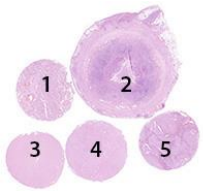
Assessment Run 64 2022
Desmin

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for Desmin, typically used in identification of the myogenic differentiation in the diagnostic work-up of cancer of unknown primary (CUP) origin. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for Desmin (see below).

Material
The slide to be stained for Desmin comprised:



1. Placenta, 2. Appendix, 3. Leiomyoma, 4. Leiomyosarcoma, 5. Malignant melanoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a Desmin staining as optimal included:

- A moderate to strong, distinct cytoplasmic staining reaction of virtually all smooth muscle cells in lamina muscularis mucosae and muscularis propria of the appendix.
- An at least weak cytoplasmic staining reaction of myofibroblasts lining the appendiceal epithelial cells.
- An at least weak to moderate staining reaction in most smooth muscle cells in the small vessels of placental villi.
- An at least weak to moderate cytoplasmic staining reaction in most smooth muscle cells of vessels in all specimens tested.
- A moderate to strong, distinct cytoplasmic staining reaction of virtually all neoplastic cells in the leiomyoma and leiomyosarcoma.
- No staining reaction of the appendiceal epithelial cells, the cytotrophoblastic and syncytiotrophoblastic cells in the placenta.
- No staining reaction of the neoplastic cells in the malignant melanoma.

Participation

Number of laboratories registered for Desmin, run 64	396
Number of laboratories returning slides	371 (94%)

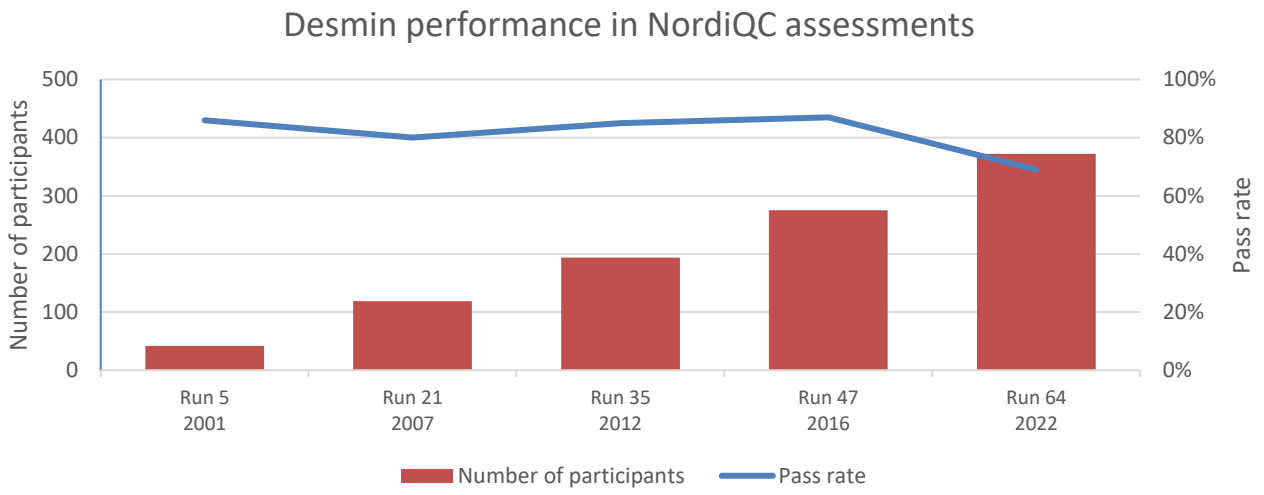
Results
At the date of assessment, 94% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

371 laboratories participated in this assessment. One laboratory was excluded from the data due to submission of an inappropriate slide. 69% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3).

The most frequent causes of insufficient staining reactions were:
- Poor performance of mAb clone D33 on the Dako Omnis platform.
- Omission of HIER
- Proteolytic pre-treatment for mAb clone DE-R-11
- Too low concentration of the primary antibody
- Use of less sensitive detection systems

Performance history
This was the fifth NordiQC assessment of Desmin. A significant fall in pass rate was observed compared to previous runs (see Graph 1). The decreased pass rate was mainly caused by less successful performance of the mAb clone D33 on Dako Omnis and application of enzymatic pre-treatment for mAb clone DE-R-11.

Graph 1. **Proportion of sufficient results for Desmin in the four NordiQC runs performed**



Conclusion
The mAb clone **D33** could produce optimal results on 3 of the main IHC platforms but is not recommended for the Dako Omnis neither as a concentrate or RTU format. A significant number (n=37) of participants transferred the Dako/Agilent RTU format with intended use for Autostainer to Omnis and only 5% provided a sufficient result, none being optimal. The **DE-R-11** and **BS21** showed promising results and were able to produce optimal results on the fully automated IHC platforms on which they were stained on. For the RTU product **DE-R-11** (Ventana/Roche, 760-2513) HIER either as a single pre-treatment or combined with proteolysis in P3 was found to be preferable as epitope retrieval providing higher sensitivity compared to the use of enzymatic pre-treatment as single retrieval method being recommended by the vendor.
Appendix and placenta are recommendable as positive and negative tissue controls. Virtually all the smooth muscle cells in the lamina muscularis mucosae and muscularis propria must show a moderate to strong cytoplasmic staining reaction, while an at least weak to moderate staining reaction must be seen in most smooth muscle cells in the vessels and in dispersed myofibroblasts lining the epithelial cells. In the placenta most smooth muscles cells in the small vessels of villi must display a low to moderate staining reaction.
No staining reaction must be seen in the appendiceal epithelial cells or the trophoblasts in the placenta.

Table 1. **Antibodies and assessment marks for Desmin, Run 64**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **D33**	79 5 2 2 2 1 1 1	Dako/Agilent Cell Marque Epredia Monosan Zytomed BioGenex Biolynx Biotech Diagnostic Biosystems	28	45	17	3	78%	30%
mAb clone **DE-R-11**	15	Leica Biosystems	9	4	2	-	87%	60%
mAb clone **BS21**	8 1	Nordic Biosite Optibodies	8	1	-	-	100%	89%
mAb clone **GTM2**	1	Gene Tech	1	-	-	-	-	-
mAb clone **IHC561**	1	GenomeMe	-	-	1	-	-	-
rmAb clone **EP15**	2	Cell Marque	-	2	-	-	-	-
rmAb clone **ZR240**	1	Zeta Corporation	1	-	-	-	-	-
Conc total	122		47	52	20	3	81%	39%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **DE-R-11[3] 760-2513**	1	Ventana/Roche	-	-	1	-	-	-
mAb clone **DE-R-11[4] 760-2513**	139	Ventana/Roche	75	23	32	9	71%	54%
mAb clone **D33 IR/IS606[3]**	21	Dako/Agilent	3	9	8	1	57%	14%
mAb clone **D33 IR/IS606[4]**	50	Dako/Agilent	2	9	18	21	22%	4%
mAb clone **DE-R-11 PA0032[3]**	12	Leica Biosystems	11	1	-	-	100%	92%
mAb clone **DE-R-11 PA0032[4]**	9	Leica Biosystems	7	2	-	-	100%	88%
mAb clone **GM007 8311-C010**	2	Sakura	2	-	-	-	-	-
mAb clone **D33 243M-17**	5	Cell Marque	1	3	-	1	-	-
mAb clone **D33 E057**	2	Linaris	-	2	-	-	-	-
mAb clone **D33 MAD-001011QD**	2	Master Diagnostica	1	1	-	-	-	-
mAb clone **D33 IP036G10**	1	Biocare Medical	-	1	-	-	-	-
mAb clone **D33 BSB5457**	1	BioSB	-	1	-	-	-	-
mAb clone **D33 MS-376-R7**	1	Immunologic	1	-	-	-	-	-
mAb clone **D33 MAD-0010011QD**	1	Vitro SA	1	-	-	-	-	-
mAb clone **C3B7 CDM-0023**	1	Celnovte	1	-	-	-	-	-
RTU total	248		105	52	59	32	63%	42%
Total	370		152	104	79	35	256	
Proportion			41%	28%	21%	10%	69%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).

Detailed analysis of Desmin, Run 64
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **D33**: Protocols with optimal results were typically based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS, Dako/Agilent) pH 9 (3-in-1) (4/9)*, Cell Conditioning 1 (CC1, Ventana/Roche) (9/43), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (13/16), Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (1/2) or TRIS-EDTA/EGTA pH 9 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 58 of 70 (83%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **DE-R-11**: Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (Dako/Agilent) (3/5), CC1 (Ventana/Roche) (2/6), BERS2 (Leica Biosystems) (4/4) or TRIS-EDTA/EGTA pH 9 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 13 of 15 (87%) laboratories produced a sufficient staining result.

mAb clone **BS21**: Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (Dako/Agilent) (8/8) or TRIS-EDTA/EGTA pH 9 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 8 of 8 (100%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for Desmin for the most commonly used antibodies as concentrate on the four main IHC systems***

Concentrated antibody	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica Biosystems 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 **D33**	4/9** (44%)	0/1	0/14	-	9/43 (21%)	-	13/16 (81%)	1/2
mAb clone **DE-R-11**	-	-	3/5 (60%)	-	2/6 (33%)	-	4/4 (100%)	-
mAb clone **BS21**	-	-	7/7 (100%)	-	-	-	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 **DE-R-11,** product no. **760-2513**, Ventana/Roche, BenchMark GX/XT/ULTRA:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 16-64 min. at 95-100°C) as single pre-treatment or in combination with either Protease 2 or 3, 8-44 min. incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings, 82 of 86 (95%) laboratories produced a sufficient staining result.

mAb clone **D33,** product no **IR/IS606**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 96-99°C), 15-20 min. incubation of the primary Ab and EnVision FLEX (K8000) as detection system. Using these protocol settings, 14 of 23 (61%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **DE-R-11,** product.no. **PA0032**, Leica Biosystems, BOND III/MAX:
Protocols with optimal results were typically based on HIER in BERS2 (efficient heating time 10-20 min. at 98-100°C), 15-20 min. incubation of the primary Ab and BOND Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 17 of 17 (100%) laboratories produced a sufficient staining result.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly according 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 3. **Proportion of sufficient and optimal results for Desmin for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT mAb DE-R-11 **760-2513**	-	-	70% (97/138)	53% (74/138)
Dako AS mAb D33 **IR/IS606**	57% (12/21)	14% (3/21)	100% (6/6)	17% (1/6)
Leica Bond III/MAX mAb **DE-R-11** **PA0032**	100% (12/12)	92% (11/12)	100% (5/5)	100% (5/5)

* 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, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments

In this assessment and in concordance with the previous NordiQC assessments for Desmin, the prevalent feature of an insufficient staining results was a too weak or false negative staining reaction of the cells expected to be demonstrated. Too weak or false negative staining reaction was seen in 87% of the insufficient results (99 of 114). Virtually all the laboratories were able to demonstrate Desmin in high-level antigen expressing structures such as smooth muscle cells in muscularis propria in the appendix and in the neoplastic cells of the leiomyoma, whereas demonstration of Desmin in neoplastic cells in the leiomyosarcoma, the myofibroblast in the appendix and the small vessels were more challenging and required an optimally calibrated protocol. In the remaining 13% of the insufficient results, these were characterized by poor signal-to-noise ratio, impaired morphology or false positive results.

33% (122 of 370) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for Desmin which is a decrease compared to the previous Run 48 where 48% used concentrated formats.

The mAb clone D33 was the most widely used antibody as concentrate and provided optimal results on three of the main IHC platforms from Leica Biosystems, Ventana/Roche and Dako/Agilent (Autostainer) as listed in Table 2. Used as a concentrate within a LD assay, mAb clone D33 gave an overall pass rate of 78% (73 of 93) of which only 30% were optimal (see Table 1).
mAb clone D33 was found successful on both the Dako/Agilent Autostainer and Leica Biosystem Bond platforms providing efficient HIER in high pH in combination with a 3-step detection system was applied. In contrast, the mAb clone D33 was found less successful on the BenchMark platform (Ventana/Roche). In total 43 participants used this clone on BenchMark, but only 9% received an optimal mark despite using HIER in high pH and a 3-step detection system as OptiView and UltraView with amplification. On BenchMark the titre of D33 typically was used in the range of 1:25-50, compared to 1:50-400 on Autostainer and Bond.
In brief, the mAb clone D33 showed an inferior performance on Omnis (Dako/Agilent) as none of 14 protocols used generated an optimal result. See section for performance of the corresponding RTU format below for a more elaborated evaluation.

The mAb clone DE-R-11 from Leica/Novocastra was used by 15 laboratories on 3 different main IHC instruments. It was able to produce optimal results on Omnis (Dako/Agilent), BenchMark (Ventana/Roche) and Bond (Leica Biosystems). On all 3 instruments HIER in high pH combined with 3-layer detection systems seemed preferable.

The mAb clone BS21 showed promising results in both this and the last run. Only 9 laboratories used this clone, but all 9 provided a sufficient result, of which 8 scored as optimal and only 1 scored as good which was mainly due to technical issues. This clone was primarily used on Omnis (Dako/Agilent) with HIER in TRS high pH for 20-30 min. The Ab was diluted 1:50 with an incubation time at 30 min. and using a 3-step detection system (EnVision FLEX+).

Ready-To-Use (RTU) antibodies were used by 67% (248 of 370) of the laboratories.

The Ventana/Roche RTU system based on mAb clone DE-R-11 (760-2513) was the most widely used RTU system applied by 140 laboratories. One laboratory used it on the Dako Omnis with a sufficient result. An overall pass rate of 71% was seen and 54% optimal.
Optimal results could only be obtained by use of laboratory modified protocol setting using HIER in CC1 as single retrieval method or a combined method using HIER in CC1 followed by proteolysis in either P2 or P3 (see Table 4). If the protocols were performed accordingly to the recommendations provided by Ventana, using proteolysis as a single retrieval method the 49 protocols submitted only provided a pass rate of 22%, none being optimal. Laboratories using modifications for pre-treatment using HIER +/- proteolysis improved the pass rate to 96% (86/90).

Table 4. **Pass rates of Ventana/Roche RTU DE-R-11 antibody on the Benchmark platform for different epitope retrieval methods.**

	Pass rate							
	Total		**HIER**		**Proteolysis**		**HIER + proteolysis**	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb DE-R-11 760-2513	139	98 (71%)	74	73 (99%)	49	11 (22%)	16	13 (81%)

When using proteolysis as single pre-treatment both the morphology was impaired and the low-level expression as smooth muscle cells in vessels, myofibroblasts and especially the neoplastic cells of the leiomyosarcoma were difficult to demonstrate, whereas smooth muscle in e.g. appendiceal lamina muscularis and neoplastic cells of the leiomyoma was strongly stained (see Figs 4a and b). The performance of the RTU product seemed to be highly impacted of the choice of detection system. Overall, 69 participants used the RTU format in combination with UltraView and a proportion of 33% (23/69) optimal was observed, compared to 74% (51/69) when OptiView was used.

The Leica Biosystems RTU system also based on mAb clone DE-R-11 was very successful providing a pass rate of 100%. Using the RTU as recommended by vendor with 20 min. HIER in BERS2 and 15 min. Ab incubation 12 of 12 laboratories received sufficient results, 92% being optimal. The RTU product was used by 9 laboratories both with minor modifications on the Bond platform and also mitigated to other instruments still providing sufficient results.

The Dako/Agilent RTU system IR/IS606 based on the mAb clone D33 is only available for the Autostainer but was frequently used by participants on the Dako Omnis. In total 71 laboratories submitted protocols using the IR/IS606 format and only 27 used the product on the Autostainer platform. 21 of the 27 used it with the vendor protocol recommendations of which 57% obtained a sufficient mark but only 14% optimal, which is inferior to the last run.
37 laboratories used the RTU format on the Dako Omnis and only 2 laboratories received a result assessed as sufficient, none being optimal. All results were commented as weak, false negative or poor-signal-to-noise-ratio due to a high rate of background combined with difficulties to demonstrate low-level antigen expressing structures. Especially the neoplastic cells of the leiomyosarcoma were only showing a weak staining reaction. It was observed that adding the mouse linker to Envision FLEX to increase the analytical sensitivity an enhanced background reaction was induced (Figs. 6a and b). This complicated the interpretation.
Overall, it must be concluded that, the mAb clone D33 clone neither as concentrated format or as the present Dako/Agilent RTU format cannot be recommended to be used on the Dako Omnis, whereas it can produce optimal results on the Autostainer platform.

In this run a declined pass rate was seen (see Graph 1) going from 87% to 69%. The number of participants has almost increased by 35% and especially the number of laboratories using RTU formats has increased from 52% to 67%. As shown in Table 1 the pass rate of the concentrated formats for Abs towards Desmin was 81% compared to the RTU formats with a pass-rate of only 63%.
The mAb clone D33 clone was widely used and both as a concentrate and as an RTU format did not perform as well as the last run (48, 2016). This is primarily related to the fact that D33 has a very poor performance on the Omnis platform (Dako/Agilent), and it was observed that an increased number of participants mitigated the use from Autostainer to Omnis in this run and with inadequate success. The DE-R-11 RTU format from Ventana/Roche was the most widely used RTU format but application of the protocol recommendations from the vendor seemed to have a significant negative impact on the overall performance of the clone. As shown in Table 4 this product should be used with either stand-alone HIER as retrieval method or with a combination of HIER and proteolysis in e.g Protease 3 (Ventana/Roche) being much more successful and preferable used with a 3-layer detection system.

The mAb clone BS21 seems to be promising especially on Omnis (Dako/Agilent) - no protocols stained on either Autostainer (Dako/Agilent) or Benchmark (Ventana/Roche) has been submitted.
The Leica Biosystems Bond platform was very successful, and no inferior performance was observed with any of the clones submitted in this run.

Controls
In this assessment and as observed in the previous runs for Desmin, appendix and placenta is recommendable as positive and negative tissue controls. Virtually all the smooth muscle cells in the lamina muscularis mucosae and muscularis propria must show a moderate to strong cytoplasmic staining reaction, while an at least weak to moderate staining reaction must be seen in most smooth muscle cells in the vessels and in dispersed myofibroblasts lining the appendiceal epithelial cells. In the placenta the smooth muscles cells in the small vessels should display a low to moderate staining reaction.
No staining reaction must be seen in the appendiceal epithelial cells or the trophoblast in the placenta.

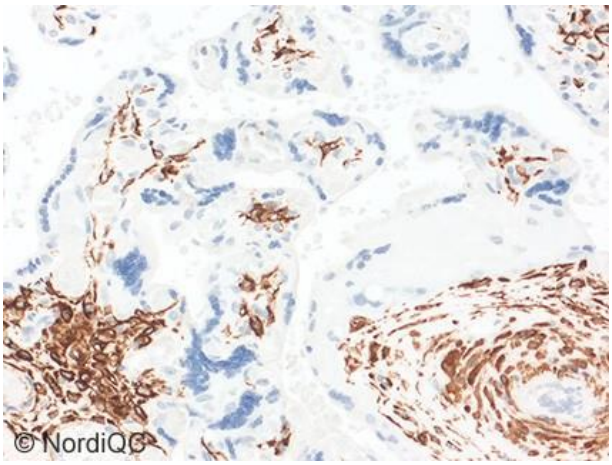


Fig. 1a (x200)
Optimal staining for Desmin of the placenta using the mAb clone BS21 as a concentrated format in a dilution 1:50 on the Omnis (Dako/Agilent). TRS high and Flex+ protocol was applied. Same protocol as in Figs. 2a-4a.
The vast majority of smooth muscle in vessels in the stromal compartment of villi show a moderate to strong cytoplasmic staining reaction. No reaction of the cytotrophoblastic and syncytiotrophoblastic cells in the placenta was seen.

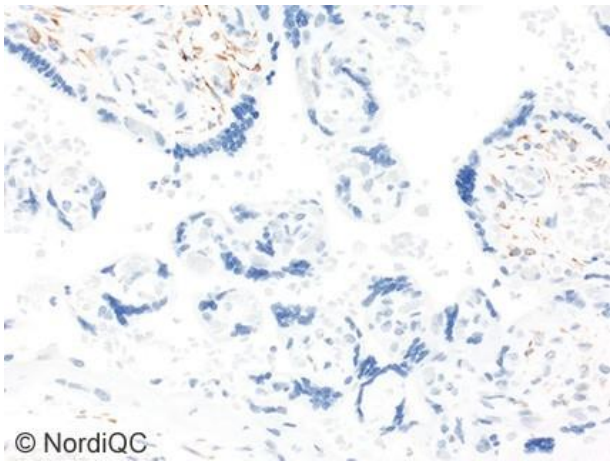


Fig. 1b (x200)
Insufficient staining for Desmin of the placenta with conc. D33 in a dilution 1:50 on the Omnis (Dako/Agilent). TRS high and Flex+ protocol was applied.
A significantly reduced intensity and proportion of positive cells is seen compared to the result expected and shown in Fig. 1a.

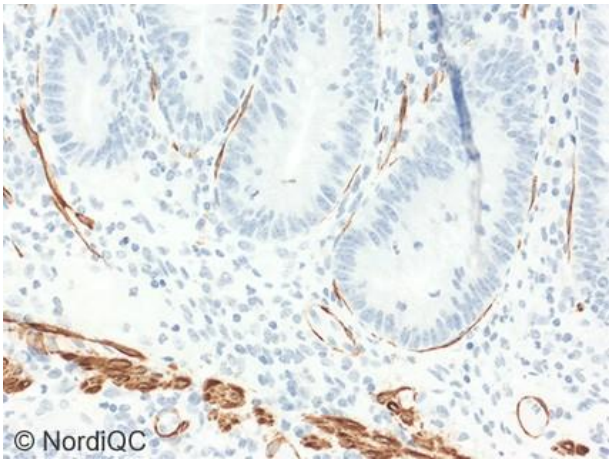


Fig. 2a (x200)
Optimal staining for Desmin of the appendix using same protocol settings as Figs. 1a-4a.
The smooth muscle cells of lamina muscularis propria and myofibroblasts lining the epithelial cells show a moderate to strong staining reaction. No background staining is seen.

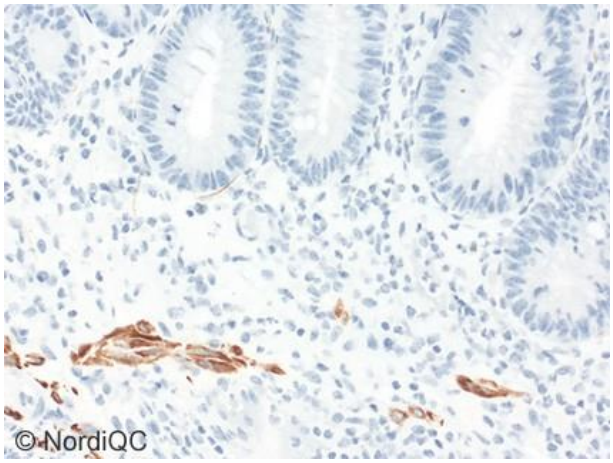


Fig. 2b (x200)
Insufficient staining for Desmin of the appendix using the same protocol as in Figs. 1b-4b.
The smooth muscle cells of lamina muscularis propria are demonstrated, while the myofibroblasts with low level Desmin expression are unstained.

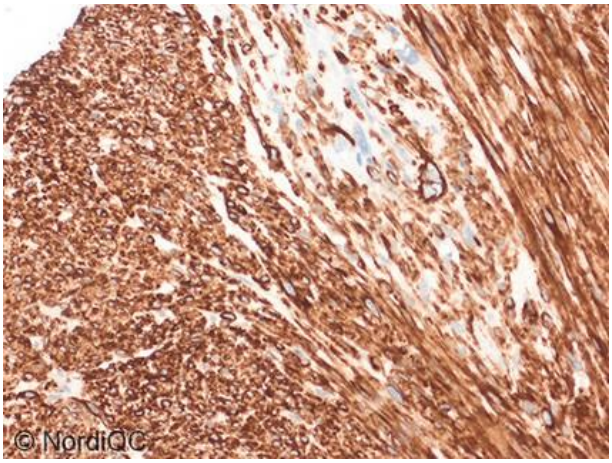


Fig. 3a (x200)
Optimal staining for Desmin of the leiomyosarcoma using the same protocol as in Figs 1a.-4a. Virtually all the neoplastic cells show a moderate to strong cytoplastic staining reaction, whereas the stroma cells are negative. Also note the smooth muscle cells in normal vessels (center, top) show a distinct staining reaction.

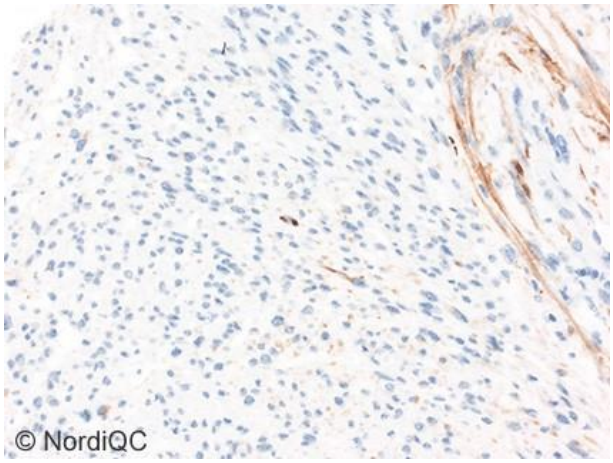


Fig. 3b (x200)
Insufficient staining for Desmin of the leiomyosarcoma using the same protocol as in Figs. 1b-4b. Almost all the neoplastic cells are negative, and only a moderate staining reaction is seen in some of the smooth muscle cells surrounding the vessels - right. Also compare with Fig. 3a. same field.

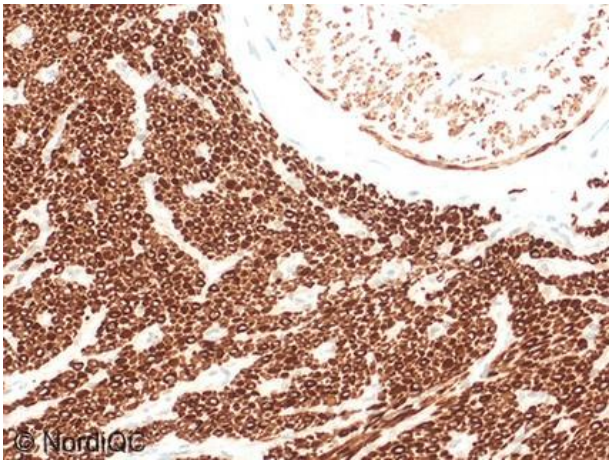


Fig. 4a (x200)
Optimal staining for Desmin of the leiomyoma using the same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a strong cytoplasmic staining reaction. The stromal cells are negative.

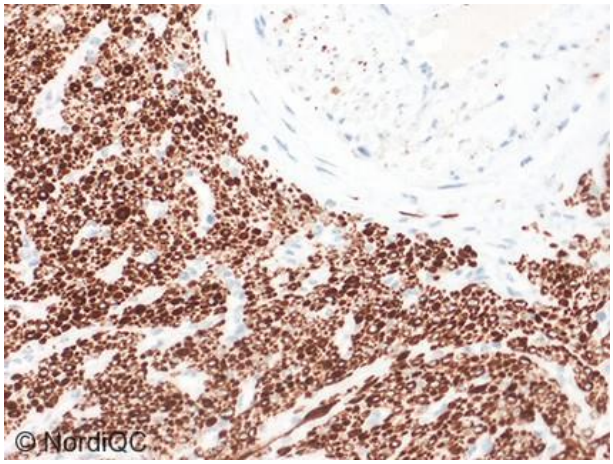


Fig. 4b (x200)
Insufficient protocol for Desmin on the leiomyoma using the same protocol as in Figs. 1b - 3b - same field as Fig. 4a. The neoplastic cells display a strong staining reaction however with a slightly reduced intensity compared to Fig. 4a. same field.

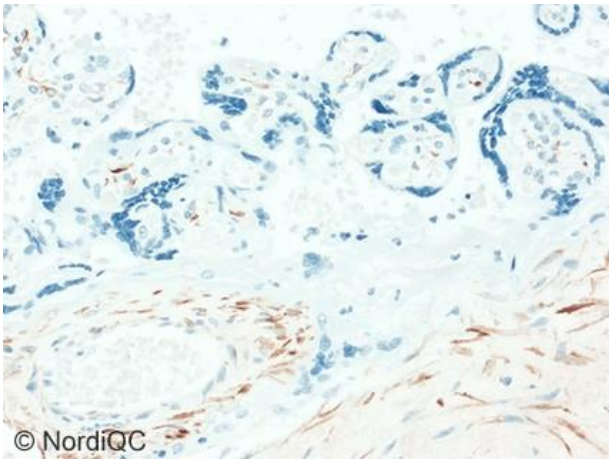


Fig. 5a (x200)
Insufficient staining reaction of Desmin in the placenta using the DE-R-11 RTU (Ventana/Roche, 760-2513) with proteolytic pre-treatment in P1 as single retrieval method and OptiView as detection system. Same protocol used in Fig. 5b.
A significantly reduced intensity and proportion of positive cells is seen compared to the result expected. Only smooth muscle cells of the larger vessels display a moderate to strong staining reaction. Compare to Fig. 1a and b same field.

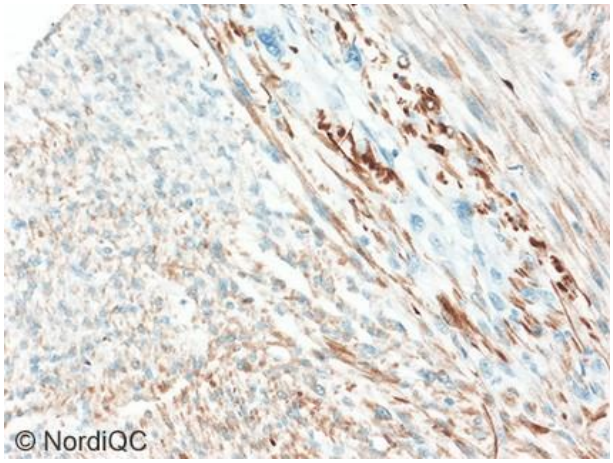


Fig. 5b (x200)
Insufficient staining for Desmin of the leiomyosarcoma using the same protocol as in Fig. 5a. The neoplastic cells have a significantly reduced intensity.
Compare with Fig. 3a, same field.

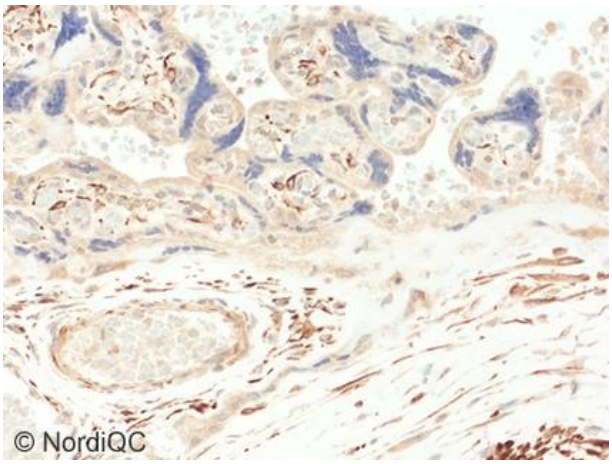


Fig. 6a (x200)
Insufficient staining for Desmin of the placenta using the RTU product D33 (IR/IS606, Dako/Agilent) on the Dako Omnis with the Flex+ protocol.
A significantly increased background reaction is observed and the number of smooth muscle cells in vessels is reduced compared to Fig. 1a.

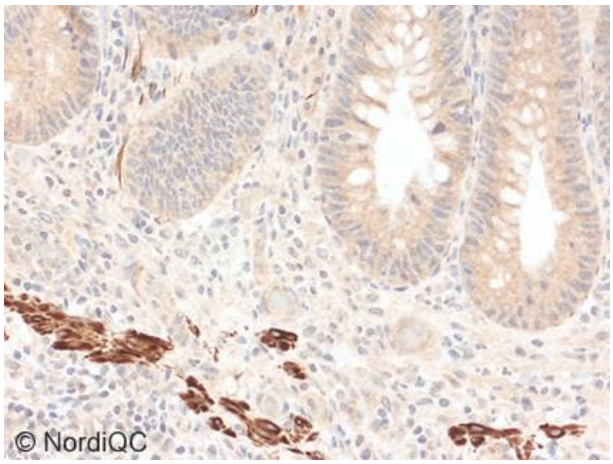


Fig. 6b (x200)
Insufficient staining for Desmin of the appendix using the same protocol as in Fig. 6a.
The smooth muscle cells of lamina muscularis propria are demonstrated, while the myofibroblasts with low level Desmin expression are only barely demonstrated. The background display a weak reaction in all cells expected to be negative and overall the scoring is compromised.

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