas a MHT of 29 min. (range 20-64 min.) was seen in protocols with insufficient results. In addition, the titre of the primary Ab

had a significant impact on the general performance and pass rate. Using the same protocol conditions as mentioned above, the Median Dilution Value (MDV) for optimal results was 1:52 (range 1:20-1:200), whereas MDV of 1:132 (range 1:100-1:225) was seen in protocols with insufficient results. Therefore, efficient HIER (time and temperature in an alkaline buffer) in combination with a careful calibration of the titre of the primary Ab, are the basic requirement for an IHC protocol to demonstrate SOX10 in structures with both low-level and high-level SOX10 expression, which is the range seen in different melanocytic lesions (e.g. malignant melanomas).

The LD assays based on rmAB clone EP268 as concentrate provided a pass rate of 73% (19 of 26) of which 50% were optimal. A weak to moderate cytoplasmic staining reaction of neurons was seen. This was fully accepted as the interpretation of the specific nuclear staining reaction was not compromised. The prerequisites for obtaining an optimal result were use of efficient HIER in alkaline buffer and a 3-step polymer / multimer based detection system. Using rmAb clone EP268 as concentrate at the optimal dilution range 1:50-1:200, HIER in an alkaline buffer in combination with a 3-step detection system, a pass rate of 83% (15 of 18 protocols) was obtained of which 67% (12 of 18) were optimal. In comparison, the pass rate for laboratories using a 2-step multimer/polymer system was 60 % (3 of 5 protocols) of which only 20% (1 of 5 protocols) were optimal.

Within a LD assay, the mAb clone BS7 was most successful providing an overall pass rate of 100% (5 of 5 protocols) of which all were optimal. The basic foundation for optimal performance was use of HIER in alkaline buffer, dilution range 1:200- 1:300 of the primary Ab and a 3-step polymer based detection system.

In this assessment, all protocols based on the RTU system, 760-4968 (Ventana/Roche) were assessed as optimal (see table 1). Optimal results were primarily obtained by using the RTU system accordingly to the protocol recommendations provided by the vendor. Laboratory modified protocol settings (typically adjusting HIER and incubation time of the primary Ab) also provided optimal results.

Two laboratories used the RTU system API 3099 AA or H based on the mAb clone BC34 (Biocare Medical) developed for the IHC stainer IntelliPATH. One protocol with optimal result was based on HIER using Diva Decloaker (Pressure Cooker, efficient heating time 15 min. at 110°C), 30 min. incubation of the primary Ab and MACH4 Universal HRP-polymer (M4U534, Biocare Medical) with mouse probe as the detection system (3-step polymer system). The protocol assessed as insufficient used identical protocol settings except for application of MACH4 Universal HRP-Polymer without mouse probe as the detection system (2-step polymer system).
Five laboratories used the RTU format on platforms other than the IntelliPATH (Biocare), and as such off-label and by an IHC system for which the format and protocol has not been thoroughly validated and verified. Although it might produce optimal result (see table 1), it is not advisable to use a particular RTU format that is not developed for the platform in use. Essentially, a RTU format of a primary Ab is used within a system with precise information on vendor recommended protocol settings, equipment, reagents and results expected.

The RTU antibody/format, 383R-XX (Cell Marque) based on the rmAb EP268 provided as pass rate of 85% (11 of 13), of which 77% (10 of 13) of the protocols were optimal. However, this RTU antibody is not developed for a particular automatic system/platform but mostly used by laboratories on the Ventana Benchmark Ultra/XT. Protocols performed on the Ventana Benchmark Ultra/XT with optimal results, were based on HIER in CC1 (efficient heating time 32-64 min. at 95-100°C), 16-32 min. incubation time of primary Ab and UltraView with or without amplification (Ventana,760-500 + 760-080) or OptiView with or without amplification (Ventana, 760-700 + 760-099/860-099) as detection systems. Using these protocol settings, 10 of 11 (91%, see table 1) laboratories produced a sufficient result. Two laboratories used this RTU format on the BOND III (Leica/Novocastra) and Autostainer Link+ (Agilent/Dako) none of which were optimal.

This was the second assessment of SOX10 in NordiQC with a pass rate of 68%. This is a significant improvement compared to the result obtained in run 45, 2015 (table 2). The extended use of mAbs clone BC34 or BC7 and rmAbs clone EP268 or SP267 both within LD assays and RTU assays accounted for the overall improvement. Grouped together, a pass rate of 75% (77 of 102) was seen for laboratories using these monoclonal primary antibodies.
Several parameters influenced the overall performance. For the mAbs insufficient HIER, too low concentration of the primary Ab and use of a less sensitive detection system were the main issues. In particular, the persistent use of less successful pAbs (see table 1) contributed to the high proportion of insufficient results. In 28% (11 of 39) of all insufficient results, protocols were based on a pAb. None of the protocols based on a pAb (11 of 11) provided a sufficient result (good or optimal). Compared to the

previous run for SOX10, the proportion of laboratories using pAbs has significantly decreased from 45% (39 of 86) in run 45, 2015 to 9% (11 of 120) in this run. The staining results based on pAbs were typically characterized by too weak specific staining reaction combined with aberrant and excessive background staining compromising interpretation. Thus, to improve the performance in laboratories still using a protocol based on a pAb, it is advisable to change the primary antibody to e.g. BC34 or EP268.

Controls

Skin and colon are recommended as positive and negative tissue controls for SOX10. In skin, a 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 colon, virtually all Schwann cells must display an as strong as possible nuclear staining reaction without any staining reaction of epithelial and smooth muscle cells. At present, and as specified in run 45, 2015, no tissue with consistent low-level expression of SOX10 has been identified reliable as positive tissue control to monitor technical sensitivity. Due to this issue both skin and colon are needed as tissue controls for SOX10.

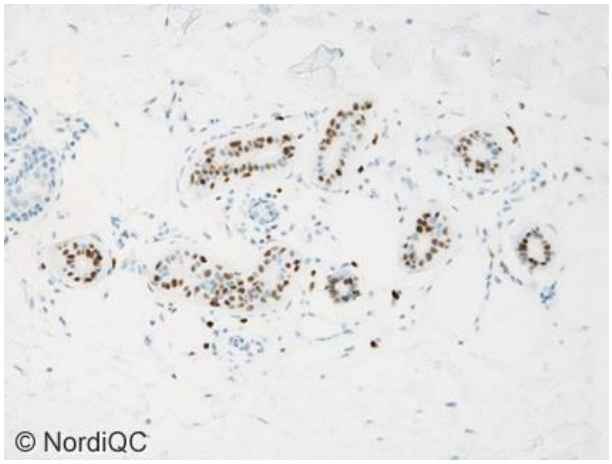

Fig. 1a (x200)
Optimal staining for SOX10 of the skin using the mAb BC34 as a concentrate, HIER in an alkaline buffer (CC1) and the multimer based detection system (OptiView, Ventana) - same protocol used in Figs. 2a - 6a. The majority of myoepithelial cells lining the sweat glands in the skin show a distinct, moderate to strong nuclear staining reaction.

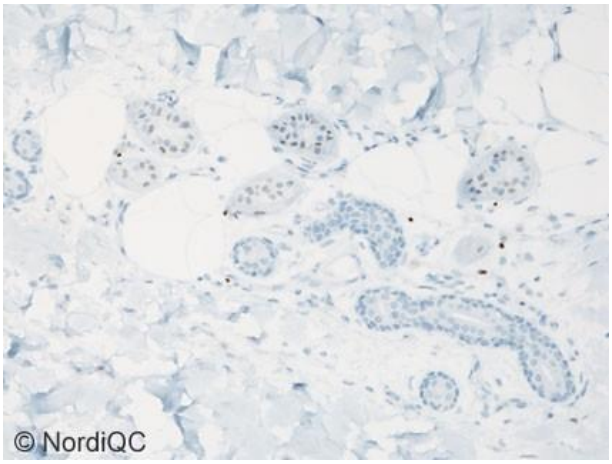

Fig. 1b (x200)
Insufficient staining for SOX10 of the skin using the mAb clone BC34 as concentrate by protocol settings giving a too low technical sensitivity - too diluted and too short efficient HIER time in CC1 - same protocol used in Figs. 2b – 4b. The proportion of positive cells and the intensity of the staining reaction is significantly reduced - compare with Fig. 1a.

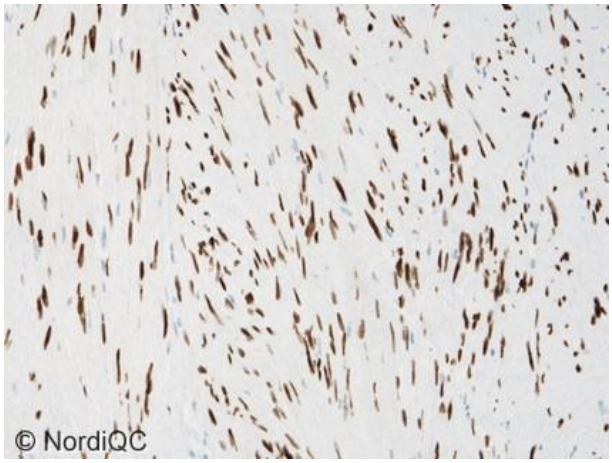

Fig. 2a (x200)
Optimal staining for SOX10 in the Schwannoma using same protocol as in Fig. 1a. Virtual all neoplastic cells show a distinct and strong nuclear staining.

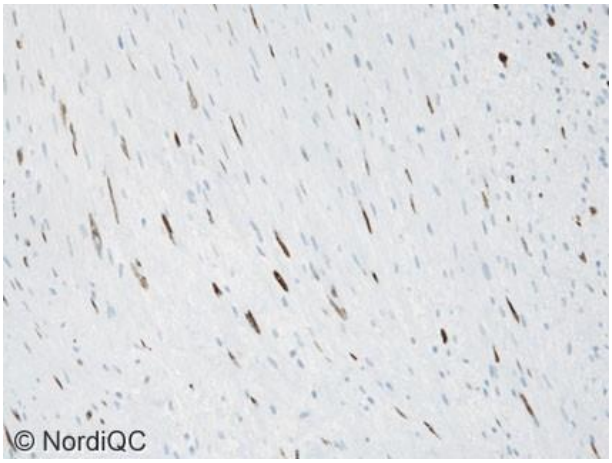

Fig. 2b (x200)
Insufficient staining for SOX10 in the Schwannoma using same protocol as in Fig. 1b. Staining intensity of the neoplastic cells is too weak and proportion of positive cells is reduced - compare with Fig. 2a.

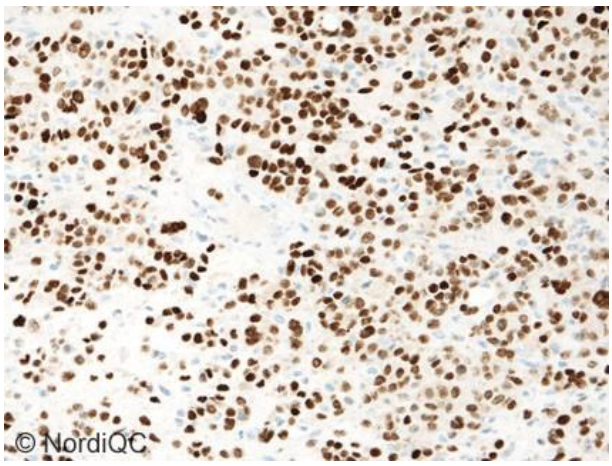

Fig. 3a (x200)
Optimal staining for SOX10 of the malignant melanoma, tissue core no. 5 using same protocol as in Figs. 1a & 2a. All the neoplastic cells show a strong and distinct nuclear staining reaction.

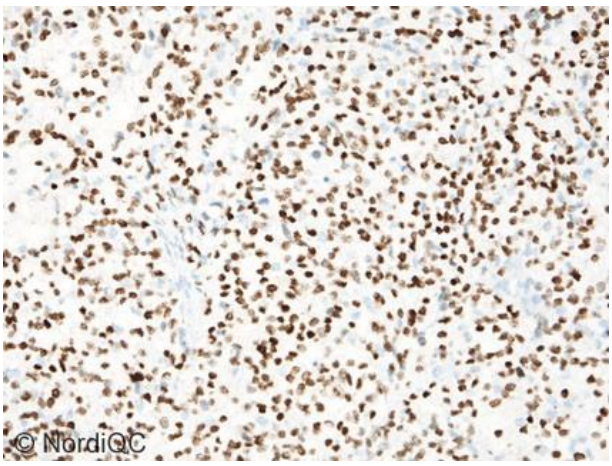

Fig. 3b (x200)
Insufficient staining for SOX10 of the malignant melanoma, tissue core no. 5 using same protocol as in Figs. 1b & 2b. The intensity and proportion of stained neoplastic cells is comparable to the result obtained in Fig. 3a (same field). However, also compare with results in Figs. 4a and 4b.

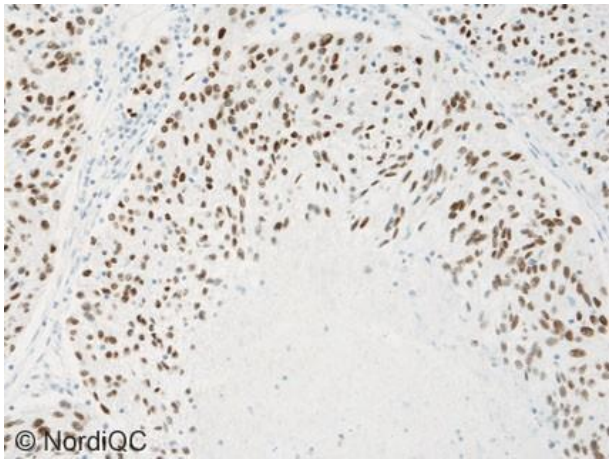

Fig. 4a (x200)
Optimal staining for SOX10 of the malignant melanoma, tissue no. core 6 using same protocol as in Figs. 1a - 3a. Virtually all neoplastic cells show a moderate to strong nuclear staining reaction.

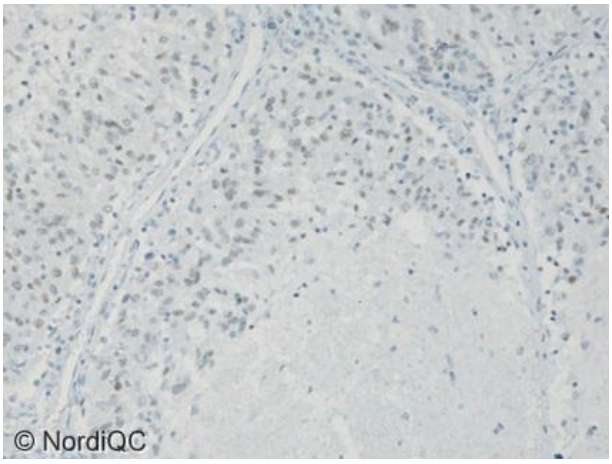

Fig. 4b (x200)
Insufficient staining for SOX10 of the malignant melanoma, tissue core no. 6 using same protocol as in Figs. 1b -3b. The neoplastic cells are false negative or only faintly stained - compare with Fig. 4a (same field).

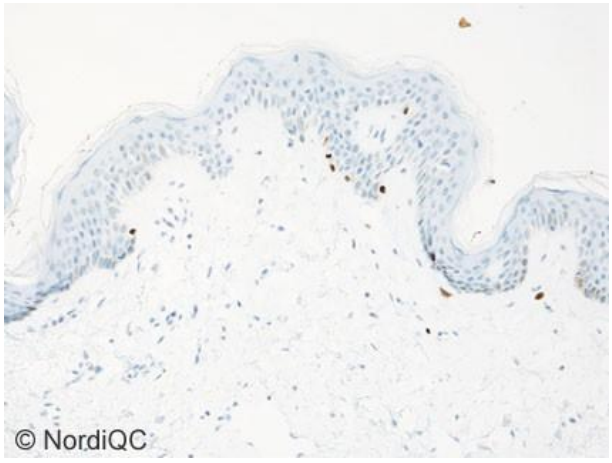

Fig. 5a (x200)
Optimal staining for SOX10 of the skin using same protocol as in Figs. 1a - 4a. Virtual all melanocytes show a strong, distinct nuclear staining reaction. No background staining is seen.

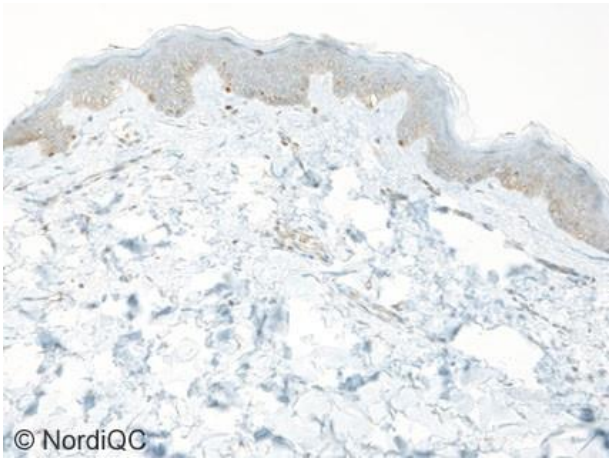

Fig. 5b (x200)
Insufficient staining of the skin using the pAb 383A-76 (Cell Marque) diluted 1:25, HIER in CC1 pH 8.5 for 52 min., UltraView with amplification as the detection system and performed on the Benchmark Ultra (Ventana/Roche). The melanocytes are difficult to identify and the squamous epithelial cells displays an aberrant cytoplasmic staining reaction, a pattern typical seen with all pAbs - compare with Fig. 5a.

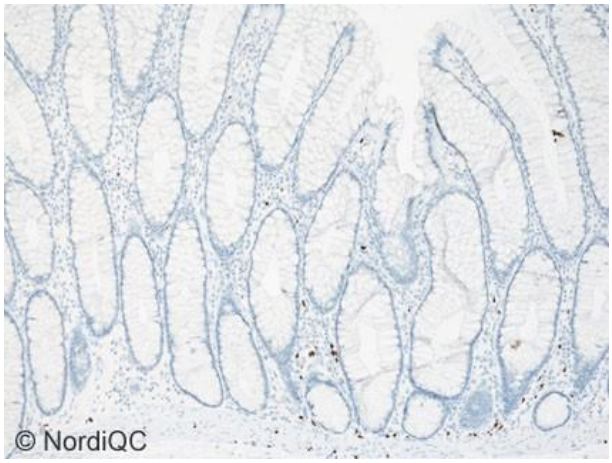

Fig. 6a (x100)
Optimal staining for SOX10 of the colon using same protocol as in Figs. 1a - 5a. The Schwann cells show a strong, distinct nuclear staining reaction. No background staining is seen.

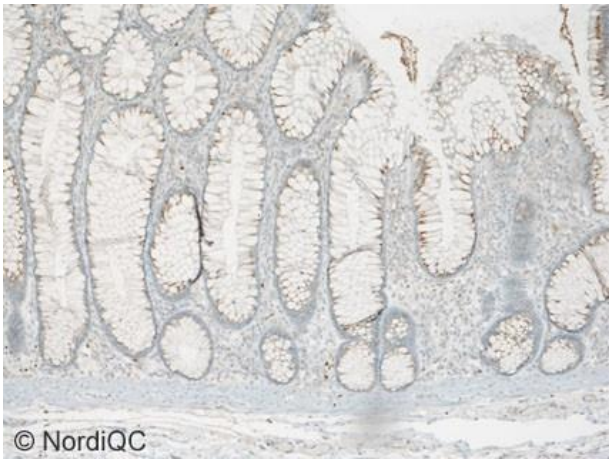

Fig. 6b (x100)
Insufficient and aberrant staining for SOX10 of the colon using the same protocol settings as in Fig. 5b. Both epithelial and stromal cells are displaying an aberrant cytoplasmic staining reaction and the Schwann cells are only weakly labelled or completely false negative – compare with Fig. 6a (same field).

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