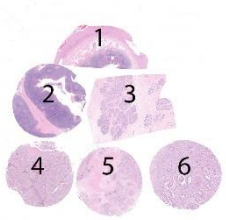
Assessment Run 45 2015

S-100 protein (S100)

[Recommended S100 protocols](#) [Recommended S100 control tissue](#)

Material

The slide to be stained for S100 comprised:



1. Appendix, 2. Tonsil, 3. Breast hyperplasia, 4-5. Malignant melanoma, 6. Colon adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing S100 staining as optimal included:

- A strong, distinct nuclear and cytoplasmic staining reaction of the vast majority of macrophages in lamina propria, Schwann cells of peripheral nerve fibres and ganglionic satellite cells in the muscularis propria and submucosa in the appendix.
- A moderate to strong, distinct nuclear and cytoplasmic staining reaction of the vast majority of myoepithelial cells in the breast, and no more than a moderate reaction in the epithelial cells.
- A weak to moderate, distinct nuclear and cytoplasmic staining reaction of the majority of neoplastic cells of the melanoma (core 4).
- A strong, distinct nuclear and cytoplasmic staining reaction of all neoplastic cells of the melanoma (core 5).
- A moderate to strong, distinct nuclear and cytoplasmic staining reaction of adipocytes and macrophages in all specimens.
- No staining of other cells. Especially all neoplastic cells in the colon adenocarcinoma, squamous epithelial cells in tonsil, smooth muscle cells and columnar epithelial cells in the appendix should be negative

In addition, for the polyclonal antibodies (Abs) Z0311 (Dako), NCL-L-S100p (Leica) and 760-2523 (Ventana), a weak cytoplasmic and nuclear staining reaction of the follicular dendritic cells in the germinal centres of the tonsil and the Peyer's plaques in the appendix was expected and accepted.

Participation

Number of laboratories registered for S100, run 45	296
Number of laboratories returning slides	251 (85%)

Results

251 laboratories participated in this assessment. 169 (68%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Insufficient HIER (too low temperature and/or too short heating time)
- Proteolytic pre-treatment or omission of epitope retrieval
- Low sensitive detection systems
- Unexplained technical issues

Performance history

This was the fourth NordiQC assessment of S100. The overall pass rate was relatively low and comparable with the result obtained in run 34, 2012 (see table 2).

Table 2: **Proportion of sufficient results for S100 in the four NordiQC runs performed**

	Run 7 2003	Run 20 2007	Run 34 2012	Run 45 2015
Participants, n=	63	106	200	251
Sufficient results	71%	75%	64%	68%

Conclusion

The concentrated pAbs **Z0311, NCL-L-S100p,** Ready-To-Use (RTU) formats **760-2523, IR/GA504** and the mAb clone **4C4.9** as RTU could all be used to obtain optimal staining results for S100. Irrespective of the primary Ab applied, efficient HIER, appropriate titre and incubation time tailored to the choice of IHC system were the most important prerequisites for an optimal staining result. The RTU systems (IR/GA504) for S100 from Dako provided the highest proportion of sufficient results. For all primary Abs applied, inferior results were obtained if HIER was omitted. Appendix is recommended as positive and negative tissue controls: The Schwann cells of peripheral nerves, macrophages/dendritic cells and adipocytes must show a moderate to strong distinct cytoplasmic and nuclear staining reaction. No staining reaction must be seen in other cell types including smooth muscle cells in lamina propria and columnar epithelial cells of the appendix.

In order to comply with the guidelines and requirements for an optimal S100 staining reaction given by the International Ad Hoc Expert Committee[1] it is recommended also to use tonsil as positive tissue control. In the tonsil (and Peyer's plaques of appendix), follicular dendritic cells must display an at least weak to moderate distinct nuclear and cytoplasmic staining reaction (only seen with the pAbs).

Table 1: **Antibodies and assessment marks for S100, run 45**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **4C4.9**	2 1 1 1	Immunologic Thermo/NeoMarkers Zytomed Systems Unknown	0	4	1	0	80%	-
mAb clone **15E2E2**	1	Biogenex	0	0	0	1	-	-
mAb clone **S1/61/69**	1	Leica/Novocastra	0	0	0	1	-	-
mAb clone **15E2E2+4C4.9**	2	BioCare	0	0	2	0	-	-
pAb **Z0311**	123	Dako	56	39	24	4	77%	88%
pAb **NCL-L-S100p**	9	Leica/Novocastra	2	2	3	2	44%	100%
Ready-To-Use systems								
mAb clone **4C4.9 790-2914**	24	Ventana	2	8	9	5	42%	100%
mAb clone **4C4.9 330M-18**	1	Cell Marque	0	0	1	0	-	-
mAb clone **4C4.9 MAD-001221QD**	2	Master Diagnostica	1	0	1	0	-	-
mAb clone **4C4.9 MON-RTU1191**	1	Monosan/Sanbio	0	0	1	0	-	-
mAb clone **15E2E2 AM058-5M**	1	Biogenex	0	0	1	0	-	-
mAb clone **16/F5 MAB-0697**	1	Maixin	0	1	0	0	-	-
pAb **IR504**	34	Dako	3	27	4	0	88%	95%
pAb **GA504**	13	Dako	5	6	2	0	85%	90%
pAb **760-2523**	26	Ventana	1	11	12	2	46%	100%
pAb **PA0900**	5	Leica/Novocastra	0	1	3	1	20%	-
pAb **PP021**	1	BioCare	0	0	1	0	-	-
pAb **E031**	1	Linaris	0	0	1	0		
Total	251		70	99	66	16	-	
Proportion			28%	40%	26%	6%	68%	

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

Detailed analysis of S100, Run 45
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
pAb **Z0311**: Protocols with optimal results were typically based on HIER using Cell Conditioning 1(CC1, Ventana) (31/58)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) or TRS pH 9 (12/24) , Tris-EDTA/EGTA pH 9 (4/6), Tris-HCL pH 9 (1/1), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (2/7), Diva Decloaker pH 9.5 (Biocare) (1/2) or Citrate pH 6 (4/7) as retrieval buffer. The pAb was typically diluted in the range of 1:300-1:4.000 depending on the total sensitivity of the protocol employed. Using these protocol settings, 71 of 81 (88%) laboratories produced a sufficient staining result (optimal or good). One protocol with an optimal result was based on proteolytic pre-treatment using Protease 1 (Ventana).
* (number of optimal results/number of laboratories using this HIER buffer)

pAb **NCL-L-S100p**: Protocols with optimal results were based on HIER using BERS2 (Leica) (2/4) as retrieval buffer. The pAb was diluted in the range of 1:450-1:1.600 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 of 3 (100%) laboratories produced a sufficient staining result.

Table 3: **Proportion of optimal results for S100 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
pAb **Z0311**	6/12** (50%)	-	29/47 (62%)	-	1/4 (25%)	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 and corresponding systems
pAb, product no. **IR504**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 15-20 min. at 97-98°C),20-30 min. incubation of the primary Ab and EnVision FLEX (K8000/K8010/K8002) as detection system. Using these protocol settings, 20 of 21 (95%) laboratories produced a sufficient staining result.

pAb, product no. **GA504**, Dako, Omnis:
Protocols with optimal results were based on HIER using TRS High pH 9 (GV804) (efficient heating time 30 min. at 97°C),17.5 min. incubation of the primary Ab and EnVision FLEX (GV800) as detection system. Using these protocol settings, 9 of 10 (90%) laboratories produced a sufficient staining result.

pAb, product no. **760-2523**, Ventana, BenchMark XT/Ultra:
One protocol with optimal result was based on HIER using CC1 (efficient heating time 32 min.) and 32 min. incubation of the primary Ab. OptiView (760-700) was used as the detection system.

mAb clone **4C4.9**, product no. **790-2914**, Ventana, BenchMark XT/Ultra:
Two protocols with optimal result were based on HIER using CC1 (efficient heating time 20-32min.) and 8-16 min. incubation of the primary Ab. UltraView +/- amplification (760-500) were used as detection systems. Using these protocol settings, 2 of 2 (100%) laboratories produced a sufficient staining result.

mAb clone **4C4.9**, product no. **MAD-001221QD**, Master Diagnostica, LAB Vision Autostainer 480:
One protocol with an optimal result was based on HIER using EDTA/EGTA pH 8 (efficient heating time 20 min. at 95°C), 10 min. incubation of the primary Ab and Quanto (MAD-021881QK) as detection system.

Comments
In this fourth NordiQC assessment of S100, the prevalent features of an insufficient staining result was a generally too weak or completely false negative staining reaction of the cells expected to be demonstrated. This was observed in 95% of the insufficient results (78 of 82).
The majority of participating laboratories were able to demonstrate S100 in Schwan cells (peripheral nerves), peripheral macrophages and in the neoplastic cells of the melanoma (core 5), whereas the demonstration of S100 in myoepithelial cells of the breast and in particular the neoplastic cells of the melanoma (core 4) was more challenging and only seen with appropriate protocol settings. 84% (210 of 251) of the laboratories were using a pAb (Z0311 (Dako), NCL-L-S100p (Leica) or 760-2523 (Ventana)) either as a concentrate or as a Ready-To-Use format.

The pAb Z0311 was the most widely used antibody for the demonstration of S100. Applied as a concentrate by a laboratory developed (LD) assay, pAb Z0311 gave an overall pass rate of 77% (95 of 123). As shown in table 3, optimal results could be obtained on all three main IHC platforms from Dako, Leica and Ventana.
HIER, preferably in an alkaline buffer, in combination with a careful calibration of the primary Ab seems to be the most critical parameters for a sufficient result. Omission of HIER or performing proteolytic pre-treatment was found to be less successful. 50% (7/14) of protocols omitting HIER or using proteolytic pre-treatment gave an insufficient staining result and only one (7%) provided an optimal result.
The concentration of the primary Ab and the choice of detection system also had a great impact on the performance. For the pAb Z0311 it was observed that applying HIER (any retrieval buffer), a titer in the range 1:300-1:4.000 and the use of a detection system of the laboratories own choice, 87% (72 of 83) of the protocols were assessed as sufficient and 60% (49 of 83) were marked as optimal. If the same condition was applied but using a titer in the range of 1:5.000-13.000, both the proportion of sufficient and optimal results was reduced giving an overall pass rate of 75% (30 of 40) of which 42% (17 of 40) were assessed as optimal.
Both 2- and 3-step multimer/polymer based detection systems could be used to obtain an optimal result. However the combination of HIER in alkaline buffers, titer range 1:300-4.000 of the pAb Z0311 and the use of 3-step multimer/polymer detection systems as Flex+ (Dako) or OptiView (Ventana) provided an improved performance. Using a 3-step system, an overall pass rate of 93% (14 of 15) was seen and 87% (13 of 15) were marked as optimal. In comparison, using same protocol settings for Z0311 but with a 2-step multimer/polymer detection systems as Flex (Dako) or UltraView (Ventana) a lower pass rate of 72% (41 of 57) was observed with only 58% (33 of 57) evaluated as optimal.

Nine laboratories used the pAb NCL-L-S100p within a LD assay and 44% (4 of 9) produced a sufficient result of which 22% (2 of 9) were assessed as optimal. The pAb was mainly applied on the Leica BOND platforms, BOND-III/MAX, and the parameters essential for an optimal performance were HIER in BERS2 pH9 (Leica), a dilution of the pAb NCL-L-S100p in the range of 1:450-1:1600 and using BOND refine (DS9800, Leica) as the detection system. Using these settings, 100% (3 of 3) were producing a sufficient result of which 66% (2 of 3) were evaluated as optimal. Two of remaining protocols stained on a BOND-III/MAX were using the same conditions except for pre-treatment in BERS1 pH6 (Leica) or performing proteolytic digestion with "Bond Enzyme Concentrate" for 2 min at 37°C. These two protocols were assessed as insufficient.

In this assessment, the Ready-To-Use (RTU) system from Dako, IR504 (Autostainer) based on a pAb, provided a higher pass rate but lower proportion of optimal results compared to laboratory developed (LD) assays using the pAb Z0311 as concentrate (see table 1).
Optimal results for this RTU format were typically obtained by using the official protocol recommendations given by the company. Laboratory modified protocol settings (typically adjusting HIER, incubation time of the primary Ab and/or choice of detection system) could also provide sufficient and optimal result. The vast majority of protocols based on this RTU system were applied as plug-and-play following the basic recommendations given by the vendor. In general, the result was fully sufficient but also revealed a less sensitive IHC system at its limit for optimal performance.
Compared to the RTU system IR504, the proportion of optimal results was higher with the RTU system GA504 (Dako, Omnis) although the pass rate was slightly lower (although the number of protocols was low and the data must be interpreted with care). Following the instructions given by the vendor, 50% (5 of 10) of the protocols were assessed as optimal and 90% (9 of 10) were evaluated as sufficient (optimal or good).

The two RTU systems from Ventana, 760-2523 and 790-2014 based on a pAb and the mAb 4C4.9, respectively, gave a low proportion of sufficient and optimal results (see table 1).
Optimal results could only be achieved with laboratory modified protocol settings for both RTU formats. For the RTU format 760-2523 on the Benchmark Ultra/XT, 19% (5 of 26) of the protocols were based on omission of epitope retrieval as recommended in the package insert from the vendor and all were assessed as insufficient. This RTU format was shown to require efficient HIER in CC1, incubation of the primary Ab for at least 24 min. in combination with a high sensitive detection system as OptiView to provide the sensitivity needed to demonstrate S100 in challenging tissue structures such as interfollicular dendritic cells in the tonsillar tissue and the melanoma tissue core 4. Using these laboratory modified settings, 100% (3 of 3) of the protocols obtained a sufficient mark.
Exactly the same pattern was seen with the RTU format 790-2914 based on the mAb 4C4.9. For participants following the recommendation (omission of epitope retrieval) given by the vendor, 90% (9 of 10) of the protocols were evaluated as insufficient and none was assessed as optimal. In comparison, for protocols based on HIER in CC1, the pass rate was significantly increased as 79% (9 of 12) were given a sufficient mark and 17% (2 of 12) were assessed as optimal. For this particular RTU system, it is also

strongly advisable to apply a high sensitive IHC system e.g. performing efficient HIER in CC1, prolonged incubation time with primary Ab and use of a sensitive 3-step labelled multimer system such as UltraView with amplification or OptiView. The official recommendation for the product is based on omission of epitope retrieval, reduced incubation time of the primary Ab and iView as detection system and cannot be recommended.

The RTU system from Leica, PA0900 based on a pAb, gave a pass rate of 20% (1 of 5). The protocol with a sufficient result was assessed as good. According to the vendors package insert, proteolytic pre-pretreatment should be applied before staining. For laboratories following this recommendation and using the Bond Enzyme 1 (10-15 min incubation), 66% (2 of 3) were giving an insufficient mark. The two remaining protocols, both assessed as insufficient, were based on omission of epitope retrieval or HIER in BERS1 pH 6 (Leica). As mentioned above and specified under concentrated Abs, this primary Ab may require HIER in BERS2 pH9 (Leica) for optimal performance.

This was the fourth assessment of S100 in NordiQC (table 2). A pass rate of 68% was obtained, which only is a minor improvement compared to 64% in run 34, 2012. The frequent use of non-HIER methods (proteolytic pre-treatment or omission of retrieval) by participants together with poorly calibrated IHC systems accounts for the overall disappointing low pass rate in this run. In addition, the guidelines (package inserts) from certain companies both in relation to their respective RTU systems but also as concentrates, especially concerning the epitope retrieval procedures (no or proteolytic pre-treatment), is misleading and should prompt companies to change their spec sheets. Altogether, 16% (40 of 251) of the protocols assessed were based on non-HIER methods and 23% (9 of 40) were giving a sufficient mark of which only 3% (1 of 40) were giving an optimal mark. Best performance was obtained with the RTU systems IR504 (Autostainer, Dako) or GA504 (Omnis, Dako), giving the highest proportion of sufficient results.

Controls

Appendix is recommended as positive and negative tissue controls for S100. Virtually all adipocytes, satellite cells, Schwann cells and dendritic cells must be stained as strongly as possible without any staining reaction of the smooth muscle and epithelial cells.

As a supplement to appendix, tonsil can be used as positive tissue control. This in concordance with previous runs and the new guidelines recommended by the International Ad Hoc Expert Committee[1] to use tissue and structures with low level of antigen expression to secure and monitor an appropriate technical and analytical sensitivity of the test. To comply with this recommendation follicular dendritic cell in germinal centres must show a distinct weak to moderate nuclear and cytoplasmic staining reaction. This staining pattern is only seen for pAbs towards S100 and most likely caused by demonstration of a S100 alpha unit. From the Committee it is also recommended to use pancreas as positive tissue control in which endocrine cells of islets of Langerhans must show weak to strong cytoplasmic and nuclear reaction (all Abs for S100).

In coming runs for S100, the NordiQC organization will take the new recommendation given by the International Ad Hoc Expert Committee into consideration and include relevant tissue for assessment.

[1]Torlakovic EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18. doi: 10.1097/PAI.0000000000000163. Review. PubMed PMID: 25474126.

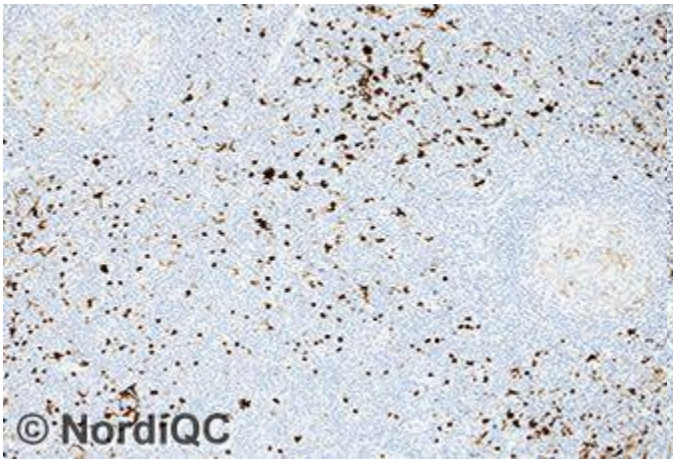


Fig. 1a (x100)
Optimal S100 staining of the tonsil using the pAb Z0311 as concentrate, HIER in an alkaline buffer (BERS2, pH 9) and a 3-step polymer based detection system (BOND Refine, Leica) – same protocol used in Figs. 2a – 4a. The majority of the interfollicular dendritic cells /macrophages are strongly stained, while the follicular dendritic cells in germinal centers show a weak but distinct cytoplasmic and nuclear staining reaction (this pattern were seen with all pAbs included in this assessment) – also compare with Fig. 1b.

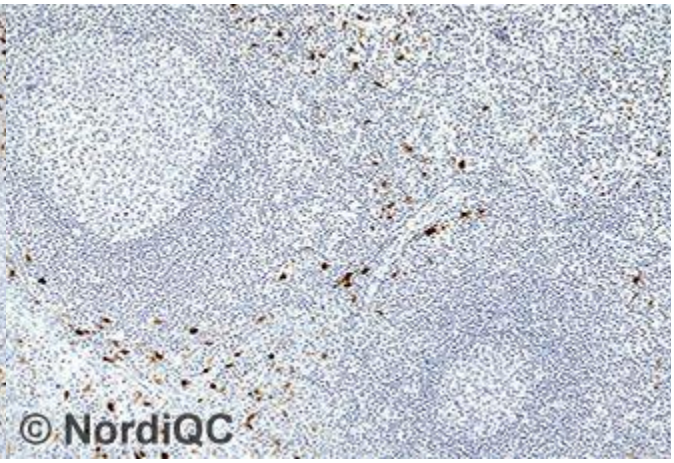


Fig. 1b (x100)
Insufficient S100 staining of the tonsil using the pAb Z3011 as a concentrate, proteolytic pre-treatment (BOND Enzyme 1), and a 3-step polymer based detection system (BOND Refine, Leica) – same protocol used in Figs. 2b-4b. The proportion and intensity of cells expected to be stained is significantly reduced and at the same time the morphology is impaired due to excessive digestion – compare with Fig. 1a (same field).

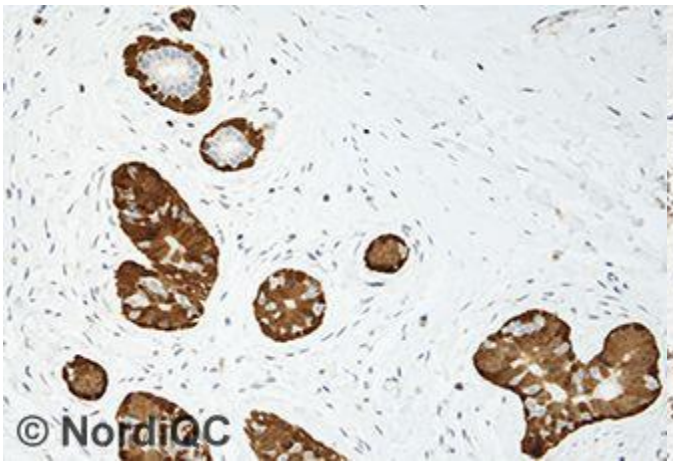


Fig. 2a (x200)
Optimal S100 staining of the breast hyperplasia using same protocol as in Fig. 1a. The myoepithelial cells show a strong cytoplasmic and nuclear staining reaction. A weaker intensity of secretory cells is seen – also compare with Fig. 2b.

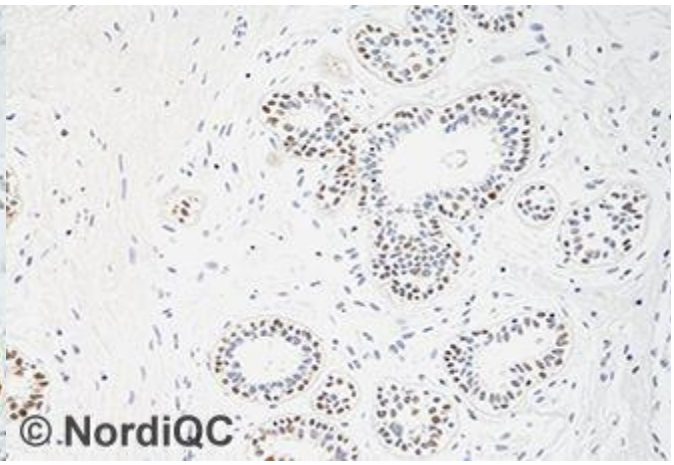


Fig. 2b (x200)
Insufficient S100 staining of the breast hyperplasia using same protocol as in Fig. 1b. The myoepithelial cells only show a weak and equivocal predominately nuclear staining reaction – compare with Fig. 2a (same field).

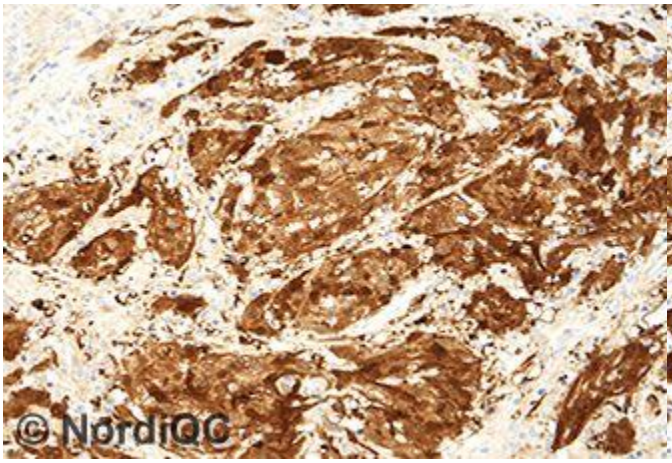


Fig. 3a (x200)
Optimal S100 staining of the melanoma (tissue core 5, high expressor) using same protocol as in Figs. 1a & 2a. All neoplastic cells show a strong cytoplasmic and nuclear staining reaction. – compare with Figs. 3b, 4a and 4b.

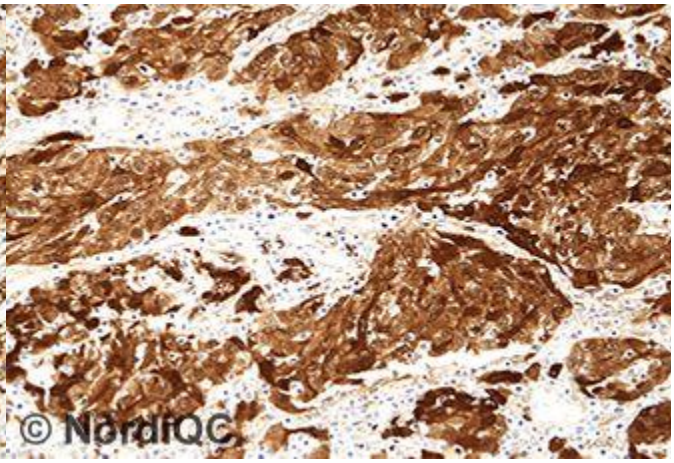


Fig. 3b (x200)
Insufficient S100 staining of the melanoma (tissue core 5, high expressor) using same protocol as in Figs. 1b & 2b. Although the staining reaction is comparable with the optimal result in Fig. 3a, the protocol provided a general too low sensitivity – compare with Figs. 3a, 4a and 4b (same fields).

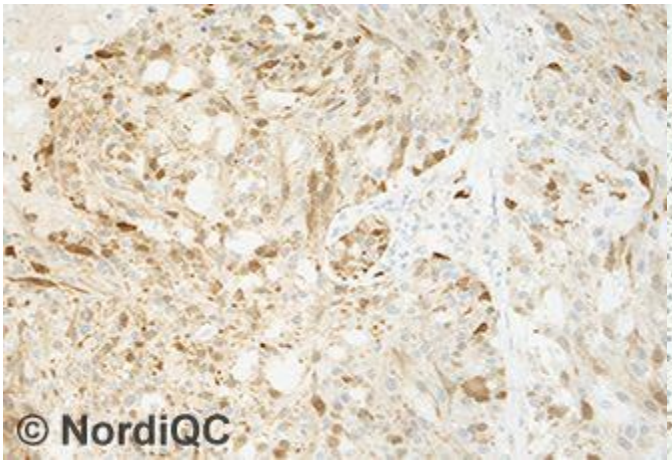


Fig. 4a (x200)
Optimal S100 staining of the melanoma (tissue core 4, low expressor) using same protocol as in Figs. 1a-3a. The vast majority of the neoplastic cells show a weak to moderate cytoplasmic and nuclear staining reaction – compare with Fig. 4 b.

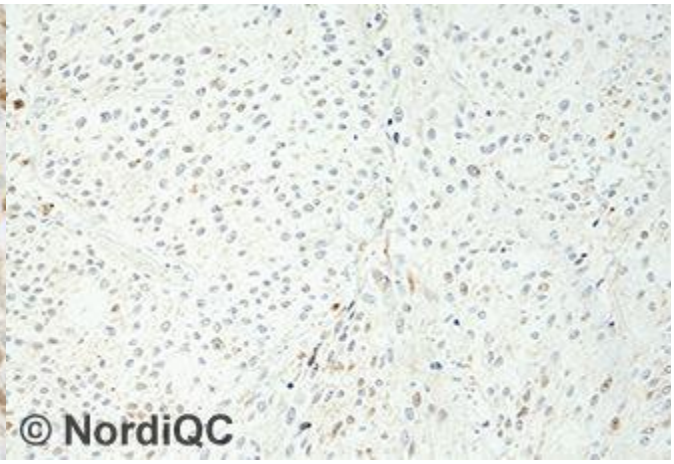


Fig. 4b (x200)
Insufficient S100 staining of the melanoma (tissue core 4, low expressor) using same protocol as in Figs. 1b-3b. Both the proportion and staining intensity of the neoplastic cells is significantly reduced. Only a faint nuclear staining reaction can be identified in a minor subset of the neoplastic cells in the melanoma – compare with Fig. 4a (same field).

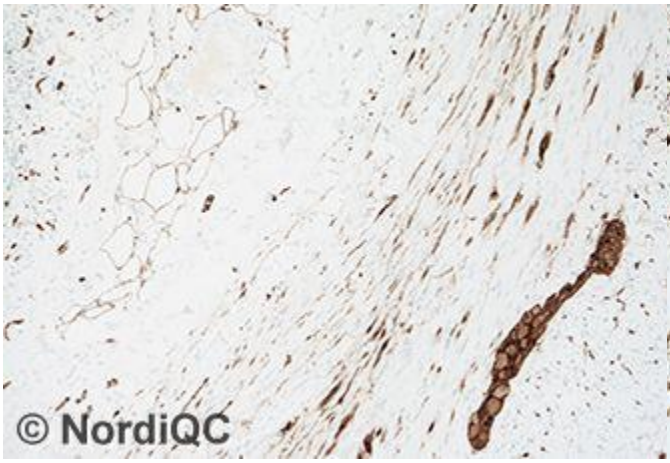


Fig. 5a (x100)
Optimal S100 staining of the appendix using the pAb Z0311 as concentrate, HIER in an alkaline buffer (CC1 pH 8.5, Ventana) and a 3-step multimer based detection system (OptiView, Ventana) – same protocol used in Fig. 6a. The adipocytes, the Schwann cells of the peripheral nerves and the ganglionic satellite cells show a moderate to strong staining reaction without any background staining - compare with Fig. 5b.

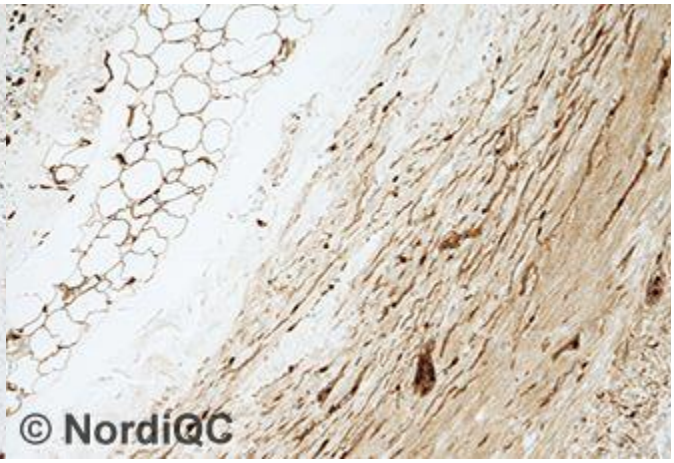


Fig. 5b (x100)
Poorly calibrated and insufficient S100 staining of the appendix using the pAb Z0311 as concentrate, proteolytic pretreatment (Protease 1, Ventana) and a 3-step multimer based detection system (OptiView, Ventana) – same protocol used in Fig. 6b. Note the false positive staining of the smooth muscle cells in the muscularis propria of the appendix – compare with Figs. 5a and 6B.

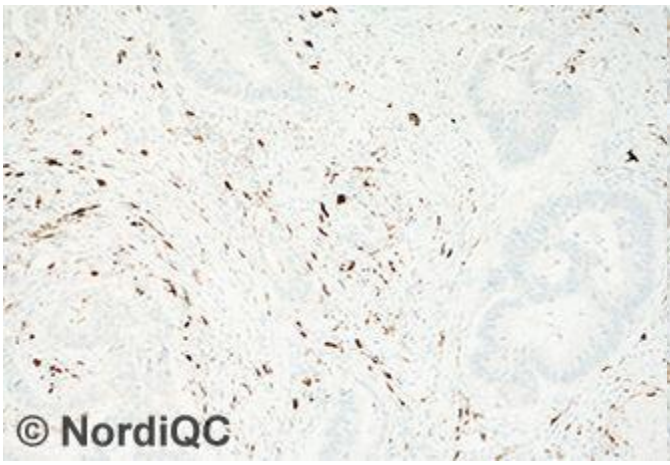


Fig. 6a (x100)
Optimal S100 staining of the colon adenocarcinoma using the same protocol as in Fig. 5a. A moderate to strong staining reaction of the dendritic cells/macrophages in the stromal compartment is seen, while the neoplastic cells of the colon adenocarcinoma is negative – compare with Fig. 6b.

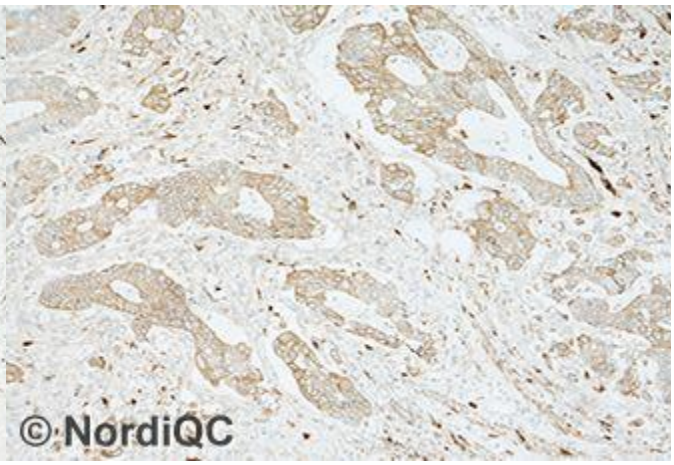


Fig. 6b (x100)
Poorly calibrated and insufficient S100 staining of the colon adenocarcinoma using the same protocol as in Fig. 5b. The dendritic cells/macrophages are labelled, but the neoplastic cells of the colon adenocarcinoma are false positive – compare with Fig. 6a.

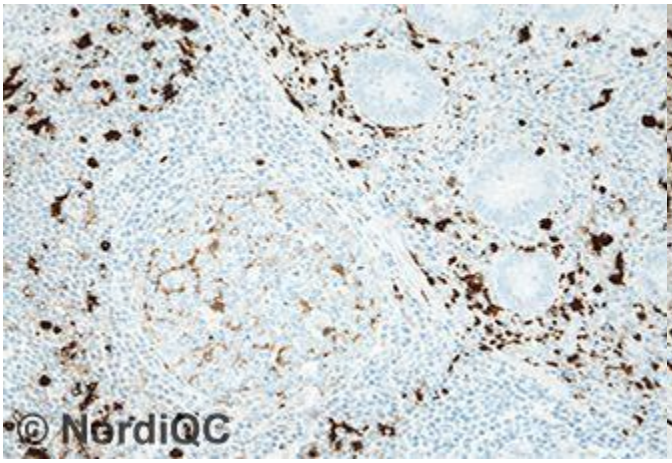


Fig. 7a (x100)
Optimal S100 staining of the appendix using the pAb 760-2523 (Ventana) as RTU format, HIER in an alkaline buffer (CC1 pH 8.5, Ventana) and a 3-step multimer based detection system (OptiView, Ventana). Note that follicular dendritic cells of the Peyer`s plaques is weakly labelled (seen with all pAbs in this assessment), whereas interfollicular dendritic cells and Schwann cells of peripheral nerves show a strong cytoplasmic and nuclear staining reaction. The epithelial cells are negative – compare with Fig. 7b.

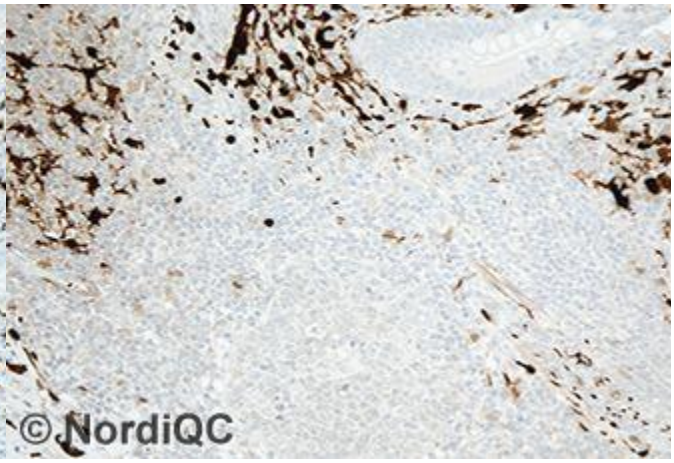


Fig. 7b (x100)
Optimal S100 staining of the appendix using the mAb clone 4C4.9 as RTU format, HIER in an alkaline buffer (CC1 pH 8.5, Ventana) and a 3-step multimer based detection system (UltraView with amplification, Ventana). Exactly the same staining pattern as in Fig. 7a is seen except that the mAb clone 4C4.9 will not demonstrate follicular dendritic cells in Peyer`s plaques in the appendix or in germinal centres of the tonsil. This staining pattern was only seen for pAbs to S100.

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