

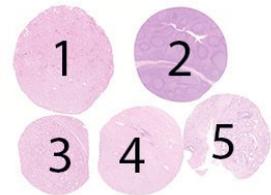
Purpose

Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for AMACR, typically used in the diagnostic work-up of differentiation of normal prostate glands and prostate intraepithelial neoplasia (PIN) from prostate adenocarcinomas. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for AMACR (see below).

Material

The slide to be stained for AMACR comprised:

1. Kidney, 2. Tonsil, 3. Prostate adenocarcinoma, 4. Prostate adenocarcinoma, 5. Prostate hyperplasia.



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a AMACR staining as optimal included:

- A moderate to strong, distinct granular cytoplasmic staining reaction of virtually all epithelial cells lining the renal proximal tubules.
- An at least weak, distinct granular cytoplasmic staining reaction in most of the epithelial cells lining the renal distal tubules and dispersed parietal epithelial cells lining Bowman’s capsule.
- A weak to strong, distinct granular cytoplasmic staining reaction of virtually all neoplastic cells in the prostate adenocarcinomas, tissue core no. 3 and 4.*
- A negative or only focal, weak granular cytoplasmic staining reaction of epithelial cells in the hyperplastic prostate glands.
- No staining of other cells including lymphocytes, macrophages and squamous epithelial cells in the tonsil. However, in case of PIN cocktails, the expected reaction for the other antibodies was accepted e.g. p63 in squamous epithelial cells and lymphocytes etc.

* The two prostate adenocarcinomas showed a slightly heterogenous AMACR expression and each participant slide was compared to the expression level observed in nearest reference slide.

In this run, and for participants using PIN-cocktails (e.g. p63+AMACR/P504s), only the specific reaction for AMACR was assessed.

KEY POINTS FOR AMACR IMMUNOASSAYS

- A high overall pass rate of 92% was observed in this fifth assessment.
- The rmAb clone **13H4** was used by 59% of all participants.
- The AMACR **RTU** systems from Dako/Agilent based on clone **13H4** and applied by vendor recommended protocols were most successful giving pass rates of 100%, 90% being optimal.
- The performance of the Ventana/Roche RTU system based on mAb clone SP116 and vendor recommendations, gave a high pass-rate of 100%, but only 27% optimal.
- The Ventana/Roche RTU system applied with OptiView and similar settings as the vendor recommended protocol provided 84% optimal results.

Participation

Number of laboratories registered for AMACR, run 63	421
Number of laboratories returning slides	379 (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.

379 laboratories participated in this assessment and 92% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3 and 4).

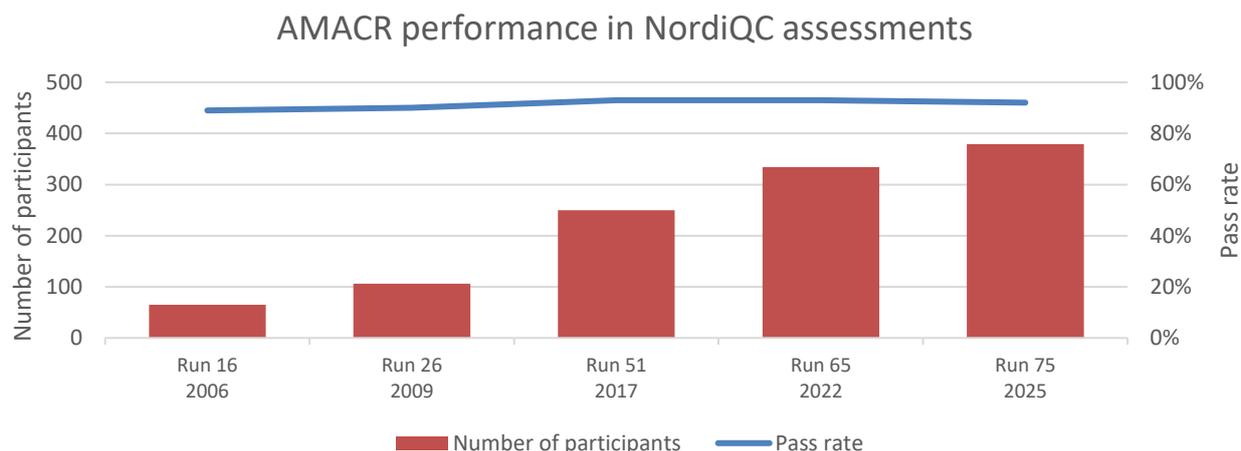
The most frequent causes of insufficient staining were:

- Inefficient HIER – too short time or use of acidic buffer.
- Too low concentration of the primary antibody or too short incubation time.
- Less sensitive detection systems used in combination with other low sensitivity protocol parameters.

Performance history

This was the fifth NordiQC assessment of AMACR. A significant increase in participants was observed with a similar pass-rate compared to the previous runs (see Graph 1). Maintaining the high pass-rate is primarily due to the access and use of robust primary antibodies and well calibrated Ready-To-Use (RTU) systems (see Tables 1b-c).

Graph 1. **Proportion of sufficient results for AMACR in the five NordiQC runs performed**



Control

Kidney is recommended as positive tissue control for AMACR: Virtually all epithelial cells of the proximal tubules must show a moderate to strong and distinct granular cytoplasmic staining, whereas most epithelial cells of the distal tubules and at least dispersed parietal epithelial cells of Bowman's capsule must display a weak granular cytoplasmic staining reaction in the main part of the tubules. Normal prostate is recommended as negative tissue control for AMACR: The epithelial cells must be negative or only show a focal granular cytoplasmic staining reaction.

Conclusion

The rmAb clones **13H4** and **SP116** were the most widely used and robust antibodies for demonstration of AMACR. Irrespective of the primary Ab applied, efficient HIER preferable in an alkaline buffer and careful calibration of the primary Ab, in combination with a sensitive IHC system (3-step polymer/multimer system), were the main prerequisites for an optimal staining result. The mAb clone **EPMU1** did produce optimal results as a concentrate and also as a RTU product for the Bond platforms, however the antibody needed very careful calibration to provide an optimal analytical sensitivity.

Table 1a. Overall results for AMACR, run 75

	n	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
Concentrated antibodies	111	69	29	13	-	88%	62%
Ready-To-Use antibodies	268	181	71	16	-	94%	68%
Total	379	250	100	29	-		
Proportion		66%	26%	8%	0%	92%	

1) Proportion of sufficient results (optimal or good).

2) Proportion of optimal results.

Table 1b. Concentrated antibodies and assessment marks for AMACR, run 75

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
rmAb clone 13H4	71	Dako/Agilent	62	23	7	-	95%	67%
	6	Cell Marque						
	6	BioSB						
	5	Zeta Corporation						
	1	Epredia						
	1	Pathnsitu						
	1	NeoBiotechnologies						
1	Immunologic							
rmAb clone QR108	2	Quartett	1	1	-	-	-	-
mAb clone EPMU1	11	Leica Biosystems	5	3	3	-	71%	46%
Unkown clone GR01	1	GeneBioSolution	-	-	1	-	-	-
pAb P504S ACA200	2	Biocare Medical	1	1	-	-	-	-
pAb P504S RP134R	1	Diagnostic BioSystems	-	-	1	-	-	-
PIN-Cocktails								
pAb P504s + mAb clone 4A4	1	Zytomed Systems	-	1	-	-	-	-
13H4+CK5/6+P63	1	Homemade	-	-	1	-	-	-
Total	111		69	29	13	-		
Proportion			62%	26%	12%	0%	88%	

1) Proportion of sufficient results (optimal or good). (≥5 assessed protocols).

2) Proportion of optimal results.

Table 1c. Ready-To-Use antibodies and assessment marks for AMACR, run 75

Ready-To-Use antibodies							Suff. ¹	OR. ²
rmAb clone SP116 790-6011 ³	15	Ventana/Roche	4	11	-	-	100%	27%
rmAb clone SP116 790-6011 ⁴	92	Ventana/Roche	68	16	8	-	91%	74%
rmAb clone 13H4 IS/IR060 ³	10	Dako/Agilent	9	1	-	-	100%	90%
rmAb clone 13H4 IS/IR060 ⁴	16	Dako/Agilent	6	9	1	-	94%	38%
rmAb clone 13H4 GA060 ³	44	Dako/Agilent	39	5	-	-	100%	89%
rmAb clone 13H4 GA060 ⁴	42	Dako/Agilent	30	9	3	-	93%	71%
mAb clone EPMU1 PA0210 ³	7	Leica Biosystems	2	5	-	-	100%	29%
mAb clone EPMU1 PA0210 ⁴	10	Leica Biosystems	6	3	1	-	90%	60%
rmAb clone 13H43	2	Sakura	2	-	-	-	-	-
rmAb clone 13H4 504R-10-ASR	4	Cell Marque	4	-	-	-	-	-
rmAb clone 13H4 MAD-000305QD	7	Master Diagnostica	2	3	2	-	71%	29%

rmAb clone 13H4 RMA-0546	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone 13H4 BSB 5059	2	Bio SB	1	1	-	-	-	-
rmAb clone 13H4 Z2001RP	2	Zeta Corporation	-	2	-	-	-	-
rmAb clone MXR012 RMA-1023	1	Fuzhou Maixin	-	1	-	-	-	-
mAb clone GM305 GT245302	1	GeneTech	1	-	-	-	-	-
mAb clone C7H4 CAM-0201	1	Celnovte	1	-	-	-	-	-
mAb clone CMA158 I1194	1	Bio Lynx	-	1	-	-	-	-
Clone DY49201 4910152	1	Dakewe	-	1	-	-	-	-
pAb P504S PP/APA200	3	Biocare Medical	3	-	-	-	-	-
pAb P504S RBG002	1	Zytomed System	-	1	-	-	-	-
pAb P504S PDR046R	1	Diagnostic BioSystems	-	1	-	-	-	-
PIN-Cocktails								
mAb clone 34βE12 + mAb clone 4A4 + rmAb 13H4 PIN002-G	1	Nordic-Mubio	-	1	-	-	-	-
mAb clone 34βE12 + mAb clone 4A4 + rmAb 13H4 API 3154DS AA	1	Biocare Medical	1	-	-	-	-	-
mAb clone LL002 + pAb P504S PPM201H	1	Biocare Medical	-	-	1	-	-	-
Unknown P63+CK5+P504S PPm 225 AA	1	Leica Biosystems	1	-	-	-	-	-
Total	268		181	71	16	-		
Proportion			68%	26%	6%	0%	94%	

1) Proportion of sufficient results (optimal or good). (≥5 assessed protocols).

2) Proportion of optimal results.

3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed 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 assessed protocols)

Detailed analysis of AMACR, Run 75

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

rmAb **13H4**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) 3-in-1 pH 9 (Dako/Agilent) (1/2)*, TRS pH 9 (Dako/Agilent) (7/9), Cell Conditioning 1 (CC1, Ventana/Roche) (31/51), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (13/15) or Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (1/2) as retrieval buffer. The rmAb was typically diluted in the range of 1:25-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings, 67 of 73 (92%) laboratories produced a sufficient staining result (optimal or good).

* (number of optimal results/number of laboratories using this buffer)

mAb **EPMU1**: Protocols with optimal results were based on HIER using BERS2 (Leica Biosystems) (4/8) or CC1 1 (Ventana/Roche) (1/2) as retrieval buffer. The mAb was typically diluted in the range of 1:50-100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 6 of 6 (100%) laboratories produced a sufficient staining result (optimal or good).

Table 2. **Proportion of optimal results for AMACR for the most commonly used antibodies 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 13H4	1/2**	-	7/9 (78%)	-	31/51 (61%)	-	13/15 (87%)	1/2
mAb clone EPMU1	-	-	-	-	1/2	-	4/8 (50%)	0/1

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.

** (number of optimal results/number of laboratories using this buffer)

1) Autostainer Classical, Link 48.

2) BenchMark GX, XT, Ultra, Ultra Plus

3) Bond III, Prime, Max

Ready-To-Use antibodies and corresponding systems

rmAb clone **SP116**, product no. **790-6011**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus: Protocols with optimal results were based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 16-32 min. incubation of the primary Ab and OptiView (760-700), UltraView DAB (760-500) or UltraView Universal Alkaline Phosphatase Red (760-501) as detection system. Using these protocol settings, 83 of 89 (93%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **13H4**, product no. **IS/IR060**, Dako/Agilent, Autostainer+/Autostainer Link: Protocols with optimal results were based on HIER using TRS High pH 9 (K8004 or S2375) (efficient heating time 20 min. at 95-98°C), 20-30 min. incubation of the primary Ab and EnVision FLEX or FLEX+ (K8000/K8002 + K8009) as detection systems. Using these protocol settings, 13 of 13 (100%) laboratories produced a sufficient staining result.

13 laboratories used product no. IR060 for staining on another platform. Data was not included in the description above

rmAb clone **13H4**, product no. **GA060**, Dako/Agilent, Omnis:

Protocols with optimal results were based on HIER using TRS High pH 9 (GV804) (efficient heating time 20-30 min. at 97°C), 10-30 min. incubation of the primary Ab and EnVision FLEX or EnVision FLEX+ (GV800 + GV809) as the detection system. Using these protocol settings, 76 of 79 (96%) laboratories produced a sufficient staining result.

3 laboratories used product no. GA060 for staining on another platform. Data was not included in the description above

mAb clone **EPMU1**, product no. **PA0210**, Leica Biosystems, Bond MAX/Bond III/Bond Prime:

Protocols with optimal results were typically based on HIER using BERS2 (efficient heating time 10-20 min. at 95-100°C), 15-20 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800/DS9284) as detection system. Using these protocol settings, 10 of 10 (100%) laboratories produced a sufficient staining result (optimal or good).

1 laboratory used product no. PA0210 for staining on another platform. Data was not included in the description above

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly according to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for AMACR for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako Autostainer Link 48+ rmAb 13H4 IR/IS060	100% (10/10)	90% (9/10)	(3/3)	(3/3)
Dako Omnis rmAb 13H4 GA060	100% (44/44)	89% (39/44)	92% (36/39)	72% (28/39)
Ventana BenchMark XT/Ultra/Ultra Plus rmAb SP116 790-6011	100% (15/15)	27% (4/15)	91% (84/92)	74% (68/92)
Leica Bond III/Prime mAb EPMU1 PA0210	100% (7/7)	29% (2/7)	90% (8/9)	55% (5/9)

* 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 are integrated.

Comments

In this assessment and in concordance with the previous AMACR assessments, the prevalent feature of an insufficient staining result was a too weak or completely false negative staining reaction of cells expected to be demonstrated. This pattern was seen in 83% (24 of 29) of the insufficient results. 17% (5/29) of the insufficient staining results was due to a false positive reaction in the apical compartment of the cytoplasm of the benign epithelial cells in the prostate hyperplasia (see Fig. 6b) or excessive background reaction. Both prostate adenocarcinomas showed a slightly heterogenous AMACR expression between and within the constructed TMAs. The staining pattern in each participant slide was carefully compared to the nearest NordiQC reference slide. In total, five TMAs were used and the AMACR expression was characterized in at least 3-5 levels in each TMA by the NordiQC AMACR reference IHC assay.

Most participating laboratories successfully detected the elevated AMACR in neoplastic cells of prostate adenocarcinoma tissue cores 3 and 4. However, identifying AMACR in kidney distal tubules, parietal epithelial cells of Bowman's capsule and neoplastic cells with low AMACR levels in core number 3 and 4 proved difficult and required optimized protocols.

29% (111 of 379) of the laboratories used a concentrated Ab format within laboratory developed (LD) assays for AMACR including PIN-cocktails. The rmAb clone **13H4** was the most widely used Ab and when used, within a LD assay, the rmAb clone 13H4 gave an overall pass rate of 95% (85 of 92) and 67% (62 of 92) optimal. The rmAb clone 13H4 could be used to obtain optimal staining results on all four of the main IHC platforms as shown in Table 2.

HIER, preferable in an alkaline buffer, in combination with a careful calibration of the primary Ab seem to be the most critical parameters for a sufficient and optimal result. In this run, optimal results could be obtained by using a 2-step multimer/polymer detection system (e.g. UltraView, Ventana/Roche, FLEX, Dako/Agilent or Bond Refine, Leica Biosystems). However, the general performance of the assays was improved by applying 3-step multimer/polymer detection systems (e.g. OptiView, Ventana/Roche or FLEX+ (Dako/Agilent) enhancing the demonstration of low-level AMACR expressing structures and providing superior signal-to-noise ratio.

Eleven laboratories used the mAb clone **EPMU1** within a LD assay on either a Bond platform (Leica Biosystems) or Ventana Benchmark platform (Ventana/Roche). The pass-rate of EPMU1 was in this run 75 inferior to the performance of rmAb clone 13H4. Optimal results were obtained on both the Ventana Benchmark system, Bond III and Bond Prime (Leica Biosystems). No protocols using mAb clone EPMU1 were performed on Dako Omnis or Autostainer (Dako/Agilent). In this run, mAb clone EPMU1 should be used in the range of 1:50-100 in combination with a 3-step polymer/multimer based detection system for an optimal result. This was a change compared to the findings observed in the previous run in which the range of primary Ab concentration for optimal results was 1:200-300. When using e.g. a more concentrated format as 1:100, an excessive background reaction was seen. This difference can either be related to change in the composition of the primary ab or tissue related variations and the final optimal titer of the mAb clone EPMU1 must be defined carefully for LD assays based on this product.

71% (268 of 379) of the laboratories used Ready-To-Use (RTU) systems for detection of AMACR including PIN-cocktails. In run 65, the number of laboratories using RTU systems was only 62%. In this run, most participants used the Dako/Agilent RTU systems based on the rmAb clone 13H4 or the Ventana/Roche RTU system based on rmAb clone SP116.

In total, 107 participants used the Ventana/Roche RTU system. As shown in Table 3, only 14% (n=15) used the Ventana/Roche RTU system **790-6011, rmAb** clone **SP116** by the vendor recommended protocol settings applying HIER in CC1 for 64 min., 16 min. incubation time of the primary Ab in combination with UltraView Alkaline Phosphatase Red (760-501) as detection system and all laboratories received a sufficient mark of which, 27% (4/11) being optimal.

It was observed that using the basic vendor recommended protocol settings for HIER and primary Ab incubation time but using OptiView as detection system instead of UltraView Red was very successful as 84% (16 of 19) of the protocols based on these settings gave an optimal result. Overall, OptiView with DAB as chromogen facilitated the demonstration of AMACR in low-level AMACR expressing structures and was superior to the Alkaline Phosphatase Red system (see Figs. 1a-4a and Figs 5a-b).

The Dako/Agilent **IS/IR060** product based on rmAb clone **13H4** produced for the Autostainer Link 48 and Classic was used by 26 laboratories. 13 laboratories applied the product on other IHC platforms. As shown in Table 3, all of the 13 laboratories using the RTU product on the intended platform received a sufficient mark, 92% optimal (12/13). The high pass rate for the RTU product for both off-label use on non-Autostainer platforms (92% sufficient) and within intended use do underline the robustness of the clone. In general, it must be emphasized that modifications of vendor recommended protocol settings for RTU systems including migration of the RTU Abs to another platform than the intended, require a meticulous validation process by the end-users. As seen in this assessment, modifications can be successful but potentially also generate aberrant results and therefore must be carefully monitored.

The Dako/Agilent **GA060** product, also based on rmAb clone **13H4**, produced for the Dako Omnis platform was used by 86 laboratories. Three used the product on other IHC platforms. Using the vendor recommendations with the EnVision FLEX+ protocol and HIER with TRS high for 30 min. and primary Ab incubation for 10 min., 100% (44/44) of the laboratories received a sufficient result, 89% (39/44) optimal. 39 participants applied a laboratory modified protocol increasing the Ab incubation time to 15-30 min. with and without linker (FLEX) and 92% (36/39) with a sufficient result. The reaction pattern using the vendor recommended protocol was characterized by a distinctive reaction pattern with a very strong reaction in cells expressing high levels of AMACR (e.g. proximal tubules) whereas cells with lower levels of AMACR expression like the epithelial cells lining the distal tubules showed a weaker intensity compared to optimal protocols stained with other RTU products on different instruments (see Figs. 6a-b). Despite the minor difference in staining intensities most likely due to the detection systems and chromogens applied, the results on Omnis were still considered fully optimal. Similar to the corresponding RTU format for Autostainer, the product seemed to be robust despite being applied within different settings.

The Leica Biosystems **PA0210** RTU system based on mAb clone **EPMU1** was used by 16 participants on the intended Bond IHC platforms. The vendor recommended protocol based on HIER in BERS2 for 20 min., 15 min. incubation time of primary Ab and Refine as detection system was used by 7 participants, but with mixed results as shown in Table 1. However, the recommended protocol is based on peroxidase blocking after the primary Ab (protocol Q). Unfortunately, the possibility for data entry of protocol does not include this information, and hence no conclusion on impact on this step can be generated. Overall, the clone EPMU1 both as RTU and as concentrated format was found slightly less successful as typically a poor signal-to-noise or excessive background reaction was observed.

This was the fifth NordiQC assessment of AMACR (see Graph 1). A pass rate of 92% was obtained which is equal to the last assessment in run 65, 2022, even though the number of participants has increased with 45 new laboratories. In this assessment, new commercially available antibodies were introduced and most were able to produce a sufficient result, which was reflected by high overall pass rates both for concentrated formats (88%) and RTU products (94%).

From the previous runs, a big increase in laboratories using the RTU products was observed going from 40% (99 of 250) in run 51 to 60% (208 of 334) in run 65. The high pass rates were supported by adequate vendor protocol recommendations for the RTU products providing optimal results on the intended IHC platforms. However, laboratory modified changes, especially seen for the Ventana/Roche RTU system changing the recommended detection system, were found very successful.

The mAb clone EPMU1 from Leica Biosystems was the least successful clone, although able to obtain optimal results. With this clone, very careful calibration was necessary to avoid poor-signal-to-noise ratio or an excessive background reaction. It still has to be investigated if endogenous peroxidase blocking before or after the primary Ab incubation step will affect the performance of this clone.

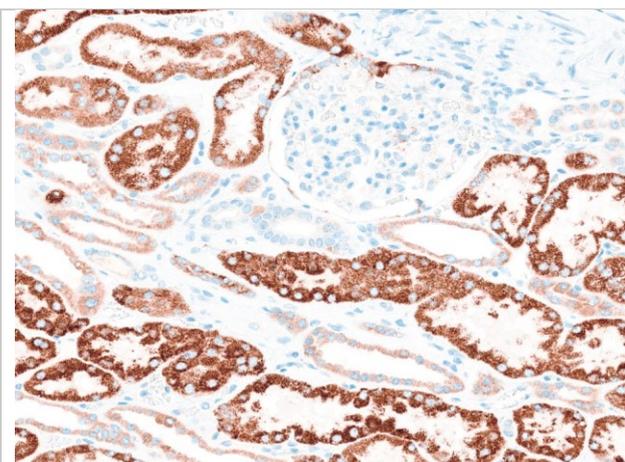


Fig. 1a (x200)
Optimal staining reaction for AMACR of kidney using the Ventana/Roche RTU system 790-6011, rmAb clone SP116, on the Ventana Benchmark, HIER for 48 min. in an alkaline buffer (CC1 pH 8,5) and a 3-step multimer detection system (Optiview, Ventana/Roche) - same protocol used in Figs. 2a-4a. The epithelial cells of the proximal tubules show a strong granular cytoplasmic staining, whereas the epithelial cells of distal tubules and parietal cells of Bowman's capsule display a weak granular cytoplasmic staining reaction.

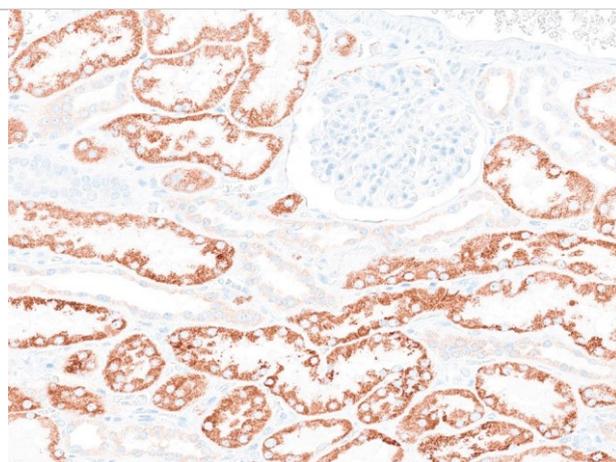


Fig. 1b (x200)
Insufficient staining reaction for AMACR of kidney using the Ventana/Roche RTU system 790-6011, rmAb clone SP116 as a RTU on the Ventana Benchmark with too short HIER in an alkaline buffer (CC1 pH 8,5) for 36 min. and a 2-step multimer detection system (UltraView, Ventana/Roche) - same protocol used in Figs. 2b-3b. The intensity of the staining reaction is significantly reduced, and especially the epithelial cells of distal tubules are almost negative - compare with Fig. 1a (same field).

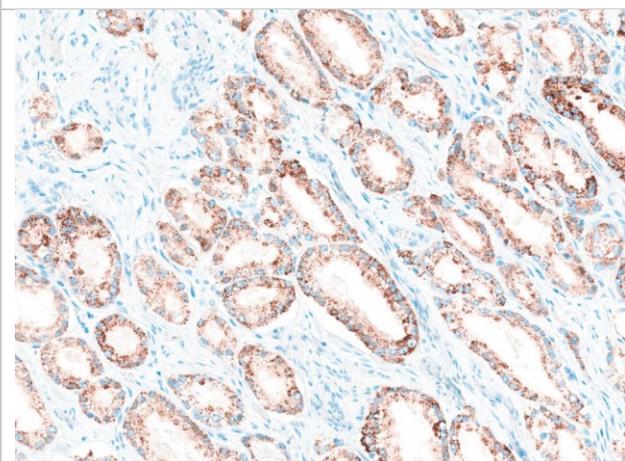


Fig. 2a (x200)
Optimal staining reaction for AMACR of the prostate adenocarcinoma (tissue core no. 3) using same protocol as in Figs. 1a - 4a. The vast majority of neoplastic cells displays a weak to moderate, distinct granular cytoplasmic staining reaction.

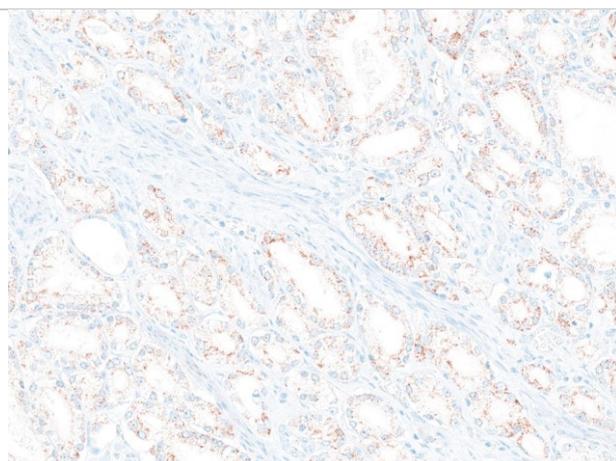


Fig. 2b (x200)
Insufficient staining reaction for AMACR of the prostate adenocarcinoma (tissue core no 3) using same protocol as in Figs. 1b - 3b. The neoplastic cells show a diffuse and faint cytoplasmic staining reaction - compare with Fig. 2a (same field).

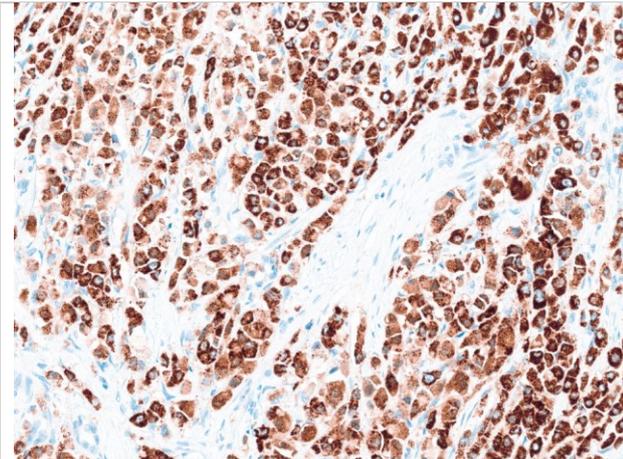


Fig. 3a (x200)
Optimal staining reaction for AMACR of the prostate adenocarcinoma (tissue core no. 4) using same protocol as in Figs. 1a - 4a. The vast majority of neoplastic cells displays a strong, distinct granular cytoplasmic staining reaction. The entire cytoplasmic compartment in the neoplastic cells is stained.

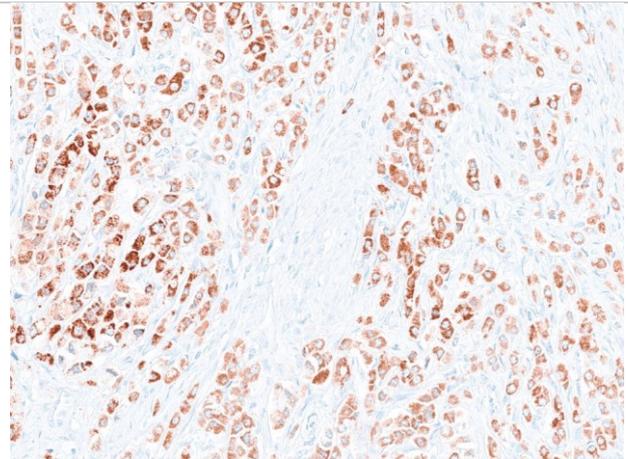


Fig. 3b (x200)
Staining reaction for AMACR of the prostate adenocarcinoma (tissue core no. 4) using same insufficient protocol as in Figs. 1b - 2b. Although the majority of the neoplastic glands are stained, the intensity is significantly reduced - compare with Fig. 3a (same field).

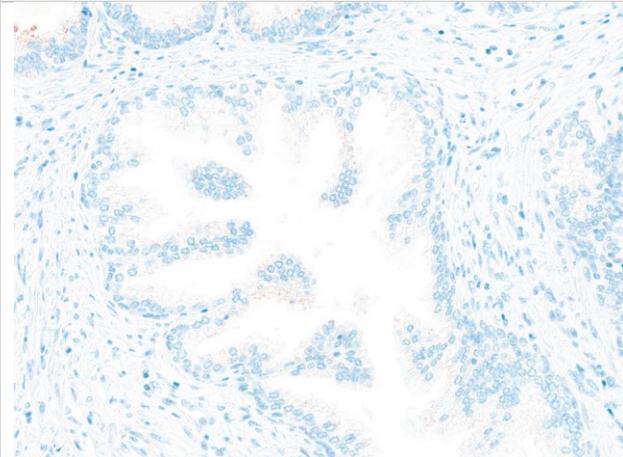


Fig. 4a (x200)
Optimal staining reaction for AMACR of the prostate hyperplasia using the same protocol as in Figs. 1a - 3a. The epithelial cells of the glands are negative or only focally, display a weak granular cytoplasmic staining reaction.

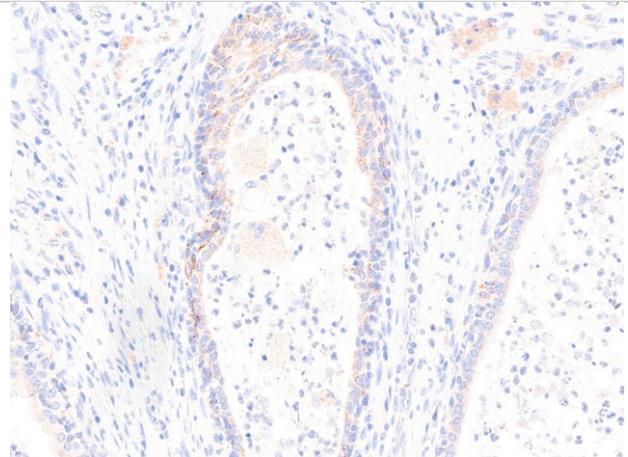


Fig. 4b (x200)
Sufficient staining reaction for AMACR of the prostate hyperplasia using the mAb clone EPMU1 (PA0210) on the Leica Bond platform with protocol F, HIER in an alkaline buffer (BERS2 pH 9,0) and a 3-step polymer detection system (Bond Refine, Leica Biosystem). An increased number of benign epithelial cells show a distinct granular cytoplasmic staining reaction and simultaneously a diffuse cytoplasmic and background staining is seen which overall compromise the scoring and final interpretation for diagnosis. - Compare to Fig. 4a.

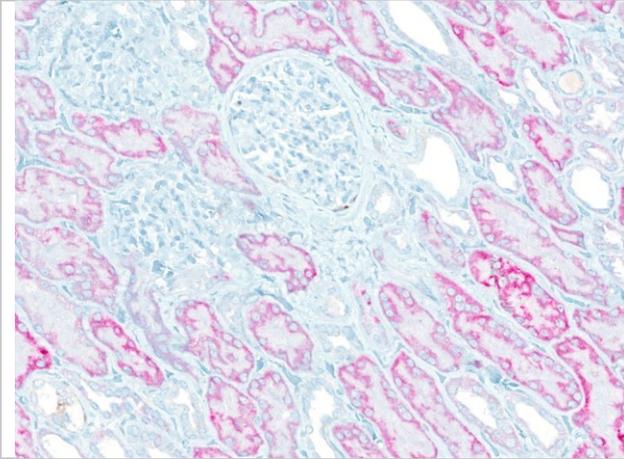


Fig. 5a (x200)
Sufficient staining reaction for AMACR of the kidney using the Ventana/Roche RTU system 790-6011 based on rmAb SP116 by vendor recommended settings with Alkaline Phosphatase detection system (Ventana/Roche) - same protocol used in Fig. 5b. The intensity of the staining reaction is reduced, and the epithelial cells of distal tubules are only faintly positive - compare with Fig. 1a-1b.

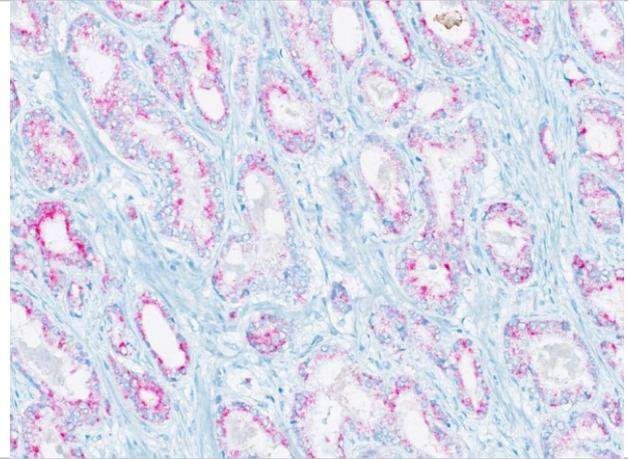


Fig. 5b (x200)
Sufficient (marked as good) staining reaction for AMACR of the prostate adenocarcinoma (tissue core no. 3) using same protocol settings as in Fig. 5a. The vast majority of neoplastic cells display a distinct granular cytoplasmic staining reaction but reduced compared to the level seen in Fig. 2a. In addition, the enhanced intensity of counterstaining complicates the scoring of AMACR in the weakly stained neoplastic cells.

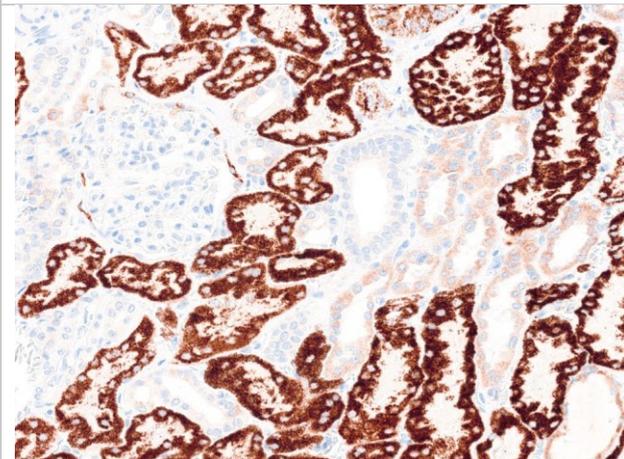


Fig. 6a (x200)
Optimal staining reaction for AMACR of the kidney by the Dako/Agilent RTU system GA060 based on rmAb clone 13H4 for the Dako Omnis by the vendor recommended protocol - same protocol used in Fig. 6b. The epithelial cells of the proximal tubules show a very strong granular cytoplasmic staining, whereas the epithelial cells of distal tubules display still show a weak granular cytoplasmic staining reaction. The contrast between the intensity of epithelial cells lining proximal tubules versus distal tubules was more pronounced for the Dako/Agilent RTU system for Omnis compared to other RTU systems providing optimal results - e.g. compare Fig. 1a and 1b using the Ventana/Roche RTU system.

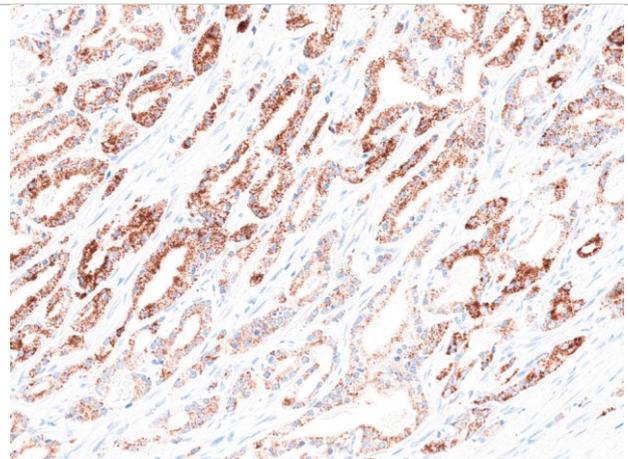


Fig. 6b (x200)
Optimal staining reaction for AMACR of the prostate adenocarcinoma (tissue core no. 3) using same protocol as in Fig. 6a. The vast majority of neoplastic cells displays a moderate to strong, distinct granular cytoplasmic staining reaction.

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