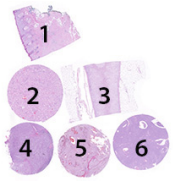
Assessment Run 56 2019

Melan A (MLA)

Material
The slide to be stained for MLA comprised:



1. Skin, 2. Kidney, 3. Adrenal gland, 4-5. Malignant melanoma, 6. Granulosa cell tumour.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing an MLA staining as optimal included:

- A moderate to strong, distinct cytoplasmic staining reaction of virtually all melanocytes in skin.
- A weak to moderate reaction in the melanocytic dendrites in most melanocytes.
- A moderate to strong, distinct cytoplasmic staining reaction of the vast majority of neoplastic cells in the malignant melanoma tissue core no 5.
- An at least weak to moderate, distinct cytoplasmic staining reaction of the majority of the neoplastic cells in the malignant melanoma tissue core no. 4.
- No or only a minimal staining in the kidney.

The criteria above are revised compared to the criteria used in previous MLA assessments. Previously – because of mAb clone A103's cross reactivity with steroid hormone producing cells – the criteria for assessing an MLA staining as optimal also included (but only for the mAb clone A103):

- *A moderate to strong, distinct granular cytoplasmic staining reaction in virtually all adrenal cortical cells (clone A103).*
- *An at least weak to moderate granular cytoplasmic staining reaction of the majority of the neoplastic cells in the granulosa cell tumour (clone A103).*

The change above was made in order to make the assessment and reporting more transparent and fairer for the different MLA antibodies with the intended use of detecting MLA in normal melanocytes and in malignant melanomas. For more details, see the Comments on page 4 of this report.

Participation

Number of laboratories registered for MLA, run 56	299
Number of laboratories returning slides	289 (97%)

Results
289 laboratories participated in this assessment. Three laboratories used an inappropriate antibody. Of the remaining 286 laboratories, 65% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:
- Less successful performance of the mAb clone A103 on the Ventana BenchMark stainer platform
- Too low concentration of the primary antibody
- Low sensitivity detection systems
- Too short efficient Heat Induced Epitope Retrieval (HIER).

Performance history
This was the eighth NordiQC assessment of MLA. A small increase in pass rate was observed (see Table 2).

Table 2. **Proportion of sufficient results for MLA in the last six NordiQC runs performed**

	Run 20 2007	Run 24 2008	Run 31 2011	Run 42 2014	Run 49 2017	Run 56 2019
Participants, n=	90	115	165	198	273	286
Sufficient results	48%	50%	66%	68%	60%	65%

Conclusion
The mAb clones **A103**, **BS52**, **M2-7C10**, and the rmAb clone **EP43** can all be used to obtain optimal staining reactions for MLA. The mAb clone **A103** was the most frequently used antibody for detection of MLA and is highly recommendable, but careful calibration of protocols is needed. Efficient HIER, preferable in an alkaline buffer, and careful calibration of antibody titre, in combination with a sensitive and specific IHC system were the main prerequisites for optimal performance.
The most successful Ready-to-Use (RTU) system was in this assessment the widely used **IR633/IS633** system (based on mAb clone A103) from Dako tailored for the Dako Autostainer. Complying to the recommended protocol settings, the pass rate was 100%. Many laboratories used the system on the Dako Omnis. Direct transfer of the recommended Autostainer-protocol to the Dako Omnis resulted in a significant drop in pass rate to 43%, whereas laboratories modifying/optimising the protocol for the Dako Omnis maintained a pass rate of 100%.
The RTU system **790-2990** from Ventana, also based on the mAb clone A103, had an overall low pass rate of 45%. The recommended protocol settings are based on the use of UltraView-AP as detection system – a system with moderate sensitivity. Using this protocol resulted in a very low pass rate of 17%. Modification based on more sensitive detection systems as OptiView-HRP with tyramide amplification or UltraView-AP with amplification resulted in a pass rate of 88%. Despite the high pass rate, the proportion of optimal staining results was only 25%, primarily due to issues with background staining most likely related to the UltraView amplification kit.
The recently introduced rmAb clone EP43 showed very promising results, indicating that it could be the most sensitive marker for MLA. Optimal results with this clone were seen on the fully automated platforms: Ventana Benchmark, Dako Omnis, Leica Bond and Sakura Genie.

Normal skin and melanomas with low MLA expression are recommendable positive tissue control for MLA. In normal skin, virtually all melanocytes should show strong positive reaction in the cytoplasm and weak to moderate reaction in the melanocytic dendrites in most melanocytes (see Fig. 7a). Kidney is recommended as negative tissue control. No staining in the epithelial cells of tubules must be seen. Scattered epithelial cells may show a granular staining reaction caused by lipofuscin.

Table 1. **Antibodies and assessment marks for MLA, Run 56**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **A103**	69 19 5 1 1 1 1	Dako/Agilent Novocastra/Leica Cell Marque Diagnostic BioSystems Immunologic Monosan Thermo Scientific	22	43	24	8	67%	73%
mAb clone **BS52**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone cocktail **HMB45+MC-7310 +M2-9E3+T311**	3	Biocare	1	2	0	0	-	-
mAb clone cocktail **M2-7C10+M2-9E3**	1	NeoMarkers	0	1	0	0	-	-
mAb clone **M2-7C10**	1	Zytomed	1	0	0	0	-	-
rmAb **EP43**	5 3 3	Nordic Biotite Cell Marque Epitomics	9	1	1	0	91%	100%
Ready-To-Use antibodies								
mAb clone **A103 790-2990**	87	Ventana/Roche	8	31	45	3	45%	66%
mAb clone **A103, IR633/IS633**	26	Dako/Agilent	10	14	2	0	92%	95%
mAb clone **A103, IR633/IS633** [3]	29	Dako/Agilent	9	12	8	0	72%	-
mAb clone **A103, IR633/IS633** [4]	9	Dako/Agilent	1	2	5	1	-	-
mAb clone **A103, PA0233**	9	Novocastra/Leica	1	8	0	0	-	-
mAb clone **A103, PA0233** [5]	1	Novocastra/Leica	0	0	1	0	-	-

mAb clone **A103, 281M-87/281M-88**	3	Cell Marque	1	0	2	0	-	-
mAb clone **A103, API3114**	1	Biocare	0	1	0	0	-	-
mAb clone **A103, MAB-0275**	1	Maixin	1	0	0	0	-	-
mAb clone cocktail **HMB45+A103+T311, 904H-08**	1	CellMarque	1	0	0	0	-	-
mAb clone cocktail **HMB45+A103+T311, 790-4677**	1	Ventana/Roche	0	1	0	0	-	-
rmAb clone **EP43, MAD-000695QD-7/N**	2	Master Diagnostica	0	1	1	0	-	-
rmAb clone **EP43, 8319-C010**	2	Sakura Finetek	1	1	0	0	-	-
Total	286		67	118	89	12	-	
Proportion			24%	41%	31%	4%	65%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer), but used by laboratories on the Dako/Agilent fully-automatic platform (Dako Omins)
4) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer), but used by laboratories on different platforms (e.g. Ventana BenchMark)
5) RTU system developed for the Leica Bond system, but used on the Ventana BenchMark system.

Detailed analysis of MLA, Run 56
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **A103**: Protocols with optimal results were all based on HIER using Cell Conditioning 1 (CC1, Ventana) (8/45)*, Target Retrieval Solution (TRS, Dako) pH 9 (3-in-1) (4/13), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (6/19), Target Retrieval Solution (TRS, Dako), High pH (3/6) or Target Retrieval Solution (TRS, Dako) pH 6 (1/2), as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 56 of 77 (73%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **BS52**: One protocol with an optimal result was based on HIER in TRS, High pH (Dako) as retrieval buffer. The mAb was diluted 1:100 and a 3-step EnVision FLEX+ (Dako K8004/DM828) detection system was used on the Dako Omnis stainer.

mAb clone cocktail **HMB45+MC-7C10+M2-9E3+T311**: One protocol with an optimal result was based on HIER using BERS2 (Bond, Leica) as retrieval buffer. The mAb was diluted 1:300 and a 3-step polymer detection kit, Bond™ Polymer Refine Red Detection (Leica, DS9390) was used on the Leica Bond.

mAb clone **M2-7C10**: One protocol with an optimal result was based on HIER in a pressure cocker using Citrate pH 6 as retrieval buffer. The mAb was diluted 1:100 and a 3-step polymer detection kit from Zytomed (POLHRP-100) was used in a manual setup.

rmAb clone **EP43**: Protocols with optimal results were based on HIER using TRS, High pH (Dako) (4/4), CC1 (Ventana) (2/3), CC1 (Ventana) followed by Protease 3 (Ventana) (2/2) or BERS2 (Leica) (1/1) as retrieval buffer. The rmAb was diluted in the range of 1:25–1:100. Using these protocol settings, 10 of 10 (100%) laboratories produced a sufficient staining result (optimal or good).

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

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark GX / 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	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **A103**	4/8** (50%)	0/1	3/6 (50%)	-	8/47 (17%)	-	-	6/19 (32%)	0/1
rmAb clone **EP43**	-	-	4/4	-	2/3	2/2	-	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 **A103**, product no. **790-2990**, Ventana/Roche, BenchMark GX/XT/ULTRA:
Protocols with optimal results were typically based on 48-64 min. HIER using CC1, 20-32 min. incubation of the primary Ab and OptiView (760-700) with amplification (760-099 / 860-099) or UltraView Universal Alkaline Phosphatase Red Detection Kit (760-501) with or without amplification (760-080) as detection system. Using these protocol settings, 19 of 29 (66%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **A103**, product no. **IR633/IS633,** Dako/Agilent, Autostainer Classic/Link:
Protocols with optimal results were all based on HIER in PT-Link using TRS pH 9 (3-in-1), 20 min. incubation of the primary Ab and 2-step EnVision Flex or 3-step Envision Flex+ as detection system. Using these protocol settings, 21 of 22 (95%) laboratories produced a sufficient staining result.

mAb clone **A103,** product.no. **PA0233**, Novocastra/Leica, BOND III/MAX:
One protocol with an optimal result was based on HIER in BERS2 (efficient heating time 30 min. at 100°C), 15 min. incubation of the primary Ab and BOND Polymer Refine Detection (DS9800) as detection system. Using similar protocol settings, 7 of 7 (100%) laboratories produced a sufficient staining result.

rmAb clone **EP43**, product no. **8319-C010**, Sakura Finetek, Tissue-Tek Genie:
One protocol with an optimal result was based on 60 min. HIER using Sakura Finetek Tissue-Tek Genie High pH Antigen Retrieval Buffer, 30 min. incubation of the primary Ab and Tissue-Tek PRO DAB Detection Kit (8826-K250) as detection system. Using similar protocol settings, 2 of 2 (100%) laboratories produced a sufficient staining result.

Table 4 summarizes the proportion of sufficient and optimal results 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 4. **Proportion of sufficient and optimal results for MLA 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 A103 **790-2990**	20% (1/5)	0% (0/5)	46% (38/82)	10% (8/82)
Dako AS mAb A103 **IR633/IS633**	100% (12/12)	58% (7/12)	86% (12/14)	21% (3/14)

* 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 integrated.

Comments
MLA (Melan-A) is a melanocyte differentiation antigen, recognized by autologous cytotoxic T lymphocytes. Melan-A is also called MART-1 (melanoma antigen recognized by T cells). The function of the protein is unknown, but MLA is expressed in all normal melanocytes, melanocyte cell lines and the vast majority of malignant melanomas. Using the monoclonal mAb clone A103, staining reaction is also seen in steroid hormone producing cells and tumours derived from these cells. This reaction is due to cross-reaction with an unknown protein (as the Melan-A/MART-1 gene is not expressed in these cells). Good IHC-markers for

steroid hormone producing cells and their tumours are rare. Consequently, in previous NordiQC MLA assessments, laboratories using the mAb clone A103 have been assessed on their ability to detect both the specific MLA and the unknown cross-reacting protein in steroid hormone producing cells and their tumours, whereas laboratories using different clones have been assessed on their ability to detect MLA only. Although the present MLA assessment (Run 56) was planned to be executed as a combined "**melanoma/melanocyte – steroid hormone**" assessment, it was decided by NordiQC to focus the assessment and report on the specific MLA data, excluding the steroid hormone producing cells cores. The reason for this decision was to make a more transparent and fair assessment of different MLA antibodies with the intended use of detecting MLA in normal melanocytes and in malignant melanomas. As a consequence, the submitted slides were assessed twice, first as a combined "**melanoma/melanocyte – steroid hormone**" assessment and finally as a "**melanoma/melanocyte**" only assessment. All data presented in Tables 1 to 4 originates from the "melanoma/melanocyte" assessment following the revised criteria given on page 1 of this report. In the following discussions a few references will be made to the unpublished data from the combined "**melanoma/melanocyte – steroid hormone**" assessment.

In this assessment and in concordance with the previous NordiQC assessments for MLA, the prevalent feature of an insufficient staining was a general too weak or false negative staining reaction of cells and structures expected to be demonstrated. This was observed in 90% of the insufficient results (91 of 101) and was almost (90 of 91 laboratories) restricted to protocols based on the mAb clone A103 either on its own or in combination with other clones. 10% (10 of 101) of the insufficient results were caused by poor signal to noise or false positive reactions. In general, almost all laboratories could detect MLA in normal skin and the malignant melanoma, tissue core no. 5, whereas demonstration of MLA in the malignant melanoma, tissue core no. 4 was much more challenging and required an optimally calibrated protocol (see Fig. 1 - 6).

40% (114 of 286) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for MLA. The mAb clone A103 was the most widely used antibody, as seen in Table 1. Optimal results could be obtained on the four most widely used IHC platforms. But as previously observed, the performance of the mAb clone A103 seemed to be related to the IHC platform applied as seen in Table 3. On the Dako Autostainer and Dako Omnis platforms optimal staining results was obtained in 50% of the laboratories, whereas the proportion of optimal staining results on the Leica Bond and Ventana BenchMark platforms was 32% (6 of 19) and 17% (8 of 47), respectively. Comparison of protocols producing optimal staining results showed that a higher concentration of the mAb clone A103 is needed on the Omnis platform compared to the Autostainer platform. With comparable protocol settings, the average dilution factor for an optimal protocol was 1:42 on the Omnis platform in contrast to 1:150 on the Autostainer. On the Leica Bond platform, all laboratories used comparable HIER and detection protocol settings, leaving variation in concentration of the primary as the main reason for differences in staining quality. For mAb clone A103 the average dilution factor for an optimal protocol was 1:70, in contrast to 1:160 for protocols producing insufficient staining results. On the Ventana BenchMark, the proportion of optimal staining results with mAb clone A103 was only 17% (8 of 47). Laboratories obtaining optimal staining results all used UltraView-AP or OptiView-HRP (both with amplification) as detection system in combination with an average dilution factor of 1:49, an average incubation time of 35 min. and an average heating time in CC1 for 61 min. Many of the protocol settings for laboratories that did not pass the assessment was surprisingly similar, but the average heating time in CC1 was significantly reduced (46 min.). Furthermore, detections systems with reduced sensitivity (UltraView and OptiView without amplification) was used by 79% (15 of 19) of these laboratories.

The recently introduced rmAb clone EP43 showed very promising results. 91% (10 of 11) of the laboratories obtained sufficient staining results of which 82% (9 of 11) were assessed as optimal. Optimal staining results could be obtained at the Leica Bond, Ventana BenchMark and Dako Omnis platforms, whereas no laboratories used the rmAb clone EP43 at the Dako Autostainer. The rmAb clone EP43, shows unlike mAb A103, no cross reaction to steroid producing cells, but in this assessment and in concordance with the previous assessment (Run 49, 2017) seems to be the most sensitive marker for MLA (see Fig. 8b)

60% (172 of 286) of the laboratories used Abs in RTU formats. The most widely used RTU systems for MLA were the Ventana **790-2990** and Dako **IR633/IS633** systems. Both are based on the mAb clone A103. The Dako IR633/IS633 system tailored for the Dako Autostainer had a high pass rate of 92% (24 of 26 laboratories) (see Table 1). Used according to the recommended protocol settings, the IR633/IS633 system had a pass rate of 100% with 58% being optimal (see Table 4). Using laboratory modified protocol settings the pass rate dropped to 86% (12 of 14) with only 21% (3 of 14) being optimal. Lacking a RTU alternative tailored for the Dako Omnis platform, 29 laboratories used the IR633/IS633 system on the Dako Omnis. Despite using similar protocol settings as recommended for the Dako Autostainer, the pass rate dropped to 43% (6 of 14) with none being optimal. Similar results were seen in previous assessment

(Run 49, 2017). In the present assessment, 15 laboratories used the IR633/IS633 system on the Dako Omnis with modified/optimized protocol settings resulting in a much-improved pass rate of 100% (15 of 15) with 60% (9 of 15) being optimal (see Fig. 1 – Fig. 3). Most significantly the majority of laboratories (11 of 15) changed protocol settings to a 3-step polymer detection system. These data – in concordance with previous findings – show that "direct" transfer of original Autostainer protocols to the Dako Omnis should be avoided. Adjustments to key protocol settings as HIER, incubation time of the primary antibody and choice of detection system might be needed. Subsequent validation is mandatory.

87 laboratories used the clone mAb A103 based RTU system (790-2990) from Ventana, but only 5 laboratories used the recommended protocol settings, and one laboratory got sufficient results (see Table 4). The recommended protocol settings are based on the use of UltraView-AP (without amplification) as detection system, but among 18 laboratories using this detection system with various protocol modifications the pass rate was only 17% (3 of 18) and none achieved optimal mark (see Figs. 4b, 5b and 6b). In contrast, 25 laboratories using UltraView-AP with amplification or OptiView with amplification, a pass rate of 88% (22 of 25) was achieved, with 28% (7 of 25) being optimal. These data support the argument that Ventana/Roche either need to revise the recommended protocol settings for the RTU system 790-2990 or reformulate the system. In the previous MLA assessment, an important finding was that the proportion of optimal staining results for using the 790-2990 system was 100% (11 of 11) provided the UltraView-AP with amplification was used as detection system. Based on these data it was recommended in the Run 49 report to use the UltraView-AP with amplification as detection system for the 790-2990 RTU system. In the present assessment 16 laboratories based the 790-2990 detecting on UltraView-AP with amplification, and although the pass rate was high (94%), the proportion of optimal scores dropped from 100% in 2017 to 25% in 2019. A similar trend was seen for the clone A103 LD-assays. Typically, many laboratories showed the expected and satisfactory specific staining reaction, but slides had a concurrent excess background staining, challenging the interpretation which resulted in a downgrade from an optimal mark to good (see Figs. 4a, 5a and 6a). The reason for the problem with excessive background staining is unclear, but it coincides with reports of problems with the quality of the UltraView amplification kit from Ventana (760-080). A number of laboratories, predominantly in Denmark and Sweden, including two NordiQC reference laboratories, have reported problems with excessive background staining when the amplification kit (760-080) is used in combination with UltraView-HRP-DAB and especially UltraView-AP-RED. The problem has been reported for numerous batch numbers of 760-080. Ventana is currently working on a new production that hopefully will solve the problem.

This was the eighth NordiQC assessment of MLA. A small increase in pass rate was observed (see Table 2) from 60% in run 49 (2017) to 65% in run 56 (2019). Due to the change in criteria, direct comparison with previous MLA assessment is obviously difficult. However, NordiQC speculates that the change of criteria is the main reason to the improved pass rate in the present assessment. The unpublished data from the combined "**melanoma/melanocyte – steroid hormone**" assessment, that was done prior to the present "**melanoma / melanocyte**" assessment, showed a significant decrease in pass rate from 60% to 29% when the "old" criteria was applied. The "**melanoma/melanocyte – steroid hormone**" assessment (unpublished data) showed, in concordance with previous MLA assessments, that sufficient steroid hormone related A103 (cross)reaction can be very difficult to obtain on the Leica Bond, Ventana BenchMark and Dako Omnis platforms.

Controls

Normal skin and melanomas with low MLA expression are recommendable positive tissue control for MLA. In normal skin, virtually all melanocytes should show strong positive reaction in the cytoplasm and weak to moderate reaction in the melanocytic dendrites in most melanocytes (see Fig. 7a). Kidney is recommended as negative tissue control. No staining in the epithelial cells of tubules must be seen. Scattered epithelial cells may show a granular staining reaction caused by lipofuscin.

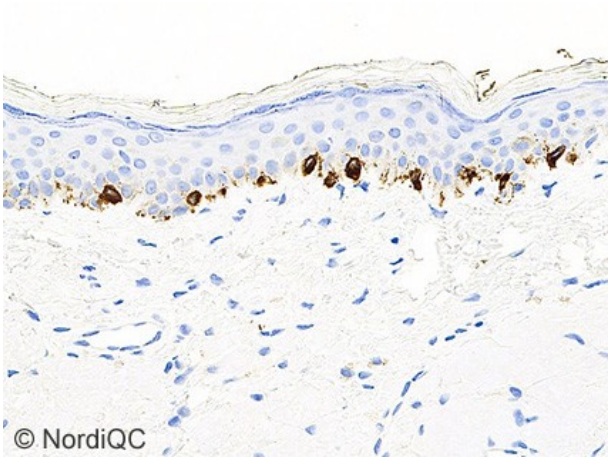


Fig. 1a
Optimal MLA staining of normal skin using the mAb clone A103 in a RTU format (Dako IR633/IS633) using **modified protocol settings** on the Dako Omnis with an incubation time of 25 min., HIER in TRS High pH 9 for 30 min., a 3-step polymer based detection kit (EnVision Flex+). Virtually all melanocytes show a strong cytoplasmic staining reaction and melanocytic dendrites are weak to moderate labelled. No background reaction is seen. Compare with Fig. 1b. Also compare with Figs. 2a – 3a, same protocol.

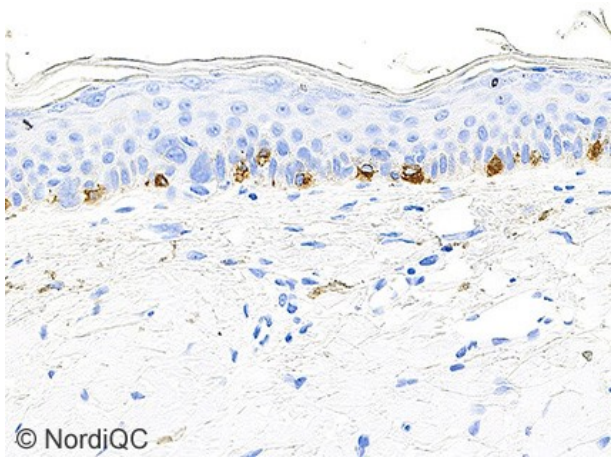


Fig. 1b
Insufficient MLA staining of normal skin using the mAb clone A103 in a RTU format (Dako IR633/IS633) on the **Dako Omnis** using the **recommended protocol settings** for the Dako Autostainer. An incubation time of 20 min, HIER in TRS High pH 9 for 30 min. and a 2-step polymer-based detection kit (EnVision Flex) results in staining of the majority of melanocytes, but the intensity is significantly reduced. Compare with Fig. 1a. – same field. Also compare with Figs. 2b - 3b – same protocol.

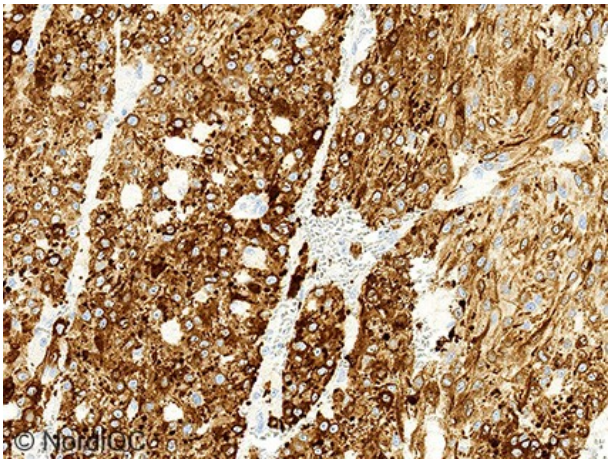


Fig. 2a
Optimal MLA staining of the malignant melanoma tissue core no. 5 (high-level expressor) using same protocol as in Fig. 1a. All the neoplastic cells show a moderate to strong cytoplasmic staining reaction. Compare with Fig. 2b.

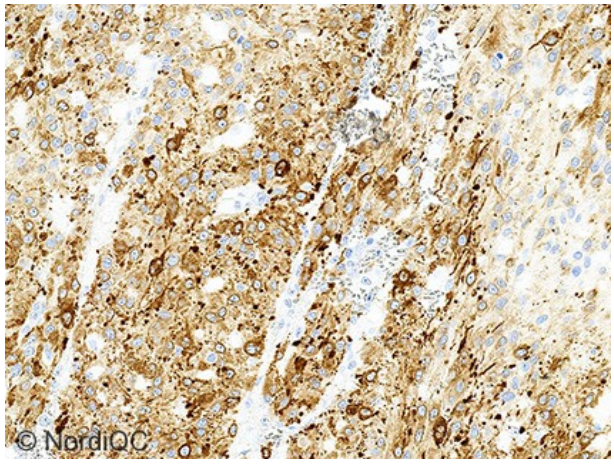
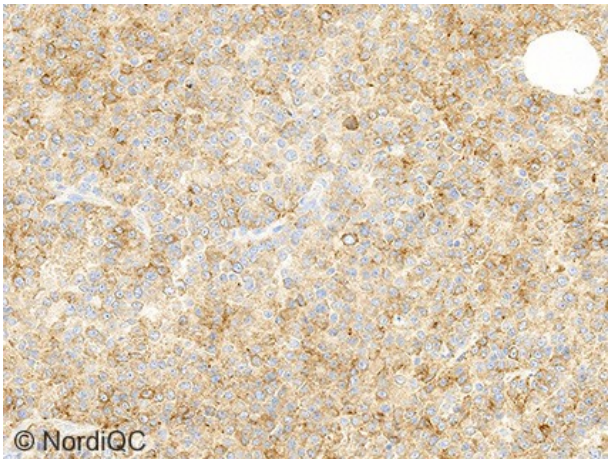


Fig. 2b
MLA staining of the malignant melanoma tissue core no. 5 (high-level expressor) using same protocol as in Fig. 1b – same field as in Fig. 2a. The majority of neoplastic cells are demonstrated, but the intensity is reduced compared to Fig. 2a. However, compare with Fig. 3b, same protocol.



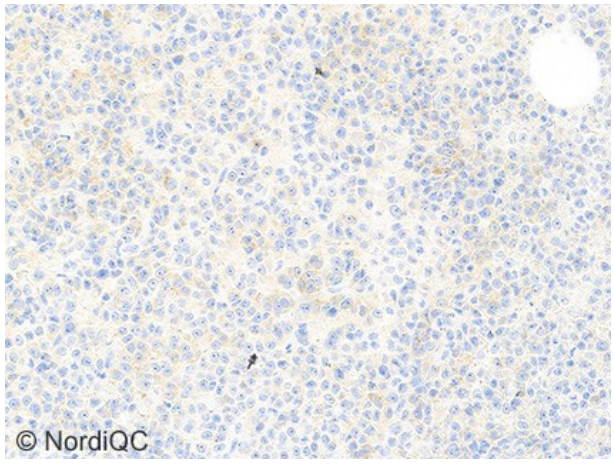


Fig. 3a
Optimal MLA staining of the malignant melanoma tissue core no. 4 (low-level expressor) using same protocol as in Figs. 1a and 2a. The majority of neoplastic cells show a weak to moderate cytoplasmic staining reaction. Compare with Fig. 3b.

Fig. 3b
Insufficient MLA staining of the malignant melanoma tissue core no. 4 (low-level expressor) using the same protocol as in Figs. 1b and 2b. Only a few scattered neoplastic cells display a very faint staining reaction making interpretation very difficult. Compare with Fig. 3a – same field.

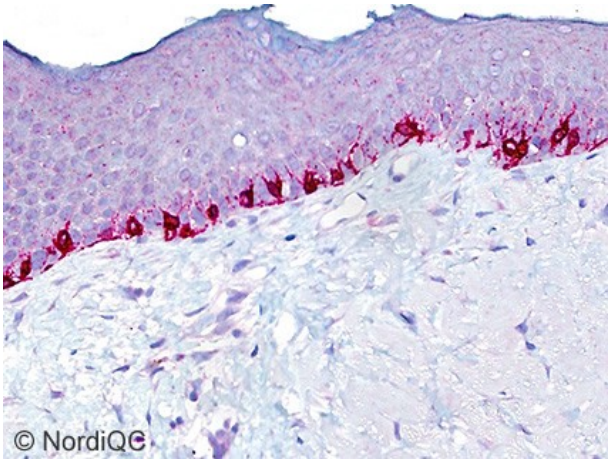


Fig. 4a
Sufficient (good) MLA staining of normal skin using the mAb clone A103 in a RTU format (790-2990) with an incubation time of 16 min., HIER in CC1 for 64 min., UltraView AP-RED (760-501) **with amplification** as detection system and performed on the BenchMark Ultra. Virtually all melanocytes show a strong cytoplasmic staining reaction. Melanocytic dendrites are weak to moderately labelled. Excessive background staining is displayed in squamous epithelial cells in epidermis and stromal cells in dermis. Also compare with Figs. 5a – 6a, same protocol.

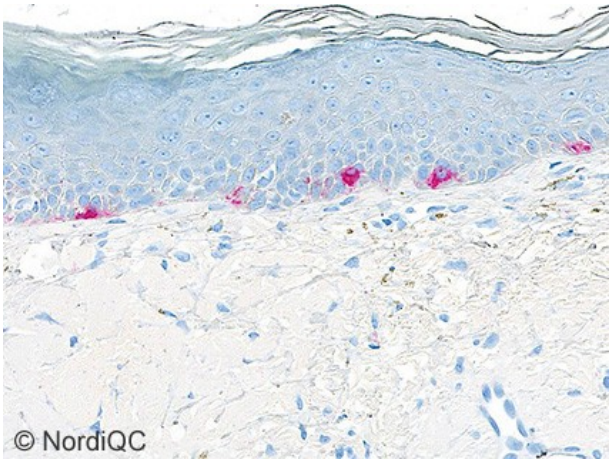


Fig. 4b
Insufficient MLA staining of normal skin using the mAb clone A103 in a RTU format (790-2990) according to Ventana's recommended protocol settings, which only differs from the protocol in Fig. 4a by using UltraView AP-RED (760-501) **without amplification** as detection system and performed on the BenchMark Ultra. Only a few melanocytes are demonstrated, and the intensity is significantly reduced. No background staining is displayed. Compare with Fig. 4a. – same field. Also compare with Figs. 5b - 6b – same protocol.

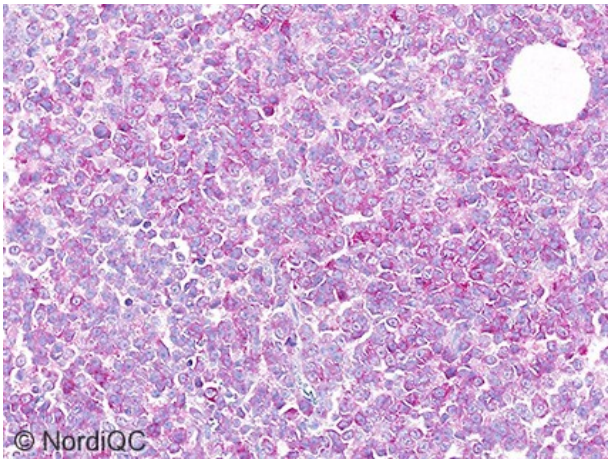


Fig. 5a
Sufficient (good) MLA staining of the malignant melanoma tissue core no. 4 (low-level expressor) using same protocol as in Fig. 4a. The majority of neoplastic cells show a weak to moderate cytoplasmic staining reaction. In this solid tumour tissue background staining is not evident but compare to Figs. 4a and 6a.

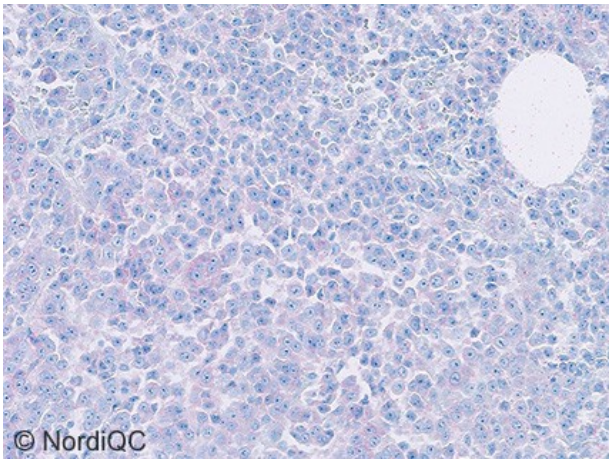


Fig. 5b
Insufficient MLA staining of the malignant melanoma tissue core no. 4 (low-level expressor) using the same protocol as in Figs. 4b. Only a few scattered neoplastic cells display a very faint staining reaction making interpretation very difficult. Compare with Fig. 5a – same field.

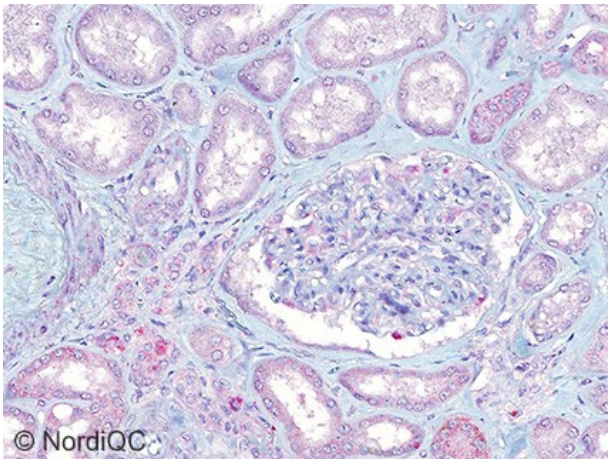


Fig. 6a
Sufficient (good) MLA staining of the normal kidney using same protocol as in Figs. 4a and 5a. Background staining is displayed in both epithelial cells and smooth muscle cells.

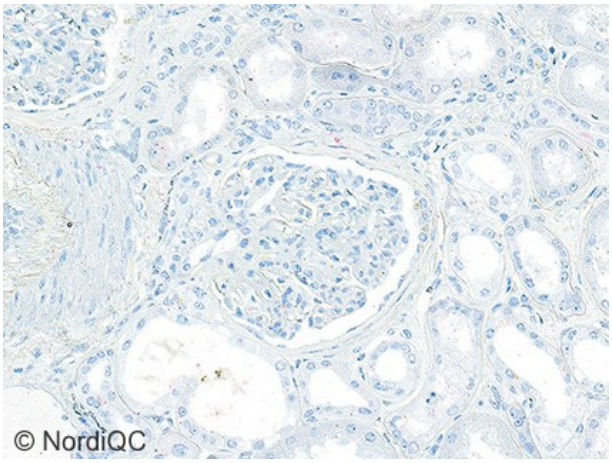


Fig. 6b
Insufficient MLA staining of the normal kidney using same protocol as in Figs. 4b and 5b. No background staining is displayed. Compare with Fig. 6a – same field.

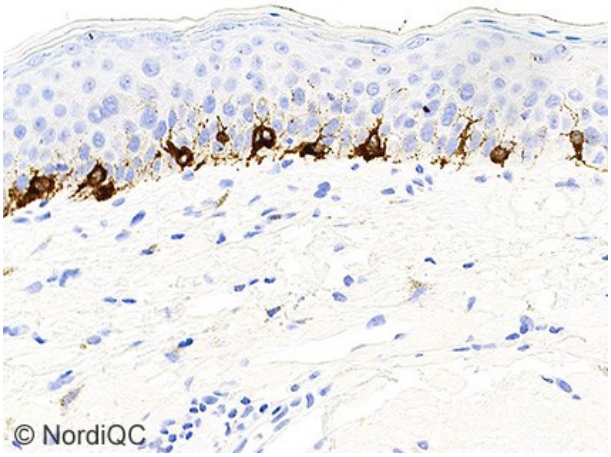


Fig. 7a
Optimal MLA staining of normal skin using the mAb clone A103 diluted 1:50 and on the Dako Omnis applying protocol settings similar to Fig. 1a. Virtually all melanocytes show a strong cytoplasmic staining reaction and melanocytic dendrites are weak to moderately labelled. No background staining is displayed. Compare with Figs. 7b and 8a, same protocol. Also compare with Figs. 4a and 4b - same field.

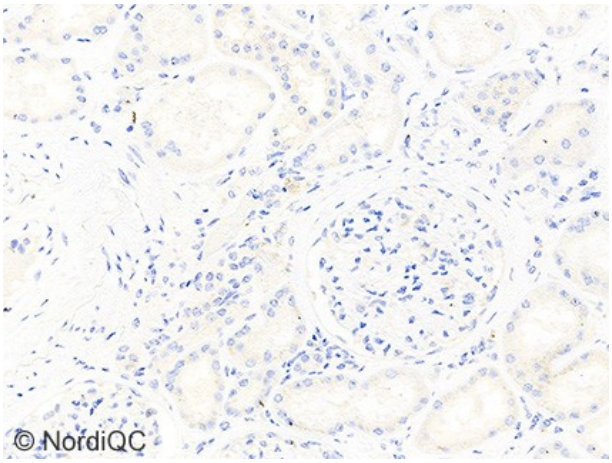


Fig. 7b
Optimal MLA staining of the normal kidney using same protocol as in Figs. 7a and 8a. Virtually no background staining is displayed. Compare with Figs. 7a and 8a, same protocol. Also compare with Figs. 6a and 6b - same field.

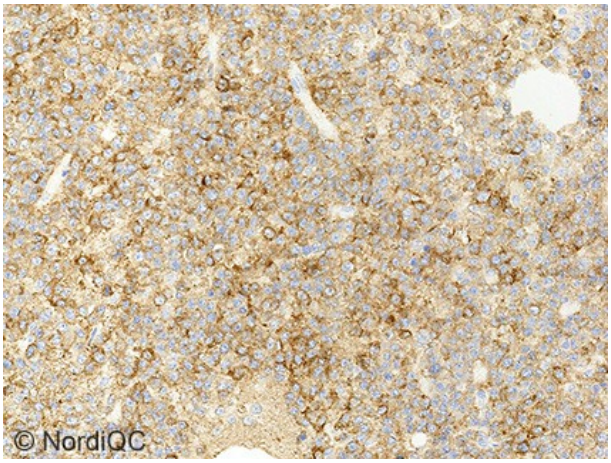


Fig. 8a
Optimal MLA staining of the malignant melanoma tissue core no. 4 (low-level expressor) using same protocol as in Figs. 7a and 7b. The majority of neoplastic cells show a weak to moderate cytoplasmic staining reaction. Compare with Figs. 7a and 8b, same protocol. Also compare with Figs. 5a and 5b - same field.

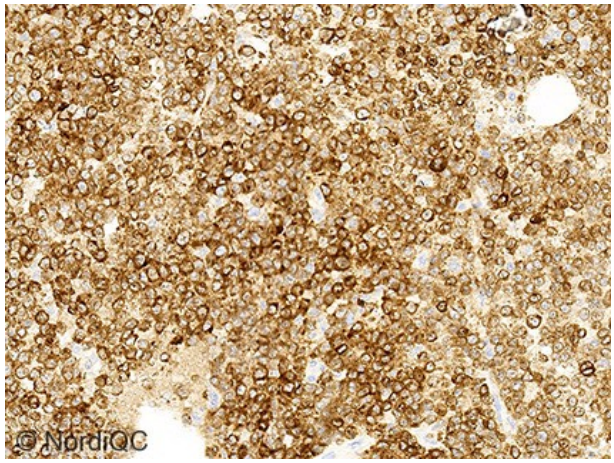


Fig. 8b
Optimal MLA staining of the malignant melanoma tissue core no. 4 (low-level expressor) using **rmAb EP43** in similar protocol settings as in Fig. 8a (and 7a and 7b) All neoplastic cells show a moderate to strong cytoplasmic staining reaction. In melanomas, rmAb EP43 produce very strong reactions. Compare with mAb A103 in Fig. 8a – same field.

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