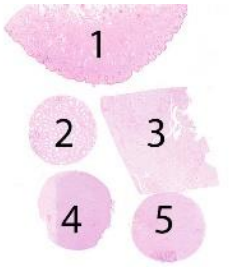
Assessment Run 68 2023

PReferentially expressed Antigen in Melanoma (PRAME)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for PRAME, typically used in the diagnostic work-up differentiating malignant melanoma from benign melanocytic lesions. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for PRAME (see below). A cut-off value of ≥76% of neoplastic cells being positive was applied in support of a malignant melanoma diagnosis.[1]
[1]Lezcano, Cecilia, et al. "PRAME expression in melanocytic tumors." *The American journal of surgical pathology* 42.11 (2018): 1456.

Material
The slide to be stained for PRAME comprised of:



1. Compound nevus, 2. Testis, 3. Adrenal gland, 4.-5. Melanoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a PRAME staining as optimal included:

- A moderate to strong nuclear staining reaction in spermatogonia close to the basal membrane of the seminiferous tubules.
- A weak to moderate, distinct nuclear staining reaction in at least dispersed germ cells of earlier stages of spermatogenesis in the seminiferous tubules in the testis
- At least a focal weak membranous staining reaction of the dispersed Leydig cells in the testis.
- No or only a weak to moderate nuclear staining reaction of <26% of melanocytes and/or dispersed keratinocytes in the compound nevus.
- Moderate to strong cytoplasmic staining in the majority of sebocytes in the compound nevus.
- Moderate to strong nuclear staining reaction in ≥76% of the neoplastic cells in the malignant melanomas, tissue cores no 4. and 5.
- A weak to moderate cytoplasmic staining reaction in malignant melanocytes with a coexisting strong nuclear staining reaction was accepted and/or a diffuse background reaction in the testicular interstitial connective tissue was accepted, provided it did not interfere with the interpretation of nuclear staining.
- At least weak focal membranous staining in epithelial cells of the adrenal gland.*

* In general, the staining reaction of the adrenal gland with respect to intensity and number of cells demonstrated did not always align with the staining patterns seen in clinically relevant tissues and therefore was not incorporated in the evaluation and the assessment score. Negative and barely perceptible staining of the adrenal gland was also accepted.

Participation

Number of laboratories registered for PRAME, run 68	240
Number of laboratories returning slides	222 (93%)

Results
At the date of assessment, 93% 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.

222 laboratories participated in this assessment and 73% 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 were:
- Inefficient Heat Induced Epitope Retrieval (HIER) – too short time or use of acidic buffer.
- Too low or too high concentration of the primary antibody.
- Less sensitive detection systems used in combination with other low sensitivity protocol parameters.
- The use of a red chromogen hindering interpretation of cells with a weak PRAME expression.

Performance history
This was the first NordiQC assessment of PRAME and the overall pass rate was 72%.

	Run 68, 2023
Participants, n=	222
Sufficient results	73%

Conclusion
The rmAb clones **EPR20330**, **QR005**, **RBT-PRAME** and **EP461** could all be used to obtain an optimal staining result for PRAME. Efficient HIER in an alkaline buffer and carefully calibrated primary Ab together with a sensitive 3- or 4-step polymer/multimer based detection system were the most important prerequisites for a sufficient staining. The most widely cited in publications and used rmAb clone EPR20330 proved to be the most robust with a pass rate of 80%, 47% optimal and provided an optimal result on all the main automated IHC platforms (Ventana/Roche, Dako/Agilent and Leica Biosystems). All other clones used by 30% (67/222) of participants had an overall pass rate of 55% (37/67), 18% (12/67) optimal, indicating the complexity and technical challenge to calibrate and implement a new IHC test in the laboratory.
Testis and skin can be recommended as a positive and negative tissue control. A moderate to strong staining reaction in the nuclei of spermatogonia located at the basal part of seminiferous tubules should be seen while a subset of germ cells, e.g. primary spermatocytes, showing a weak to moderate, distinct nuclear staining reaction. The proportion of cells demonstrated is higher at the basal part and reduced at luminal areas. Dispersed Leydig cells should show a weak to moderate membranous staining reaction. In skin a moderate to strong cytoplasmic staining reaction in the majority of sebocytes should be seen with the vast majority or keratinocytes and normal melanocytes being negative. Although PRAME expression in the membranes of scattered adrenal cortical epithelial cells is described in literature and seen with most sufficient staining protocols, this assessment revealed that an optimal result in clinically relevant tissues could also be achieved with little to no staining in the epithelial cells of adrenal gland.

Table 1. **Antibodies and assessment marks for PRAME, Run 68**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **CL5148**	1	Invitrogen	0	0	1	0	-	-
rmAb clone **EPR20330**	65	Abcam	23	27	11	4	77%	35%
rmAb clone **EPR20330**	14	Biocare Medical	7	3	1	2	77%	54%
rmAb clone **EPR20330**	5	Diagnostic BioSystems	3	1	0	1	80%	60%
rmAb clone **EPR20330**	5	Gennova	3	2	0	0	100%	60%
rmAb clone **QR005**	21	Quartett	2	10	9	0	57%	10%
rmAb clone **QR005**	1	DCS	0	1	0	0	-	-
rmab clone **RBT-PRAME**	11	Bio SB	1	3	5	2	36%	9%
rmAb clone **EP461**	5	Cell Marque	1	1	2	1	40%	20%
rmAb clone **BP6248**	1	Biolynx	0	0	0	1	-	-
rmAb clone **IHC092**	1	GenomeMe	0	1	0	0	-	-
rmAb clone **ZR383**	1	Zeta Corporation	0	0	0	1	-	-
Conc total	130		40	49	29	12	68%	31%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
rmAb clone **EPR20330 790-7149 (VRPS)[3]**	28	Ventana/Roche	16	7	5	0	82%	57%
rmAb clone **EPR20330 790-7149 (LMPS)[4]**	11	Ventana/Roche	5	5	1	0	91%	45%
rmAb clone **EPR20330 AVI/ALI/ACI3252**	22	Biocare Medical	13	5	4	0	82%	59%
rmAb clone **EPR20330 MAD-000793QD**	4	Master Diagnostica	2	1	1	0	-	-
rmAb clone **EPR20330 ZA-0700**	1	Beijing zhongshan	1	0	0	0	-	-
rmAb clone **EPR20330 RMPD109**	1	Diagnostic BioSystems	0	0	1	0	-	-
rmAb clone **QR005 P-P003**	14	Quartett	5	6	3	0	79%	36%
rmAb clone **QR005 8355-C010**	1	Sakura Finetek	0	0	1	0	-	-
rmAb clone **EP461 484R-10/17/18**	4	Cell Marque	3	1	0	0	-	-
rmab clone **RBT-PRAME BSB-2374-3/7/15**	2	Bio SB	0	2	0	0	-	-
rmAb clone **E7I1B RMA-1047**	2	Fuzhou Maixin Biotech	0	0	2	0	-	-
rmAb clone **738G4C3 PA439**	1	Abcarta	0	0	1	0	-	-
rmAb clone **PRAME/8558R AND41GP**	1	Biogenex	0	0	0	1	-	-
RTU total	92		45	27	19	1	78%	49%
Total	222		85	76	48	13		
Proportion			38%	34%	22%	6%	73%	

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 PRAME, Run 68
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
rmAb clone **EPR20330**: Protocols with optimal results were typically based on HIER using Target Retrieval Solution (TRS, Dako/Agilent) pH 9 (3-in-1) (4/9)*, TRS pH 9 (Dako/Agilent) (18/27), Cell Conditioning 1 (CC1, Ventana/Roche) (12/41), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (2/7), TRIS-EDTA/EGTA pH 9 (1/1) as retrieval buffer. The rmAb was typically diluted in the range of 1:50-1:2.500 depending on the Ab vendor (mean dilution factor for optimal results with Abcam product was 1:566 and 1:79 for Biocare product) and total sensitivity of the protocol employed. Using these protocol settings, 61 of 77 (79%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **QR005**: Two protocols with optimal results were based on HIER using BERS2 (Leica Biosystems) (2/9) as retrieval buffer and the dilution factor for the rmAb was 1:100. Using these protocol settings, 4 of 5 (80%) laboratories produced a sufficient staining result.

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

Concentrated antibody	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark GX / Ultra**		**Leica Biosystems Bond MAX/III**	
	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
rmAb clone **EPR20330**	5/9** (44%)	-	18/27 (67%)	-	12/41 (29%)	0/1	2/7 (29%)	-
rmAb clone **QR005**	0/2	-	0/4***	-	0/7	-	2/9 22%	-

* 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).
*** All protocols were assessed as Good.

Ready-To-Use antibodies and corresponding systems
rmAb clone **EPR20330**, product no. **790-7149**, 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 of the primary Ab and OptiView (760-700) as detection system. Using these protocol settings, 12 of 12 (100%) laboratories produced an optimal staining result.

rmAb clone **EPR20330**, product no. **AVI 3252**, Biocare Medical, Ventana BenchMark GX/ULTRA:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-76 min. at 95-100°C), 32 min. incubation of the primary Ab and OptiView (760-700) as detection system. Using these protocol settings, 8 of 8 (100%) laboratories produced an optimal staining result.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU system. The performance was evaluated both as a "true" plug-and-play system 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 PRAME for the most commonly used RTU IHC system**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra rmAb EPR20330 **790-7149**	82% (23/28)	57% (16/28)	100% (9/9)	44% (4/9)

* 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 first assessment of PRAME a pass rate of 72% was obtained. The prevalent features of insufficient staining results were characterized by a generally too weak/false negative staining reaction of the cells expected to be demonstrated, also in combination with a poor-signal-to noise ratio. Too weak or false negative staining reaction was observed in 74% of the insufficient results (46/62). Although very sensitive protocol settings are required for an optimal staining result, a false positive staining reaction was seen in 13% of insufficient results (8/62). It was mainly characterized by a weak but unequivocal positive nuclear

staining reaction in most of the normal melanocytes and/or keratinocytes in the skin epidermis and hair follicles of the compound nevus, tissue core no. 1 (see Fig. 6a).
Virtually all laboratories were able to detect PRAME in high-level antigen expressing cells as normal spermatogonia in the testis. In contrast the demonstration of PRAME in low-level antigen expressing cells as e.g., a subset of primary spermatocytes and Leydig cells in the testis and of diagnostic importance, the neoplastic cells of the malignant melanoma, tissue core no. 4, was more challenging and could only be obtained with optimally calibrated protocols. Although a proportion of the epithelial cells of the adrenal cortex were expected to show a weak to moderate membranous staining reaction as it is stated in the literature and demonstrated by tests done in-house with various Ab clones, it was excluded from the formation of the assessment mark. It was evident that the expected result was not conceivable with all protocol settings which otherwise showed an optimal staining result in all other cores, particularly when using a detection system with a red chromogen.

Concentrated Ab formats within laboratory developed (LD) assays for PRAME were used by 58% (129/222) of the participants with 68% (88/129) producing a sufficient result, 31% (40/129) optimal. Optimal results could be obtained on all the main automated staining platforms from Ventana/Roche, Dako/Agilent and Leica Biosystems, however a variation in pass rates was observed between the staining platforms, the Ab clones, antibody diluents and visualization systems used.
The rmAb clone EPR20330 was the most widely used antibody, being applied by 68% (88/129) of laboratories as a concentrate (see Table 1). It also proved to be the most robust as the main prerequisite for a sufficient staining was the use of a sensitive 3- or 4-step polymer/multimer based detection system which, regardless of stainer platform or any other parameters used, achieved a pass and optimal rate of 84% (58/69) and 43% (30/69), respectively. In addition, when the antibody was also diluted in diluent produced by Dako/Agilent (K8006, S0809, S2022, S3022) or Biocare Medical (PD902, PD304), the pass rate increased to 93% (39/42), 57% (24/42) optimal, with 3 insufficient results being caused by a technical issue or too high dilution factor (≥1:5.000). Optimal dilution factors were dependent on the Ab vendor as well as the antibody diluent used. All sufficient protocols based on the rmAb clone EPR20330 from Biocare Medical used a primary antibody dilution of 1:50-1:200 together with the aforementioned diluents from Dako/Agilent and Biocare Medical. However, the rmAb clone EPR20330 based concentrate from Abcam could be diluted either 1:50-1:200 in an antibody diluent from Ventana/Roche, 1:100-1:400 in the antibody diluent from Dako/Agilent (K8006) or 1:400-1:2.500 in an antibody diluent produced by Dako/Agilent (S2022, S3022) or Biocare (PD904) for an optimal staining result.

The rmAb clone QR005 was the second most used Ab concentrate and gave a pass rate of 59% (13/22), 9% (2/22) optimal. It was observed to be the most reproducible on the Dako Omnis staining platform as all 4/4 participants using the Ab on this platform received an assessment score of Good, while the pass rate for other stainer platforms was between 0-57%. The most common cause of an insufficient result with the rmAb clone QR005 was either a too weak, false positive or false negative staining reaction accompanied by a poor signal-to-noise ratio.
Of unexplained reasons, all 4 slides stained with rmAb QR005 and a DAB based detection system on a Ventana BenchMark Ultra stainer platform exhibited an aberrant staining pattern where sebaceous glands in the compound nevus showed a significantly lower level of PRAME expression compared to what would be expected based on the staining intensity seen in other tissue cores on the same slide (see Figs. 5a-5b). The significance of this staining pattern is unknown and was accepted providing the result was as expected in the melanocytic lesions.
Both rmAb clone RBT-PRAME and EP461 based antibody concentrates provided an inferior pass rate of 36% and 40%, respectively. The main reason for an insufficient result was either a too weak or false negative staining reaction.

The RTU formats of PRAME Abs were used by 42% (92/222) of laboratories, 78% (72/92) providing a sufficient result, 49% (45/92) optimal. The Ventana/Roche RTU system based on rmAb clone EPR20330 (760-7149) was the most widely used RTU system with a total pass rate of 85% (33/39), 54% (21/39) optimal. All (n=23) protocols based on OptiView or UltraView DAB as a detection system were assessed as sufficient, 87% (20/23) optimal and together with vendor recommended protocol settings (VRPS) a 100% (16/16) optimal rate was achieved. Protocols based on UltraView AP Red detection kit was mainly used according to VRPS which achieved a pass rate of 58% (7/12), none being optimal. 2 participants increased the incubation time of the primary antibody from 32 to 36-40 min. and both received an assessment score Good. All protocols stained with a red chromogen showed a generally too weak staining reaction with or without a diffuse unspecific staining reaction (poor signal-to-noise ratio).

Biocare Medical has launched different RTU products based on the rmAb clone EPR20330 intended for different IHC stainer platforms as e.g. Intellipath, Benchmark or Bond III. This induced an increased level of uncertainty and validity of the protocol data submitted by the participants regarding correct registration

and *de facto* use of the specific products. Consequently, all the Biocare RTU formats were pooled and analyzed as "the same" RTU product. With offset in this, the Biocare Medical PRAME RTU antibody worked best on the Ventana BenchMark stainer platforms, achieving an overall pass rate of 94% (15/16), 75% (12/16) optimal. The only insufficient result was most likely due to a shorter HIER time (32 min. instead of vendor recommended and most commonly used 64 min.). 50% (3/6) of laboratories using the RTU system on the Leica Bond III stainer platform received a sufficient result but only 1 (17%) was optimal. All protocols assessed as sufficient were based on HIER for 30 min. in an alkaline BERS2 buffer (AR9640).

The RTU format of the rmAb clone QR005 by Quartett outperformed the respective Ab concentrate achieving a pass rate of 79% (11/14), 36% (5/14) optimal (see Table 1). 9 participants used the RTU product on the Ventana BenchMark Ultra platform, 100% (9/9) assessed as sufficient, 33% (3/9) optimal. All protocols were based on 52-72 min. HIER in an alkaline CC1 buffer and an optimal result was achieved with either UltraView AP Red with Amplification Kit or OptiVew as detection system. The same and aberrant reaction pattern of weaker sebaceous glands that was observed for the rmAb clone QR005 as a concentrate together with UltraView DAB and OptiView was also seen with the respective RTU format as 2/3 protocols based on these detection systems showed similar results.
1/3 and 1/2 participants using the rmAb clone QR005 based RTU product on the Leica Bond III and Dako Omnis staining platforms, respectively, were assessed as optimal. The remaining results were assessed as borderline. Insufficient protocols on the Bond III showed false positive staining reaction in the normal keratinocytes (see Fig. 6a), although the only difference in protocol settings provided by the participants between these and the one optimal result was a 10 min. decrease of HIER in BERS2 to 20 min.

Also, in contrast to the inferior results seen with the antibody concentrate, the rmAb EP461 based RTU format showed a high pass rate of 100% (4/4) with a sufficient result achieved on all main fully automated stainer platforms by Dako/Agilent, Ventana/Roche and Leica Biosystems. All slides were stained using HIER in an alkaline buffer and a sensitive detection system as FLEX+, OptiView or Bond Refine. Two slides assessed as optimal and stained on the Leica Bond were based on the recently launched flip protocol for the Bond platform (endogenous peroxidase blocking step being performed after primary antibody).

The purpose of this PRAME assessment was focused on melanocytic lesions and 24% (53/222) of participants used a detection system based on a red chromogen, achieving a pass rate of 62% (33/53) and only 13% (7/53) optimal. The respective proportions for DAB based systems were 75% (127/169) and 46% (78/169), respectively. The lower rate of sufficient results for detection systems with a red chromogen was in part due to the more complicated interpretation of weakly stained cells on the hematoxylin-stained blue background, which is why this should be preferred only in highly pigmented neoplasms. An optimal result could be obtained on all main fully automatic stainer platforms from Dako/Agilent, Leica Biosystems and Roche/Ventana, but required carefully calibrated protocol settings.

This was the first assessment of PRAME in NordiQC and an overall pass rate of 72% was achieved. The majority of participants (129/222) opted for a concentrated format, although a slight increase in pass and optimal rates was achieved within participants using an RTU system. PRAME proved to be technically challenging as both false negative and false positive staining reaction was observed. The most cited and most widely used rmAb clone EPR20330 proved to be relatively robust with a high pass rate of 80% (124/155) and 47% (73/155) optimal which together with a sensitive 3- or 4-step polymer/multimer detection system increased to 85% (99/117) sufficient, 56% (65/117) optimal. Other rmAbs as QR005, RBT-PRAME and EP461 were used by 27% (59/222) of participants and achieved a lower pass rate of 61% (36/59), 20% (12/59) optimal, indicating the need for a careful optimization and thorough validation processes.

Controls
At present, according to publications and preliminary data generated in this NordiQC assessment, testis and skin can be recommended as a positive and negative tissue control. Protocols must be calibrated to provide a moderate to strong nuclear staining reaction in most spermatogonia located at the basal part of seminiferous tubules while dispersed cells of early spermatogenesis such as primary spermatocytes should show a weak to moderate, distinct nuclear staining reaction. The proportion of cells demonstrated is higher at the basal part and reduced at luminal areas. Dispersed Leydig cells should show a weak membranous staining reaction (the expression pattern in testis is still to be verified to be used as critical control). In skin a moderate to strong cytoplasmic staining reaction in the majority of sebocytes should be seen with the vast majority or keratinocytes and normal melanocytes being negative. Although PRAME expression in the membranes of scattered cortical epithelial cells is described in literature and seen with most sufficient staining protocols, this assessment revealed that an optimal result in clinically relevant tissues could also be achieved with little to no staining in the adrenal gland.

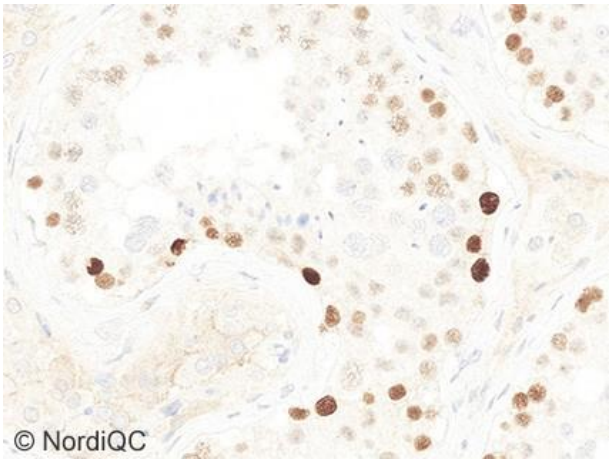


Fig. 1a
Optimal PRAME staining reaction of testis using the Ventana/Roche RTU system based on rmAb clone EPR20330 (32 min. incubation), HIER in CC1 (64 min.), UltraView with Amplification Kit as a detection system and performed on Ventana BenchMark Ultra stainer platform. Spermatogonia located at the basal part of seminiferous tubules show a strong nuclear staining while a proportion of cells of early spermatogenesis display a weak to moderate nuclear staining reaction. Dispersed Leydig cells show a weak membranous staining reaction. A weak cytoplasmic staining reaction is seen and accepted, provided it did not interfere with interpretation. Same protocol used in Figs. 2a-4a.

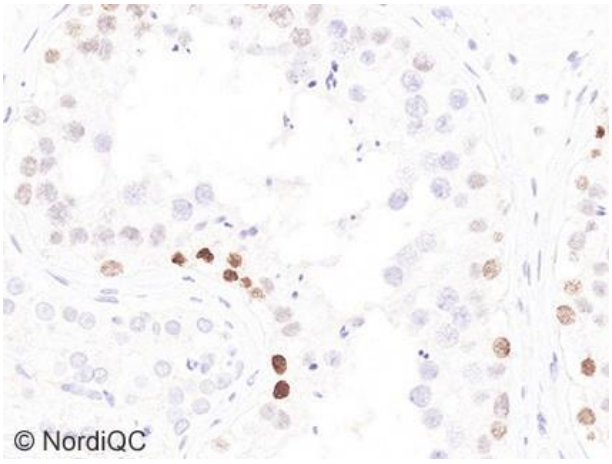


Fig. 1b
Insufficient PRAME staining reaction of testis using the rmAb clone RBT-PRAME as a concentrate (1:100) on the Dako Omnis stainer platform with a relatively short HIER time (10 min.) and a 3-step detection system FLEX+. Spermatogia being high expressors show a strong nuclear staining reaction, whereas a reduced proportion of germ cells are positive in the seminiferous tubules and Leydig cells being completely negative. Compare to Fig. 1a. Same protocol used in Figs. 2b-4b.

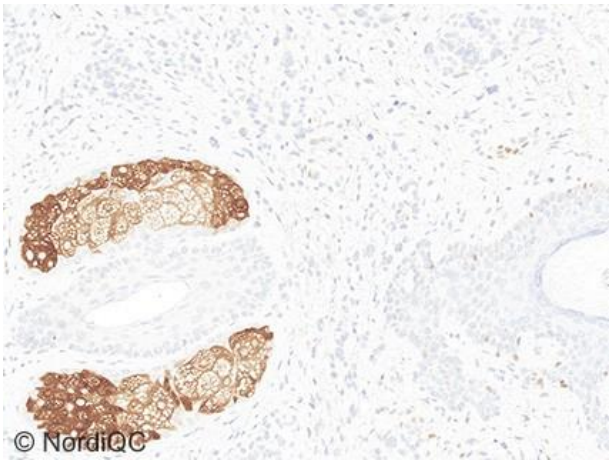


Fig. 2a
Optimal staining reaction for PRAME in the compound nevus using the same protocol as in Fig. 1a. Virtually all sebocytes display a moderate to strong cytoplasmic staining reaction, while normal keratinocytes are virtually all negative. <25% of the melanocytes in the nevus nests display a weak nuclear staining reaction. Same protocol used in Figs 3a-4a.

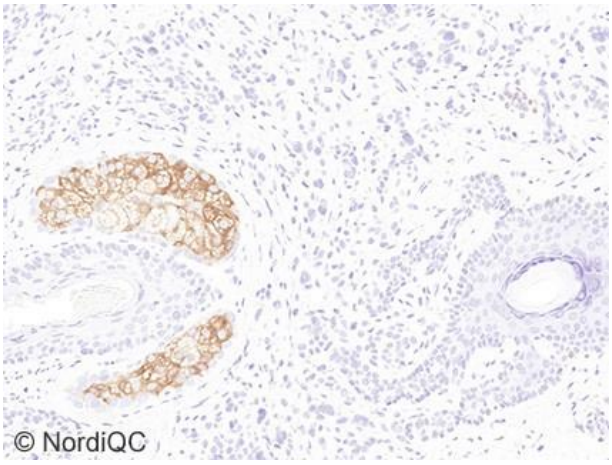


Fig. 2b
Staining reaction for PRAME in the compound nevus using the same insufficient protocol as in Fig 1b. The majority of sebocytes show a moderate cytoplasmic staining reaction while virtually all other cells are negative. Same protocol used in Figs. 3b-4b – same field as 2a.

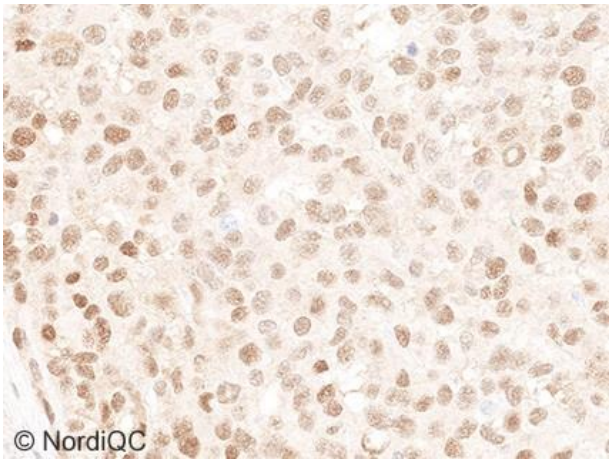


Fig. 3a
Optimal staining reaction for PRAME in the malignant melanoma, tissue core no. 4, using the same protocol as in Figs. 1a-2a. Virtually all tumor cells show a weak to moderate nuclear staining reaction.

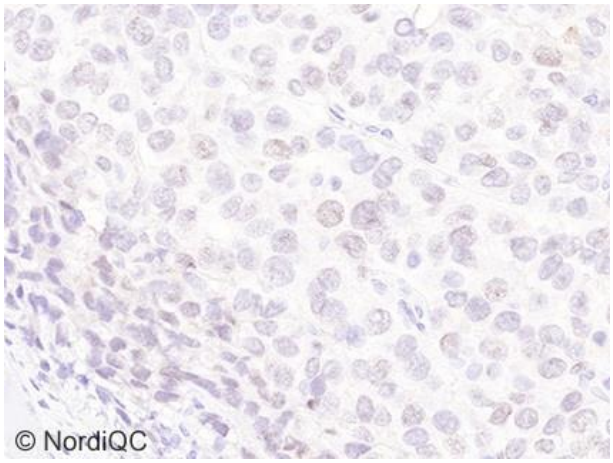


Fig. 3b
Insufficient staining reaction for PRAME in the malignant melanoma, tissue core no. 4, using the same protocol as in Figs. 1b-2b. The intensity and proportion of positive tumor cells has significantly decreased compared to the level expected, displaying mainly a weak indistinct staining reaction. Same field as 3a.

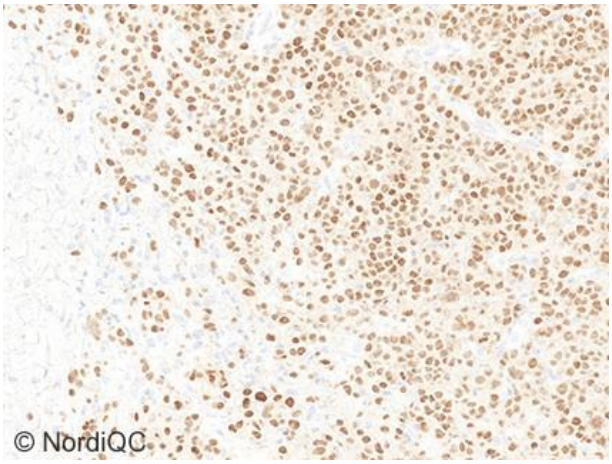


Fig. 4a
Optimal staining reaction for PRAME in the high expressor malignant melanoma, tissue core no. 5, using the same protocol as in Figs. 1a-3a. Virtually all tumor cells show moderate to strong nuclear staining reaction.

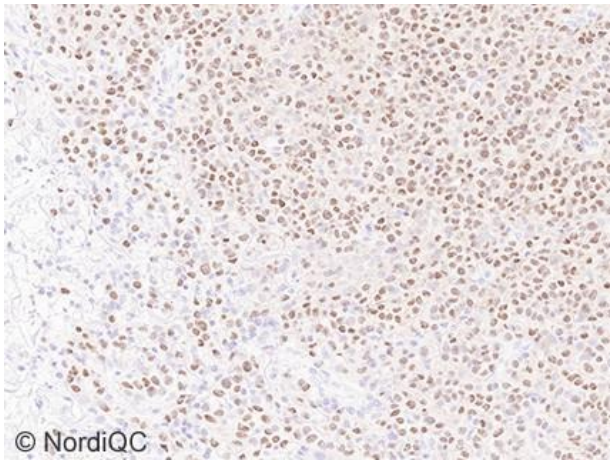


Fig. 4b
Staining reaction for PRAME in the high expressor malignant melanoma, tissue core no. 5, using the same insufficient protocol as in Figs. 1b-3b. ≥76% of tumor cells show a weak to strong nuclear staining reaction, however the general intensity of the staining is reduced. Same field as 4a.

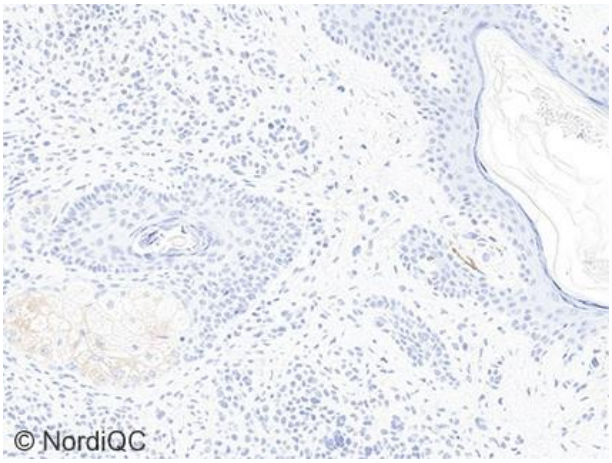


Fig. 5a
Very faint cytoplasmic staining reaction in the sebocytes of the compound nevus using rmAb clone QR005 as a concentrate (diluted 1:50) on the Ventana BenchMark Ultra platform with OptiView as a detection system. Although the staining result is insufficient, the sebocytes are stained much weaker than expected – compare to Fig. 2b, same slide as in Fig. 5b.

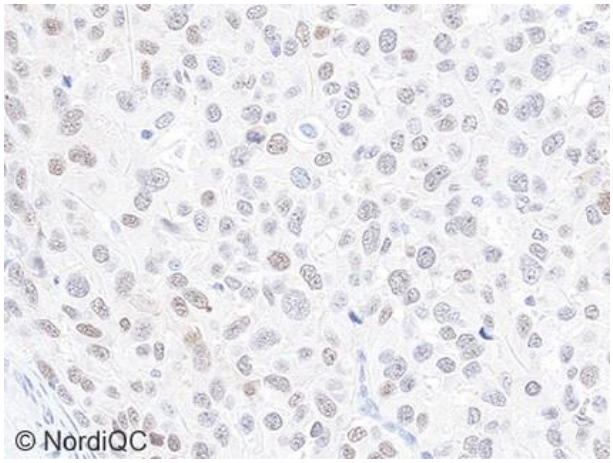


Fig. 5b
Weak distinct nuclear staining reaction in the majority of malignant melanocytes in the melanoma, tissue core no. 4, using the same protocol as in Fig. 5a. The staining is insufficient, however the level of expression in this core is comparable and even slightly stronger than what is seen in Fig. 3b (same field), while the staining in sebocytes (Fig. 5a) is aberrantly weak (see Fig. 5a compared to Fig. 2b).

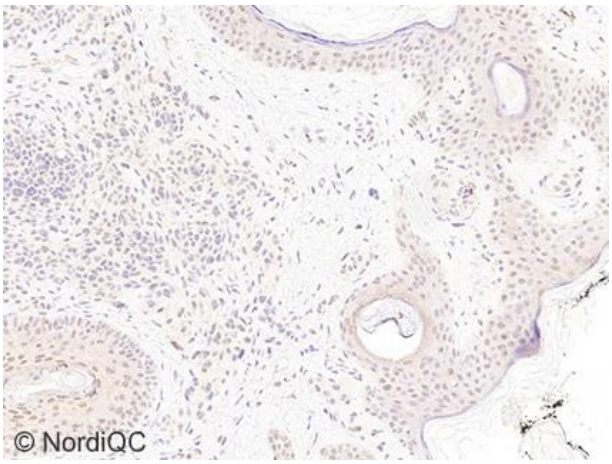


Fig. 6a
False positive staining reaction in the nuclei of normal keratinocytes in the epidermis and the hair follicle as well as in melanocytes. This aberrant staining pattern was seen in 4/6 insufficient staining results using the rmAb clone QR005 on the Leica Bond III staining platform.

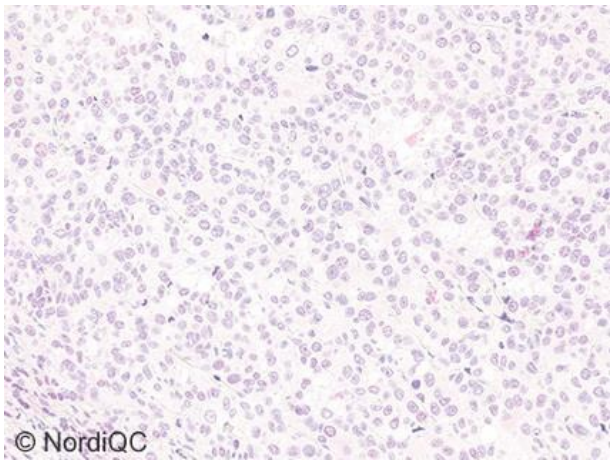


Fig. 6b
Insufficient staining reaction for PRAME in the malignant melanoma, tissue core no. 4, using a detection system based on a red chromogen. The proportion of weakly positive neoplastic cells is difficult to evaluate as the difference between a negative cell nucleus stained blue and a positive cell nucleus stained light pink/purple is not distinct. Moreover, the diffuse cytoplasmic staining reaction further hampered the readout.

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