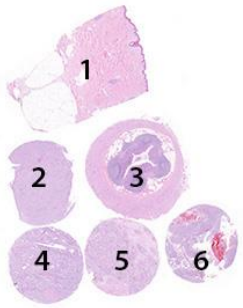
Assessment Run 55 2019

Transcription factor SOX-10 (SOX10)

Material
The slide to be stained for SOX10 comprised:



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

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a SOX10 staining as optimal included:

- A strong, distinct nuclear staining reaction of virtually all melanocytes in the skin and Schwann cells in the appendix.
- An at least moderate, distinct nuclear staining reaction of the majority of myoepithelial cells lining sweat glands in the skin.
- A strong, distinct nuclear staining reaction of virtually all neoplastic cells in the Schwannoma and the malignant melanoma, tissue core no. 6.
- An at least moderate, distinct nuclear staining reaction of the majority of neoplastic cells in the malignant melanoma, tissue core no. 5.
- 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 55	211
Number of laboratories returning slides	204 (97%)

Results
204 laboratories participated in this assessment. 181 (89%) 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 – especially polyclonal Abs (pAbs)
- Insufficient Heat Induced Epitope Retrieval (HIER) (too short heating time or HIER in acidic buffer).
- Less sensitive detection systems
- Unexplained technical issues

Performance history
This was the third NordiQC assessment of SOX10. The overall pass rate improved significantly compared to the result obtained in run 48, 2016 (see Table 2).

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

	Run 45 2015	Run 48 2016	Run 55 2019
Participants, n=	86	120	204
Sufficient results	45%	68%	89%

Conclusion
The mAb clones **BC34, BC7, ZM10**, and the rmAb clones **EP268** and **SP267** could all be used to obtain an optimal result for SOX10. Irrespective of the clone applied, efficient HIER (preferable in an alkaline buffer), a precise calibration of the primary Ab and the use of a 3-step multimer/polymer based detection system, were the main prerequisite for an optimal result. The RTU system 760-4968 (Ventana) based on the rmAb clone SP267, showed superior performance providing a pass rate of 100% of which 91% (58 of 64) were assessed as optimal.

Skin and colon/appendix are recommendable positive and negative tissue controls for SOX10. Virtually all melanocytes of the skin and Schwann cells of the appendix/colon must display a strong nuclear staining reaction, while the majority of myoepithelial cells surrounding sweat glands in skin must show an at least moderate, but distinct nuclear staining reaction. No reactions should be seen in other cells.

Table 1. **Antibodies and assessment marks for SOX10, run 55**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BC34**	45 1 1 1	Biocare Medical Abcam Zytomed Systems Menarini Diagnostics	31	7	10	0	79%	96%
mAb clone **BS7**	8	Nordic Biosite	7	0	1	0	-	-
mAb clone **ZM10**	1	Zeta Corporation	1	0	0	0		
mAb clone **A-2**	1	Santa Cruz	0	1	0	0	-	-
mAb clone **SOX10/1074**	1	Immunologic	0	0	1	0	-	-
rmAb clone **EP268**	40 4 4 1	Cell Marque Epitomics BioSB Diagnostic Biosystems	30	16	2	1	94%	93%
rmAb clone **SP267**	2	Spring Bioscience	1	1	0	0	-	-
pAb **383A-76**	5	Cell Marque	0	0	3	2	-	-
Ready-To-Use antibodies								
mAb clone **BC34** **API 3099**	1	Biocare Medical	1	0	0	0	-	-
mAb clone **BC34** **API 3099**[3]	7	Biocare Medical	3	3	1	0	-	-
rmAb clone **EP268**[4] **383R**	11	Cell Marque	3	7	1	0	-	-
rmAb clone **EP268** **MAD-000656QD**	2	Master Diagnostica	2	0	0	0	-	-
rmAb clone **EP268** **RMA-0726**	1	Maixin	1	0	0	0	-	-
rmAb clone **EP268**[4] **PR135**	1	PathSitu Biotechnologies	1	0	0	0	-	-
rmAb clone **EP268**[4] **BSB-2582**	1	BioSB	0	0	1	0	-	-
rmAb clone **EP268** **8264-M250**	1	Sakura Finetek	0	1	0	0	-	-
rmAb clone **SP267** **760-4968**	64	Ventana/Roche	58	6	0	0	100%	100%
Total	204		139	42	20	3	-	
Proportion			68%	21%	10%	1%	89%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only (see below).
3) Ready-to-use product developed for a specific semi/fully automated platform by a given manufacturer but inappropriately applied by laboratories on other non-validated semi/fully automatic systems or used manually.
4) RTU format not developed for a specific IHC system and used by laboratories on different platforms as e.g. Ventana Benchmark Ultra/XT or Leica BOND III.

Detailed analysis of SOX10, Run 55

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb **BC34**: Protocols with optimal results were all based on HIER using an alkaline buffer as Cell Conditioning 1 (CC1, Ventana) (21/25)*, Target Retrieval Solution (TRS) (3-in-1) pH 9 (Dako) (5/9), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (4/8) or Novocastra™ Epitope Retrieval Solutions pH 9 (Leica) (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings, 25 of 26 (96 %) 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 all based on HIER using either TRS (3-in-1) pH 9 (Dako) (3/3), BERS2 (Leica) (1/1), CC1 (Ventana) (1/1), Tris-EDTA/EGTA pH 9 (1/1) or TRS (3-1) pH 6 (Dako) (1/2). The mAb was typically diluted in the range of 1:100-1:200 depending on the total sensitivity of the protocol employed.

mAb **ZM10**: One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9. The mAb was diluted 1:60 and GTVision (Gene Tech) was used as the detection system.

rmAb **EP268**: Protocols with optimal results were all based on HIER using either CC1 (Ventana) (12/21), TRS High pH (3-in-1) pH9 (Dako) (15/22), BERS2 (Leica) (1/3), DBS Montage EDTA Antigen Retrieval Solution (Diagnostic Biosystem) (1/1), Tris-EDTA/EGTA pH 9 (1/1) and Bond Epitope Retrieval Solution 1 (BERS1, Leica) (1/1) as retrieval buffer. 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, 38 of 41 (93 %) laboratories produced a sufficient staining.

rmAb **SP267**: One protocol with an optimal result was based on HIER using CC1 (Ventana). The rmAb was diluted 1:100 and OptiView (Ventana) was used as the detection system.

Table 3. **Proportion of optimal results for SOX10 for the most commonly used antibodies as concentrate on the four main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	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**	0/0**	0/1	2/2	0/0	17/20 (85%)	0/0	3/4	0/0
rmAb clone **EP268**	3/5	-	8/10 (80%)	-	10/18 (56%)	-	1/3	1/1

* 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,** 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 the detection system.

rmAb clone **SP267,** product no. **760-4968,** Ventana/Roche Benchmark Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 32 min. incubation time of primary Ab, UltraView with or without amplification (760-500 + 760-080) or OptiView (760-700) as the detection system. Using these protocol settings, 49 of 49 (100%) laboratories produced an optimal result.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 asessed protocols). The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly 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 4. **Proportion of sufficient and optimal results for SOX10 for the most commonly used RTU IHC system**

RTU system	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT/GX rmAb SP267 **760-4968**	100% (36/36)	94% (34/36)	100% (25/25)	84% (21/25)

* 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 >25%, detection kit – only protocols performed on the specified vendor IHC stainer were included.

Comments
In this assessment and in concordance with the observations in previous NordiQC assessments of SOX10, the prevalent feature of an insufficient staining reaction was a too weak or false negative staining reaction of cells expected to be demonstrated. Too weak or false negative staining reaction was seen in 65% of the insufficient results (15 of 23). The majority of the laboratories were able to stain for SOX10 in Schwann cells of the appendix, the neoplastic cells of the Schwannoma and of the melanoma tissue core no. 6, whereas demonstration of SOX10 in the neoplastic cells of the melanoma tissue core no. 5, normal melanocytes and myoepithelial cells of the skin was more challenging and required a carefully calibrated protocol. In 35% (8 of 23) 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 within a laboratory developed (LD) assay. Used as a concentrate, mAb clone BC34 gave an overall pass rate of 79% (38 of 48) of which 58% was optimal (see Table 1). As shown in Table 3, optimal results could be obtained on the three main fully automated IHC platforms from Dako, Leica and Ventana. Efficient HIER in alkaline buffer, careful calibration of the primary Ab and use of a sensitive detection system were the most central parameters for optimal results. As noted in the previous run for the concentrated format of mAb clone BC34, efficient HIER time is important influencing the overall analytical sensitivity of the assays. If HIER in alkaline buffer (at 95-100°C) was applied, and a standard 2- or 3-step multimer/polymer detection system was used, the efficient Average HIER Time (AHT) for optimal results was 44 min. (range 20-64 min.), whereas an AHT of 30 min. (range 20-64 min.) was seen in protocols with insufficient results. In addition, titre of the primary Ab influenced the general performance of the analyses. Using the same protocol conditions as mentioned above, the Average Dilution Value (ADV) for optimal results was 1:57 (range 1:30-1:200), whereas a ADV of 1:93 (range 1:25-1:200) 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 basic requirements for demonstration of SOX10 in structures with both low-level and high-level SOX10 expression, which is the range seen in e.g. different melanocytic lesions.
Sensitivity of the detection system also influenced the overall performance and final outcome of the results. In protocols assessed as optimal applying the protocol settings described above, 97% (30 of 31) of the laboratories used a 3-step multimer/polymer system (e.g. OptiView/Ventana or Bond Refine/Leica). In protocols assessed either as good or borderline, only 69% (9 of 13) of the laboratories applied a 3-step multimer/polymer system.

The LD assays based on rmAB clone EP268 provided a pass rate of 94% (46 of 49) of which 61% were assessed as optimal. A weak to moderate cytoplasmic staining reaction of neurons was seen which was fully accepted as the interpretation of the specific nuclear staining reaction was not compromised. The prerequisites for obtaining an optimal result were the use of efficient HIER in alkaline buffer and the use of a 3-step polymer / multimer based detection system.

Using the concentrate format of mAb clone BS7, 88% (7 of 8) of the stainings were assessed as optimal. One laboratory with an insufficient mark, applied a protocol with too low sensitivity based on HIER in acidic buffer and too diluted primary Ab.

None of the pAbs were assessed as sufficient (5 of 5), typically providing to too weak/false negative staining results in combination with poor signal-to-noise ratio.

In this assessment, the RTU system 760-4968 (Ventana) based on the rmAb clone SP267 was the most often used assay for detection of SOX10. The number of participants using this system, has increased dramatically from the previous run 48 (2016) to this run 55 (2019) – from 5 to 64. In these two assessments, the pass rate was 100% (69 of 69) of which 91% (63 of 69) were assessed as optimal. As shown in Table 4, optimal results were primarily obtained by using the RTU system accordingly to the protocol recommendations provided by the vendor. For laboratories applying OptiView as the detection system, 100% (22 of 22) of the slides were assessed as optimal. Laboratory modified protocol settings (typically adjusting HIER and incubation time of the primary Ab) could also provide optimal results, but proportion of optimal results decreased from 94% to 84% compared to vendor recommended protocol settings. Thus, participants should follow the recommendation of the vendor, and advisable, apply OptiView as detection system acquiring an assay that, from a technical point of view, seems very robust and providing a high proportion of optimal results.

One laboratory obtaining an optimal result, used the RTU system API 3099 based on the mAb clone BC34 (Biocare Medical) developed for the IHC stainer IntelliPATH (see protocol settings above). However,

seven laboratories used the RTU format on platforms other than the IntelliPATH (Biocare). Such off-label use of an RTU format, validated for a given IHC system e.g. platform and immuno-reagents, is not advisable despite obtaining optimal results (see Table 1). 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. This inappropriate/incorrect use of an RTU product could also be seen with other RTU formats e.g. 383R based on the rmAb clone EP268 from Cell Marque.

This was the third assessment of SOX10 in NordiQC (see Table 2). The pass rate increased significantly compared to the latest run 48, 2016. Several parameters contributed to the positive development: 1) The extended use of robust primary Abs (e.g. EP268), 2) The superior performance of the RTU system 760-4968 (Ventana) based on the rmAb clone SP267 and applied by 31% of the participants, 3) The number of laboratories using pAbs, providing consistent poor results, has been reduced from 9% (11 of 120) in the previous run to 2% (5 of 204) in this assessment, 4) Laboratories following advices giving by the NordiQC organization in past runs, typical recommendations of HIER in an alkaline buffer, careful calibration of the primary Ab and the use of an 3-step multimer/polymer detection system.
Importantly, protocols must stain according to the expected antigen level of the recommended control material (see below).

Controls
Skin and colon/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 colon/appendix, virtually all Schwann cells must display an as strong as possible nuclear staining reaction without any staining reaction of epithelial cells and smooth muscle cells. At present, and as specified in previous assessments, no reliable tissue component with consistent low level expression of SOX10 has been identified, monitoring the overall analytical sensitivity of an assay. Due to this issue both skin and colon/appendix are needed as tissue controls for SOX10.

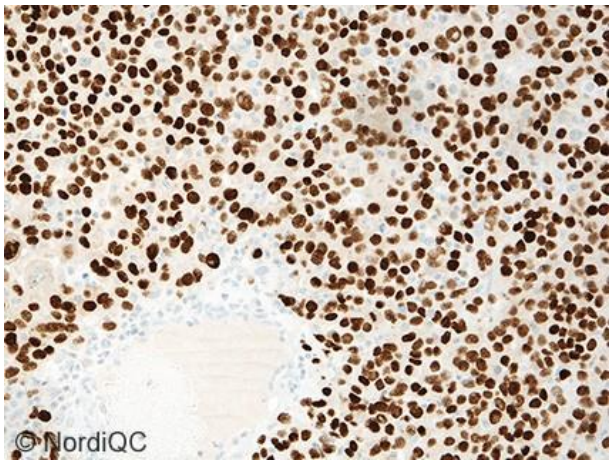


Fig. 1a (x200)
Optimal SOX10 staining of the melanoma (tissue core no. 6) using the mAb BC34 as a concentrate, HIER in an alkaline buffer (CC1) and OptiView (Ventana) as detection system – same protocol used in Figs. 2a-5a. All the neoplastic cells show a strong and distinct nuclear staining reaction (weak cytoplasmic staining reaction must be accepted).

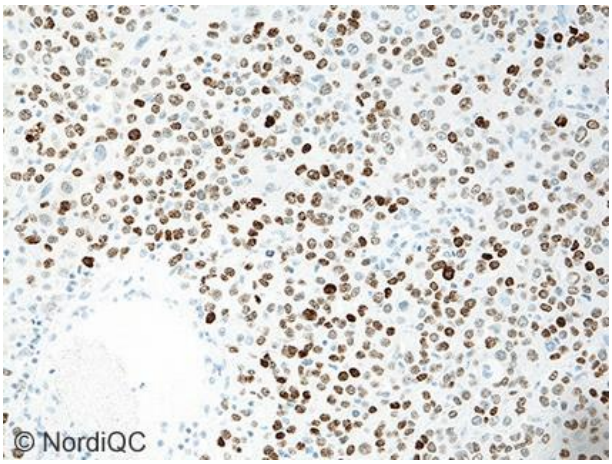


Fig. 1b (x200)
Insufficient SOX10 staining of the melanoma (tissue core no. 6) using the mAb BC34 as a concentrate (too diluted), HIER in CC1 and OptiView with amplification (Ventana) as detection system – same protocol used in Figs. 2b – 5b. Although nuclei of the neoplastic cells display relative strong staining intensity, the assay was challenged by too low analytical sensitivity - compare with Fig. 1a-5b.

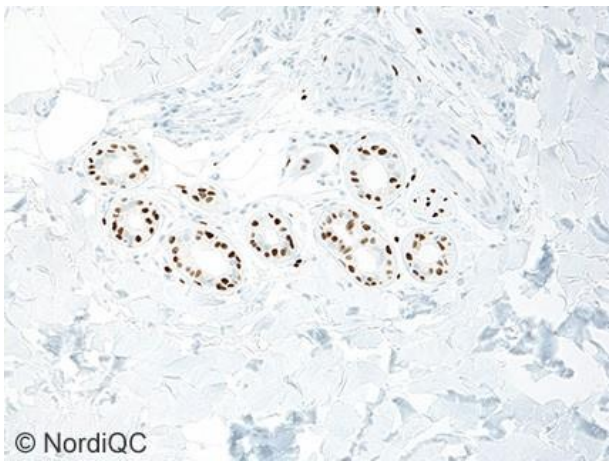


Fig. 2a (x200)
Optimal SOX10 staining of the skin using same protocol as in Fig. 1a. Virtually all myoepithelial cells of the sweat glands show a distinct, moderate to strong nuclear staining reaction.

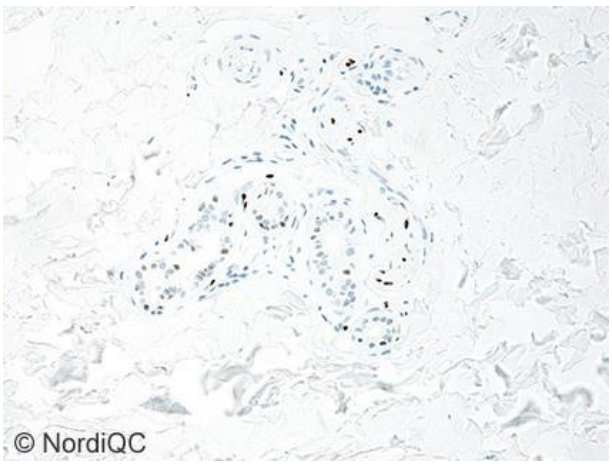


Fig. 2b (x200)
Insufficient SOX10 of the skin using same protocol as in Fig 1b. Staining intensity is reduced, and only dispersed nuclei of myoepithelial cells display weak to moderate reactions - compare with Fig. 2a.

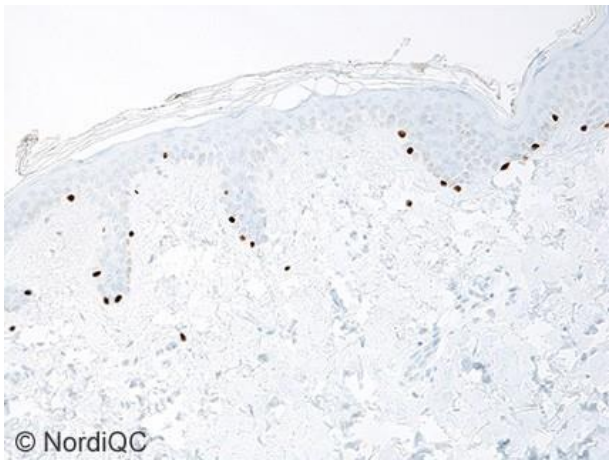


Fig. 3a (x200)
Optimal SOX10 staining of the skin using same protocol as in Figs. 1a-2a. Virtually all melanocytes display a strong, distinct nuclear staining reaction. No background staining is seen.

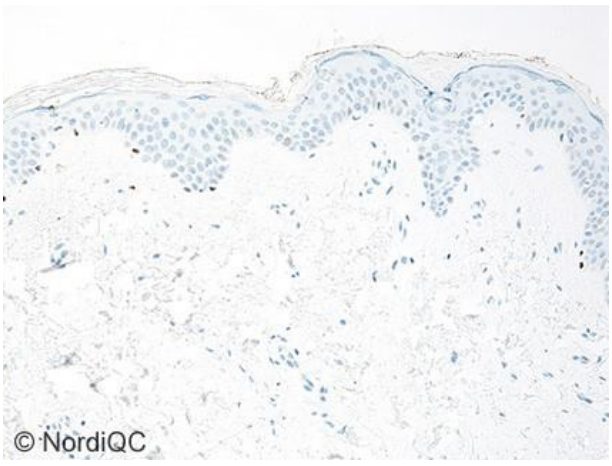


Fig. 3b (x200)
Insufficient SOX10 staining of the skin using same protocol as in Figs. 1b-2b. The intensity and proportion of positive melanocytes is significantly reduced – compare with Fig. 3a

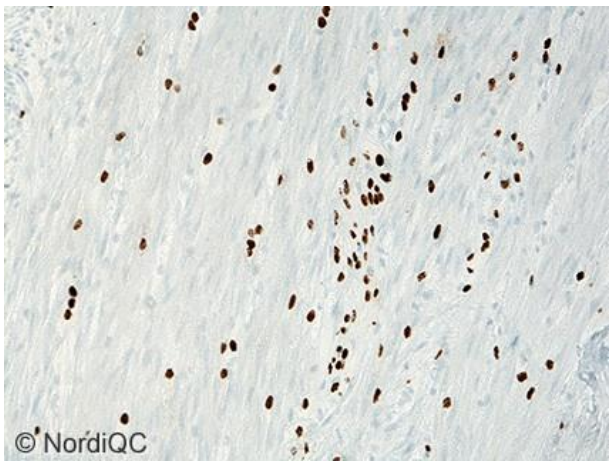


Fig. 4a (x200)
Optimal SOX10 staining of the appendix using same protocol as in Figs. 1a-3a. Virtually all Schwann cells in lamina muscularis propria show a strong nuclear staining reaction. The smooth muscle cells are negative.

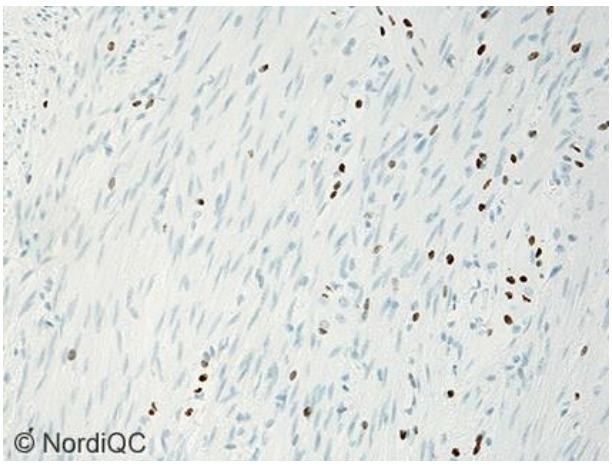


Fig. 4b (x200)
Insufficient SOX10 staining of the appendix using same protocol as in Figs. 1b-3b. The staining intensity of the vast majority of Schwann cells is reduced, displaying only weak to moderate nuclear staining reaction - compare with Fig. 4a (same field).

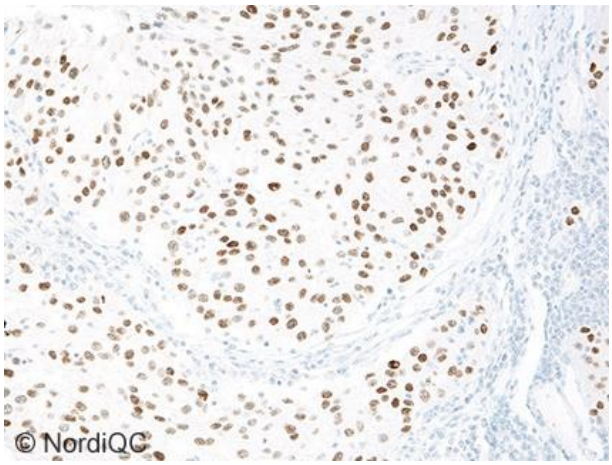


Fig. 5a (x200)
Optimal SOX10 staining of the melanoma (tissue core no. 5) using same protocol as in Figs. 1a-4a. The vast majority of the neoplastic cells show an at least moderate, distinct nuclear staining reaction.

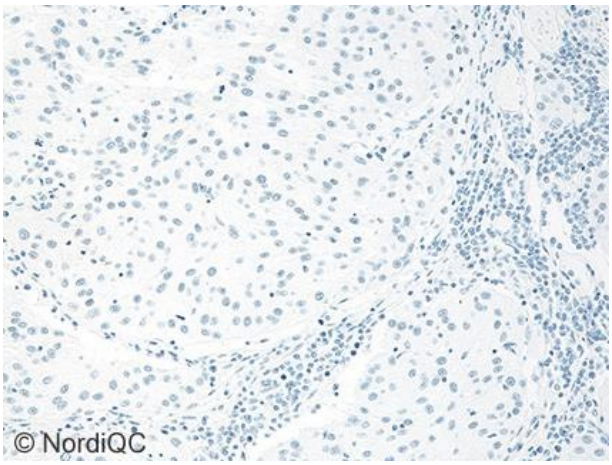


Fig. 5b (x200)
Insufficient SOX10 staining of the melanoma (tissue core no. 5) using same protocol as in Figs. 1b-4b. The neoplastic cells are false negative – compare with Fig. 5a.

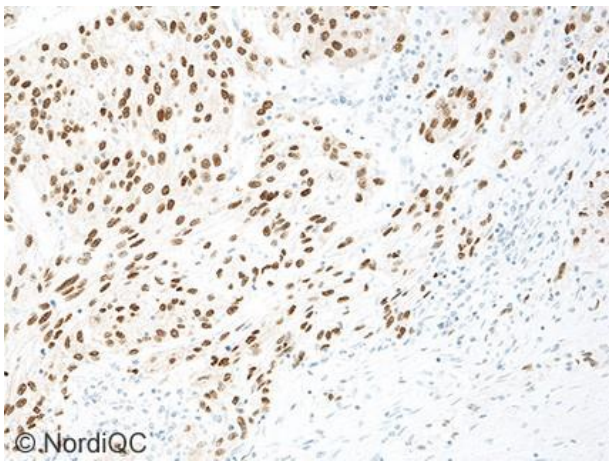


Fig. 6a (x200)
Optimal SOX10 staining of the melanoma (tissue core no. 5) using the RTU system 760-4968 (Ventana) based on the rmAb clone SP267, HIER in CC1 and OptiView as detection system. Virtually all of the neoplastic cells show moderate to strong, distinct nuclear staining reaction. Applying these protocol settings, 100% (22 of 22) of the slides were assessed as optimal.

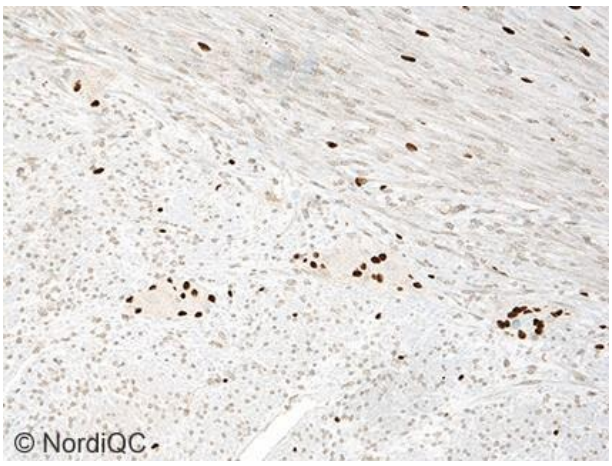


Fig. 6b (x200)
Insufficient SOX10 staining of the appendix using the mAb BC34 as a concentrate (diluted 1:25) on a Benchmark Ultra platform (Ventana), HIER in CC1 pH 8.5 (64 min.) and UltraView with amplification as the detection system. Although the protocol settings are in the optimal range, the nuclei of the smooth cells in lamina muscularis propria display a faint but distinct false positive nuclear staining reaction (Schwann cells are strongly and specific stained). No single parameters could be identified explaining this aberrant staining pattern, and thus, the laboratory is advised to repeat the staining without no further recommendations. However, the assay shown in Fig. 6a, displayed superior performance and is recommendable for participants struggling with optimization of SOX10 on the Benchmark platform (Ventana).

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