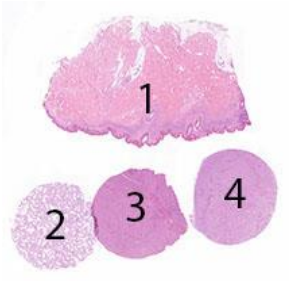
Assessment Run 70 2024

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.-4. 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 3. and 4.
- A weak to moderate cytoplasmic staining reaction in malignant melanocytes with a coexisting strong nuclear staining reaction 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.

KEY POINTS FOR PRAME IMMONUASSAYS
- The rmAb clone **EPR20330** is recommendable both as a concentrated Ab and an RTU.
- Follow vendor recommended protocol settings for the **Ventana RTU system 790-7149**.
- Use a sensitive 3-step detection system based on DAB as chromogen.
- Detection systems based on a red chromogen should be avoided or used only after a thorough optimization and validation process.

Participation

Number of laboratories registered for PRAME, run 70	289
Number of laboratories returning slides	259 (90%)

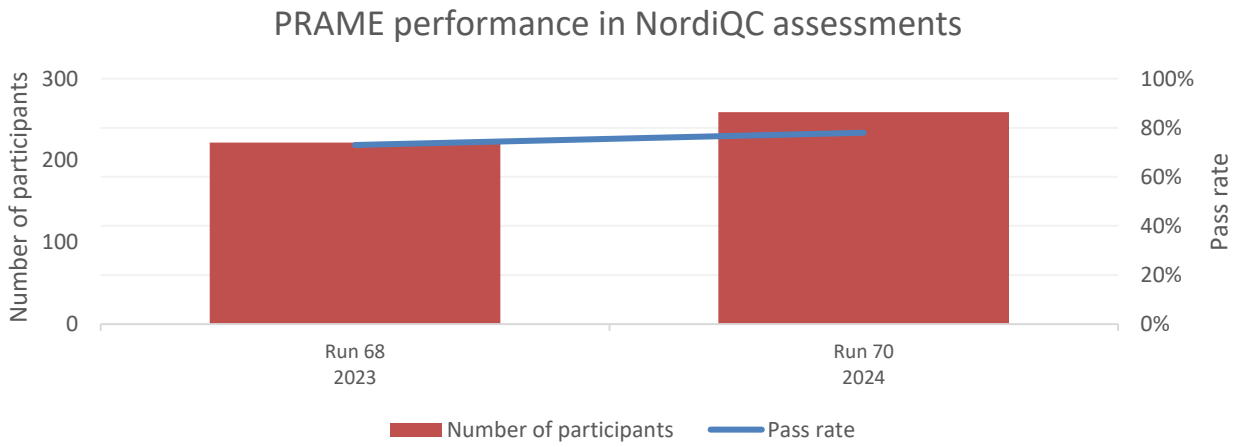
Results
At the date of assessment, 90% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

259 laboratories participated in this assessment and 78% achieved a sufficient mark (optimal or good, see Table 1a, page 2). Tables 1b and 1c summarize 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 second NordiQC assessment of PRAME and a slight increase in pass rate was observed compared to the previous run (see Graph 1).

Graph 1. Proportion of sufficient results for PRAME in the two NordiQC runs performed



Controls
At present, 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.

Conclusion
The rmAb clones **EPR20330**, **QR005**, **EP461, RBT-PRAME** and **E7I1B** 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-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%, 44% optimal and provided an optimal result on all the main automated IHC platforms (Ventana/Roche, Dako/Agilent and Leica Biosystems), similar to the level seen in previous run. Although PRAME is primarily used for melanocytic lesions, care must be taken when choosing a suitable detection system. Participants using a detection system based on a red chromogen achieved a general lower pass rate of 68% (36/53) with only 17% (9/53) being optimal, compared to a pass rate of 81% (166/206) and 50% optimal (104/106) for DAB based detection systems.

Table 1a. **Overall results for PRAME, run 70**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	108	40	32	28	8	67%	37%
Ready-To-Use antibodies	151	73	57	19	2	86%	48%
Total	259	113	89	47	10		
Proportion		44%	34%	18%	4%	78%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for PRAME, run 70**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **EPR20330**	53	Abcam	18	18	14	3	68%	34%
rmAb clone **EPR20330**	15	Biocare Medical	8	2	4	1	67%	53%
rmAb clone **EPR20330**	10	Diagnostic BioSystems	5	4	1	0	90%	50%
rmAb clone **EPR20330**	1	Gennova	0	1	0	0	-	-
rmAb clone **QR005**	19	Quartett	2	7	8	2	47%	11%
rmAb clone **EP461**	7	Cell Marque	5	0	0	2	71%	71%
rmab clone **RBT-PRAME**	3	Bio SB	2	0	1	0	-	-
Total	108		40	32	28	8		
Proportion			37%	30%	26%	7%	67%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).

Table 1c. **Ready-To-Use antibodies and assessment marks for PRAME, run 70**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **EPR20330 790-7149/7150 (VRPS)[3]**	44	Ventana/Roche	25	18	1	0	98%	57%
rmAb clone **EPR20330 790-7149/7150 (LMPS)[4]**	58	Ventana/Roche	21	23	14	0	76%	36%
rmAb clone **EPR20330 AVI/ALI/API3252**	18	Biocare Medical	10	6	2	0	89%	56%
rmAb clone **EPR20330 MAD-000793QD**	5	Master Diagnostica	3	2	0	0	100%	60%
rmAb clone **EPR20330 RMPD109**	2	Diagnostic BioSystems	0	0	1	1	-	-
rmAb clone **QR005 P-P003**	11	Quartett	5	4	1	1	82%	45%
rmAb clone **QR005 8355-C010**	1	Sakura Finetek	1	0	0	0	-	-
rmAb clone **EP461 484R-10/17/18**	6	Cell Marque	5	1	0	0	100%	83%
rmab clone **RBT-PRAME BSB-2374-3/7/15**	3	Bio SB	1	2	0	0	-	-
rmAb clone **E7I1B RMA-1047**	2	Fuzhou Maixin Biotech	2	0	0	0	-	-
rmAb clone **IHC092**	1	GenomeMe	0	1	0	0	-	-
Total	151		73	57	19	2		
Proportion			48%	38%	13%	1%	86%	

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 70

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) (3/10)*, TRS pH 9 (Dako/Agilent) (14/33), Cell Conditioning 1 (CC1, Ventana/Roche) (8/24), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (4/10) as retrieval buffer. The rmAb was typically diluted in the range of 1:25-1:2.500 depending on the Ab vendor (mean dilution factor for optimal results with Abcam product was 1:712, 1:69 for Biocare product and 1:85 for Diagnostic Biosystems product) and total sensitivity of the protocol employed. Using these protocol settings, 52 of 74 (70%) 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 CC1 (Ventana/Roche) (1/7) and BERS2 (Leica Biosystems) (1/6) as retrieval buffer and the rmAb was diluted in the range of 1:50-1:100. Using these protocol settings, 7 of 13 (54%) laboratories produced a sufficient staining result.

rmAb clone **EP461**: Protocols with optimal results were based on HIER using CC1 (Ventana/Roche) (3/3) and BERS2 (Leica Biosystems) (2/3) as retrieval buffer and the rmAb was diluted in the range of 1:20-1:100. Using these protocol settings, 5 of 6 (83%) 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[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
rmAb clone **EPR20330**	3/9** (33%)	-	14/31 (45%)	-	8/24 (33%)	0/1	4/10 (40%)	-
rmAb clone **QR005**	0/1	-	0/1	-	1/7 14%	-	1/6 17%	-
rmAb clone **EP461**	-	-	0/1	-	3/3	-	2/3	-

* 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.
1) Autostainer Link 48
2) BenchMark GX, Ultra, Ultra plus
3) Bond MAX, III, Prime

Ready-To-Use antibodies
rmAb clone **QR005**, product no. **P-P003**, Quartett:
Protocols with optimal results were based on HIER using CC1, TRS High pH or BERS2 as retrieval buffer (efficient heating time 48-64, 30 or 20 min., respectively) and OptiView (760-700), Bond Refine (DS9800) or FLEX (GV800/GV823) as detection system. Using these protocol settings, 5 of 5 (100%) laboratories produced an optimal staining result.

rmAb clone **EP461**, product no. **484R-10/17/18**, Cell Marque:
Protocols with optimal results were based on HIER using CC1, TRS High pH or BERS2 as retrieval buffer (efficient heating time 64, 30 or 20 min., respectively) and OptiView (760-700), Bond Refine (DS9800), Bond Refine Red (DS9390) or FLEX+ (GV800/GV823 + GV809) as detection system. Using these protocol settings, 6 of 6 (100%) laboratories produced a sufficient staining result, 5 of 6 (83%) optimal.

rmAb clone **EPR20330**, product no. **AVI/ALI/API3252**, Biocare Medical:
Protocols with optimal results were typically based on HIER using CC1 or BERS2 (efficient heating time 64 or 30 min., respectively, at 95-100°C), 15-32 min. incubation of the primary Ab and UltraView Red (760-501), OptiView (760-700), Bond Refine (DS9800) or Bond Refine Red (DS9390) as detection system. Using these protocol settings, 13 of 13 (100%) laboratories produced a sufficient staining result, 9 of 10 (90%) optimal.

Ready-To-Use antibodies and corresponding systems
rmAb clone **EPR20330**, product no. **790-7149/7150**, Ventana/Roche, BenchMark GX/Ultra/Ultra Plus:
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 UltraView DAB (760-500) or UltraView Red (760-501) with or without amplification or OptiView (760-700) as detection system. Using these protocol settings, 66 of 72 (92%) laboratories produced a sufficient staining result, 38/72 (53%) optimal.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. 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 GX/Ultra/Ultra Plus rmAb EPR20330 **790-7149/7150 UltraView DAB**	100% (3/3)	33% (1/3)	75% (6/8)	50% (4/8)
VMS GX/Ultra/Ultra Plus rmAb EPR20330 **790-7149/7150 UltraView AP Red**	100% (7/7)	14% (1/7)	62% (13/21)	14% (3/26)
VMS GX/Ultra/Ultra Plus rmAb EPR20330 **790-7149/7150 OptiView**	97% (33/34)	68% (23/34)	88% (23/26)	50% (13/26)

* 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 second assessment of PRAME an overall pass rate of 78% 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. Too weak or false negative staining reaction was observed in 82% of the insufficient results (47/57). Although very sensitive protocol settings are required for an optimal staining result, an increased number of positive cells or false positive staining reaction was seen in 11% of insufficient results (6/57) indicating that the PRAME IHC assay must be carefully calibrated for diagnostic accuracy as a semi-quantitative scoring is applied in the final interpretation. The reduced analytical specificity was mainly characterized by a positive nuclear staining reaction in most of the melanocytes and keratinocytes in the skin epidermis and hair follicles in the nevus, tissue core no. 1. Virtually all laboratories were able to detect PRAME in high-level antigen expressing cells as normal spermatogonia in basal parts of the seminiferous tubules in 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.

Concentrated Ab formats within laboratory developed (LD) assays for PRAME were used by 42% (108/259) of the participants with 67% (72/108) producing a sufficient result, 37% (40/108) optimal. Optimal results for the most commonly applied Abs could be obtained on all the main automated staining platforms from Ventana/Roche, Dako/Agilent and Leica Biosystems – see Table 2.
The rmAb clone **EPR20330** was the most widely used antibody, being applied by 73% (79/108) of laboratories as a concentrate (see Table 1b). It also proved to be the most robust as the main prerequisite for a sufficient staining was the use of a sensitive 3-step polymer/multimer based detection system which, regardless of stainer platform or any other parameters used, achieved a pass and optimal rate of 77% (46/60) and 43% (26/60), respectively. Similar to the previous PRAME assessment run 68, an improved pass rate of 90%, 50% being optimal was observed if the clone was diluted in diluent produced by Dako/Agilent (K8006, S0809, S2022, S3022) or Biocare Medical (Renoir Red PD904) and regardless of the dilution factor applied.

The rmAb clone **QR005** was the second most used Ab concentrate and gave an inferior pass rate of 47% (9/19), 11% (2/19) optimal. The main reason for an insufficient result was either a too weak or false negative staining reaction seen in 90% (9/10) of insufficient results. The main prerequisites for a successful assay were the use of a sufficient HIER procedure in an alkaline buffer for at least 30 min. on all main staining platforms from Ventana/Roche, Dako/Agilent and Leica Biosystems together with the use of a sensitive 3-step detection system. Using this combination, a pass rate of 70% (7/10) was achieved, however only one result was optimal.

In this assessment, the rmAb clone **EP461** achieved a significantly better pass rate of 71% (5/7) with all being scored as optimal compared to a pass rate of 40% (2/5) and optimal rate of 20% (1/5) achieved in the previous PRAME assessment run 68 (2023). All optimal results were achieved with a sensitive 3-step detection system. The two protocols providing a result assessed as Poor were based on the same antibody

LOT (171270). NordiQC internal data on same lot has revealed an inferior performance of selected vials from this lot, suddenly causing a too weak or false negative staining result. This was also the staining pattern seen in the insufficient results. Nevertheless, it cannot be concluded with certainty if this was the main reason for the poor staining results in this assessment run due to limited data points.

The RTU formats of PRAME Abs were used by 58% (151/259) of laboratories, showing a significant increase compared to 42% (92/222) in the previous assessment run 68 (2023). Within all RTU protocols 86% (130/151) provided a sufficient result, 48% (73/151) optimal. The Ventana/Roche RTU system based on rmAb clone **EPR20330** (**760-7149/7150**) was the most widely used RTU system with a total pass rate of 85% (87/102), 46% (45/102) optimal. The most successful staining results were seen when vendor recommended protocol settings (VRPS) were applied, with the highest proportion of optimal results obtained using OptiView as detection system (see Table 3, Figures 1a-4a). 28 participants applied the UltraView AP Red detection kit, producing an overall pass rate of 71% (20/28) and a low optimal rate of 14% (4/28). Although the majority of participants could correctly categorize the clinical samples, 64% (18/28) of all laboratories using the UltraView AP red detection kit received a comment describing a lower technical quality such as excessive background, poor signal-to-noise ratio and indistinct nuclear staining most likely caused by the visualization system used. Extra care must be taken when using the UltraView AP red detection kit with Amplification kit as 89% (8/9) laboratories exhibited excessive background and/or an increased proportion of positive cells/false positive result in the compound nevus (see Figures 5a and 5b).

Biocare Medical RTU products based on the rmAb clone **EPR20330** (**AVI/ALI/API3252**) proved again to work best on the Ventana BenchMark stainer platforms, with all (10/10) laboratories achieving a sufficient result, 70% (7/10) optimal. 8 participants applied the RTU system on the Leica Bond stainer platforms with a pass rate of 75% (6/8), 38% (3/8) optimal. Based on the data from this and the previous PRAME assessment run (68, 2023) the main prerequisite for a sufficient staining result on Leica Bond stainer platforms was HIER for 30 min. in an alkaline BERS2 buffer (AR9640).

As seen in the previous PRAME assessment, the RTU format of the rmAb clone **QR005** (**P-P003**) by Quartett outperformed the corresponding Ab concentrate achieving a pass rate of 82% (9/11), 45% (5/11) optimal (see Tables 1b and 1c). Insufficient results were characterized as exhibiting a too weak or a false negative staining reaction. All optimal results were achieved with the use of a DAB based detection system as OptiView, EnVision FLEX and Bond Refine together with a sufficient HIER in an alkaline buffer for at least 48, 30 and 20 min. on the three respective stainer platforms (Ventana BenchMark, Dako Omnis, Leica Bond).

The rmAb **EP461** based RTU format **484R-10/17/18** showed a high pass rate of 100% (6/6) 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.

The purpose of this PRAME assessment was focused on melanocytic lesions and 20% (53/259) of participants used a detection system based on a red chromogen, achieving a general lower pass rate of 68% (36/53) and only 17% (9/53) were optimal. The respective proportions for DAB based systems were 81% (166/206) and 50% (104/206). 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 (see Figures 1b-4b). In this context, DAB based detection systems should be the preferred choice whereas the red chromogen based systems can be beneficial in highly pigmented neoplasms. 75% (40/53) of participants used the UltraView AP Red detection kit, which can cause an overall granular precipitate over relevant parts of the tissue sections that can hinder the interpretation. An optimal result using a detection kit based on a red chromogen 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 second NordiQC assessment of PRAME and an overall pass rate of 78% was obtained, which is a slight increase from run 68 in 2023 (73%, see Graph 1). Opposite to what was seen in the previous assessment, the majority of participants (151/259) opted for an RTU system, which overall achieved a superior performance regarding the proportion of sufficient and optimal results (see Table 1a). The most cited and widely used rmAb clone EPR20330 proved to be relatively robust with a high pass rate of 80% (164/206) and 44% (90/206) optimal. Other rmAbs as QR005, RBT-PRAME and EP461 were used by 19% (50/259) of participants and achieved a pass rate of 70% (35/50), 42% (21/50) optimal, demonstrating an increase compared to 61% (36/59) and 20% (12/59), respectively, obtained in the previous PRAME assessment.

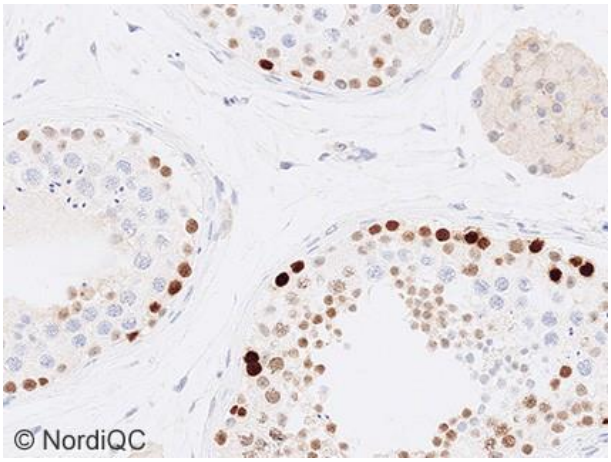


Fig. 1a
Optimal PRAME staining reaction of testis using the Ventana/Roche RTU system based on rmAb clone EPR20330 (32 min. incubation), HIER in CC1 (64 min.), OptiView as a detection system and performed on Ventana BenchMark Ultra stainer platform. Spermatogonia located at the basal part of seminiferous 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.

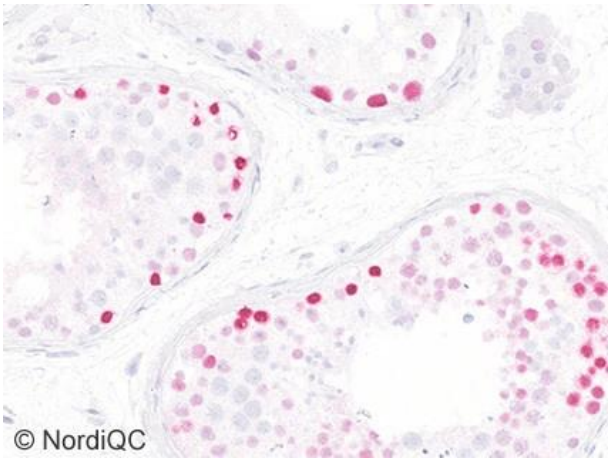


Fig. 1b
Insufficient PRAME staining reaction of testis using the rmAb clone EPR20330 as a concentrate (1:500) on the Ventana BenchMark Ultra stainer platform with a relatively short HIER time (10 min.) and a 2-step detection system UltraView AP Red. Spermatogonia being high expressors show a strong nuclear staining reaction, whereas a reduced proportion of germ cells are positive in the seminiferous tubules and Leydig cells showing no or only very faint membranous staining reaction. Compare to Fig. 1a. Same protocol used in Figs. 2b-4b.

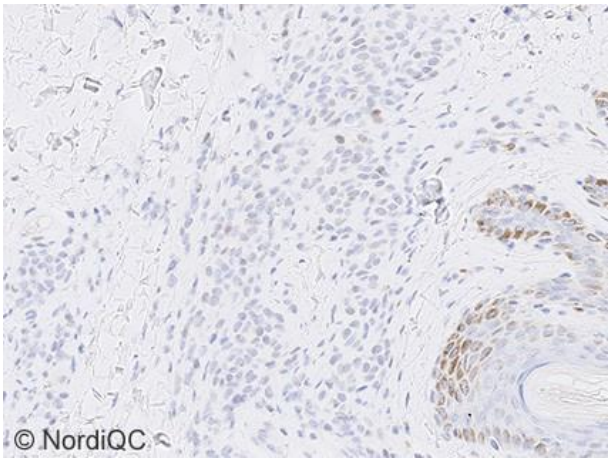


Fig. 2a
Optimal staining reaction for PRAME in the compound nevus using the same protocol as in Fig. 1a. <26% of the melanocytes in the nevus nests display a weak to moderate nuclear staining reaction. No nuclear staining reaction is seen in the epidermis. Same protocol used in Figs 3a-4a.

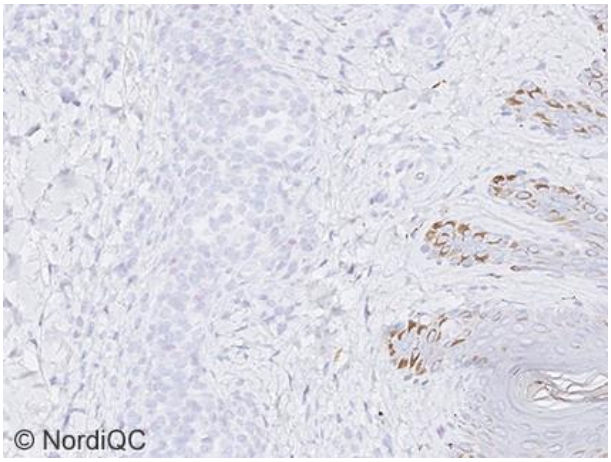


Fig. 2b
Staining reaction for PRAME in the compound nevus using the same insufficient protocol as in Fig 1b. Only a few scattered nevus cells exhibit a weak nuclear staining reaction while all other cells are negative. Same protocol used in Figs. 3b-4b – same field as 2a.

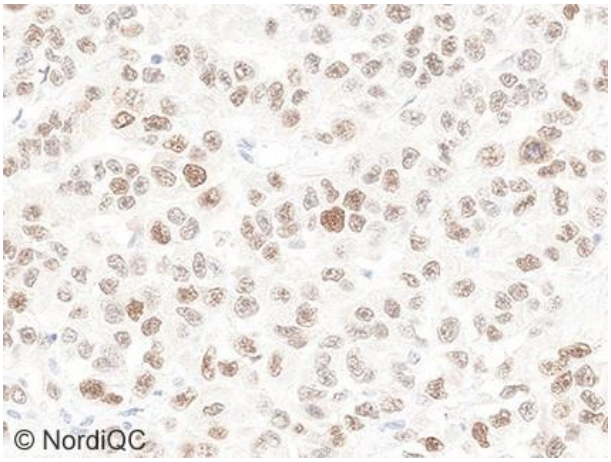


Fig. 3a
Optimal staining reaction for PRAME in the malignant melanoma, tissue core no. 4, using the same protocol as in Figs. 1a-2a. Virtually all tumor cells show a weak to moderate nuclear staining reaction.

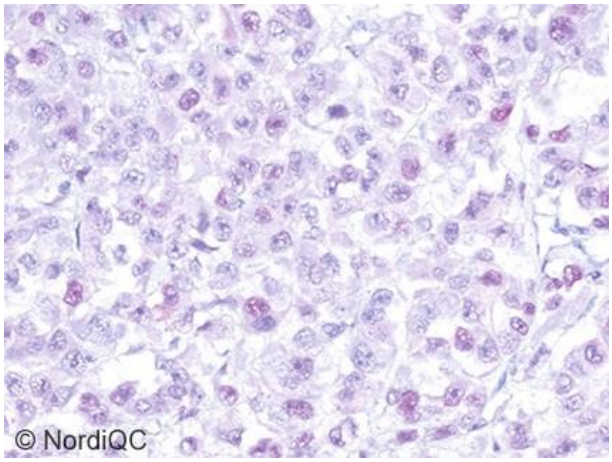


Fig. 3b
Insufficient staining reaction for PRAME in the malignant melanoma, tissue core no. 4. It is extremely difficult to distinguish between negative and weakly positive nuclei and estimate the proportion of positive tumor cells. Mainly an overall weak indistinct staining reaction is seen and <76% of neoplastic cells being demonstrated. Same field as 3a, same protocol as in Figs 1b-2b.

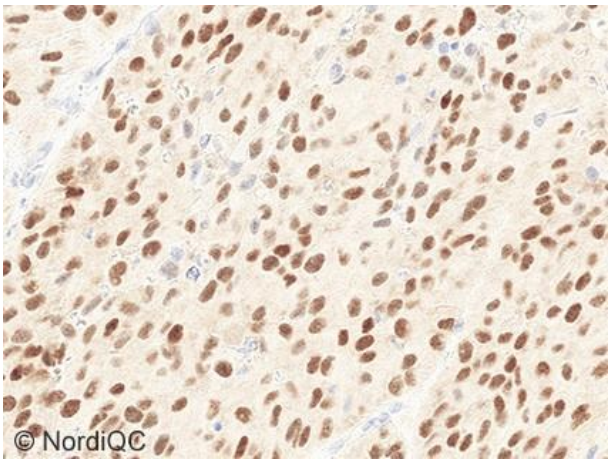


Fig. 4a
Optimal staining reaction for PRAME in the high expressor malignant melanoma, tissue core no. 3, using the same protocol as in Figs. 1a-3a. Virtually all tumor cells show moderate to strong nuclear staining reaction

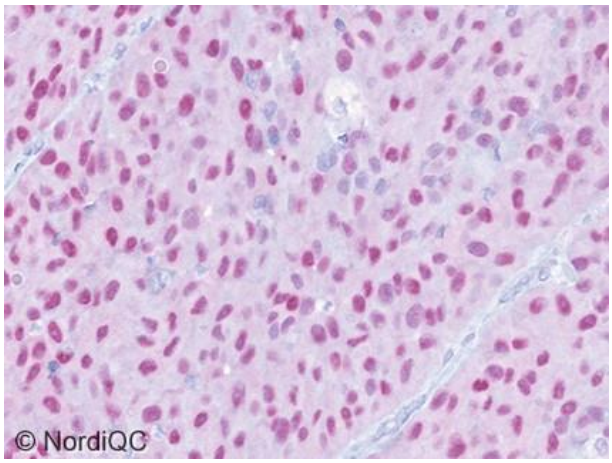


Fig. 4b
Staining reaction for PRAME in the high expressor malignant melanoma, tissue core no. 3, using the same insufficient protocol as in Figs. 1b-3b. ≥76% of tumor cells show a nuclear staining reaction. Same field as 4a.

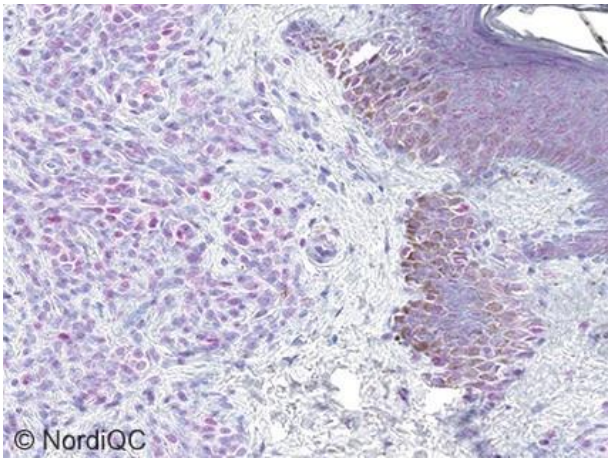


Fig. 5a
Insufficient staining reaction (borderline) for PRAME in the nevus, tissue core no. 1, using UltraView AP red detection system together with UltraView Amplification Kit. An increased proportion of positive nevus cells and a clear granular red precipitation is seen in the epidermis layer.

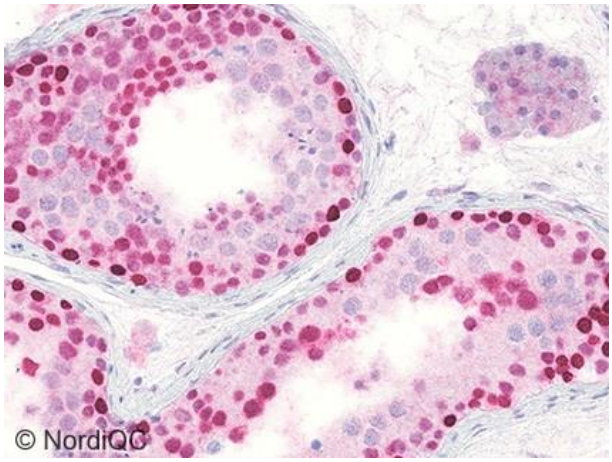


Fig. 5b
Insufficient staining reaction for PRAME in testis, tissue core no. 2. A moderate diffuse cytoplasmic staining is seen in virtually all the cells in seminiferous tubules and Leydig cells. Same insufficient protocol as in Fig 5a.

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