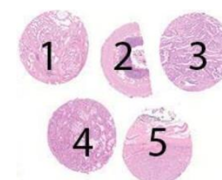
NordiQC

Assessment Run 69 2023

Prostate Specific Antigen (PSA)

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

Material
The slide to be stained for PSA comprised:



1. Prostate hyperplasia, 2. Appendix, 3. Colon adenocarcinoma, 4. Prostate adenocarcinoma, 5. Prostate adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a PSA staining as optimal included:

- As strong as possible, predominantly cytoplasmic staining reaction of all epithelia cells of the hyperplastic prostate glands. Background reaction in the surrounding stroma was accepted.
- An at least weak to moderate, predominantly cytoplasmic staining reaction of virtually all the neoplastic cells of the prostate adenocarcinoma, tissue core no 5.
- A moderate to strong, predominantly cytoplasmic staining reaction of virtually all the neoplastic cells in the prostate adenocarcinoma, tissue core no 4.
- No staining reaction in the appendix.
- No staining reaction of the neoplastic cells in the colon adenocarcinoma.

Participation

Number of laboratories registered for PSA, run 69	401
Number of laboratories returning slides	378 (94%)

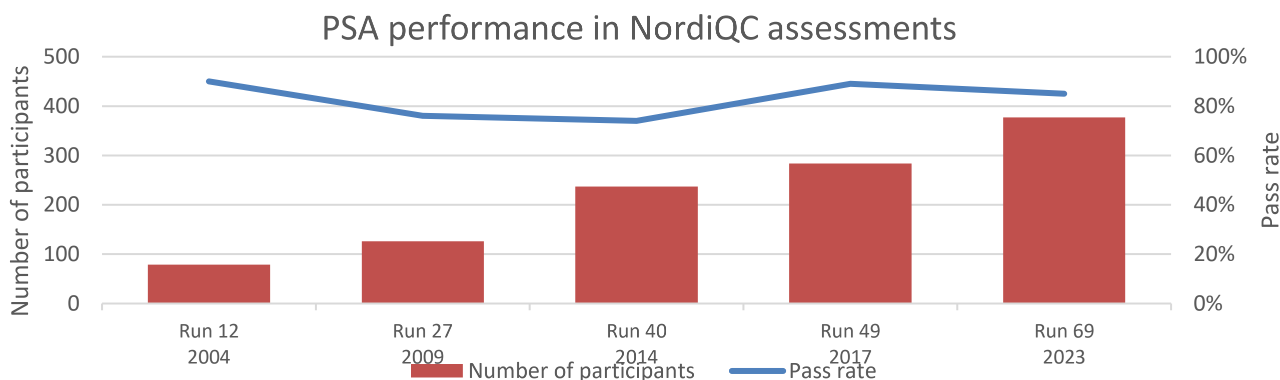
Results
At the date of assessment, 94% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

378 laboratories participated in this assessment and 85% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3).
One laboratory used an inappropriate antibody and was not included in the statistic.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Use of less sensitive detection systems
- Too short incubation time in primary Ab
- Insufficient Heat Induced Epitope Retrieval (HIER) (too short heating time).
- Unexplained technical issues

Performance history
This was the fifth NordiQC assessment of PSA. A slight decrease in pass rate was observed compared to previous run (see Graph 1), which both could be related to many new participants (see graph 1) and termination of the successful Dako/Agilent Ready-To-Use systems IR/GA514 providing a pass rate of 100% (n=53 participants) in the latest run 49, 2017 and subsequent need to implement a new IHC assay for PSA.

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



Conclusion
The mAb clones **35H9**, **ER-PR8,** rmAb clone **EP109** and **pAb 0562** could all be used to obtain an optimal staining for PSA. As concentrated format within a laboratory developed test, mAb clone **35H9** and rmAb **EP109** were most successful. mAb clone 35H9 was most widely used and provided optimal results on the three main full-automated IHC systems (Omnis, Benchmark and BOND). Whereas the ER-PR8 proved to be more challenging and needed a carefully calibration of both Ab titer and choice of detection system. Insufficient results in general were mainly characterized by too weak or completely false negative staining reaction of neoplastic cells one of the two prostate adenocarcinomas.
The Ready-To-Use (RTU) systems from Ventana/Roche (**760-2506** and **760-4271**) and Leica Biosystems (**PA0431**) both provided a high proportion of sufficient and optimal results.
Prostate hyperplasia is recommended as positive tissue control provided that the epithelial cells show an as strong as possible cytoplasmic staining reaction (a weak to moderate staining of the stroma must be accepted). Kidney/appendix is recommended as negative tissue control, as no staining reaction must be seen in the epithelial cells.

Table 1. **Antibodies and assessment marks for PSA, Run 69**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **ER-PR8**	73 7 2 1	Dako/Agilent Cell Marque Zytomed Systems BioGenex	37	25	18	3	75%	45%
mAb clone **35H9**	34 1	Leica Biosystems JIYIN	25	8	2	-	94%	71%
mAb clone **IHC654**	2	GenomeMe	2	-	-	-	-	-
mAb clone **28A4***	1	Leica Biosystems	-	1	-	-	-	-
rmAb clone **EP109**	3 2	Biocare Medical Cell Marque	2	3	-	-	100%	40%
rmAb clone **RBT-PSA**	2	BioSB	1	-	1	-	-	-
rmAb clone **EP1588Y**	1	Epredia	1	-	-	-	-	-
rmAb clone **ZR232**	1	Zeta Corporation	-	-	-	1	-	-
rmAb clone **QR038**	1	Quartett	1	-	-	-	-	-
pAb **0562**[5]	34	Dako/Agilent	12	17	4	1	85%	35%
pAb **P07288**	1	Other	-	1	-	-	-	-
Conc total	166		81	55	25	5	82%	49%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **ER-PR8 760-4271**[3]	-	Ventana/Roche	-	-	-	-	-	-
mAb clone **ER-PR8 760-4271**[4]	6	Ventana/Roche	2	2	2	-	67%	33%
pAb **760-2506**[3]	19	Ventana/Roche	10	9	-	-	100%	53%
pAb **760-2506**[4]	116	Ventana/Roche	69	39	8	-	93%	60%
pAb **IS/IR514**[3,5]	4	Dako/Agilent	1	-	2	1	-	-
pAb **GA514**[4,5]	3	Dako/Agilent	-	2	1	-	-	-
mAb clone **35H9 PA0431**[3]	16	Leica Biosystems	5	10	1	-	94%	31%
mAb clone **35H9 PA0431**[4]	19	Leica Biosystems	10	7	2	-	90%	53%
rmAb clone **EP109**[3] **8244-C010**	1	Sakura	1	-	-	-	-	-
mAb clone **ER-PR8 324M-17/18**	7	Cell Marque	2	3	1	1	71%	29%
rmAb clone **EP109 324R-18-RUO**	1	Cell Marque	-	1	-	-	-	-
mAb clone **ER-PR8 AM014-10M**	3	BioGenex	2	-	1	-	-	-
mAb clone **ER-PR8 MAD-000532QD**	3	Master Diagnostica	-	-	3	-	-	-
mAb clone **35H9 PDM548R**	2	Diagnostic BioSystems	-	-	2	-	-	-
mAb clone **ER-PR8 MSG051**	1	Zytomed Systems	-	1	-	-	-	-
rmAb clone **EP109 APR390AA**	1	Biocare Medical	-	-	1	-	-	-
rmAb clone **BY038 BFM-0260**	1	Bioin Biotechnology	-	1	-	-	-	-
rmAb clone **EP109 MRH1155**	1	Path N situ	1	-	-	-	-	-
mAb clone **C2C12 CPM-0404**	1	Celnovte	1	-	-	-	-	-
rmAb clone **BP6043 I10222E**	1	Biolynx Biotechnology	1	-	-	-	-	-

rmAb clone **QR038** **P-P014-70**	1	Quartett	1	-	-	-	-	-
mAb clone **IHC654** **IHC654-7**	1	GenomeMe	1	-	-	-	-	-
mAb clone **MX030** **MAB-0719**	1	Fuzhou Maixin	1	-	-	-	-	-
Ab clone **DA103** **RMA1A027**	1	Dartmon Biotechnology	-	1	-	-	-	-
Ab clone **DGR055**	1	Other	1	-	-	-	-	-
RTU total	211		109	76	24	2	88%	52%
Total	377		190	131	49	7	-	
Proportion			50%	35%	13%	2%	85%	

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).
5) Terminated by vendor.

Detailed analysis of PSA, Run 69
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb **ER-PR8**: The protocols giving an optimal result were all based on HIER using an alkaline buffer as Cell Conditioning 1 (CC1, Ventana/Roche) (7/16)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (9/21), TRS High pH (Dako/Agilent) (20/35) or Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (1/4) as retrieval buffer. The mAb was typically diluted in the range of 1:10 – 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 53 out of 70 (76 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb **35H9**: The protocols giving an optimal result were based on HIER using either an alkaline buffer as CC1 (Ventana/Roche) (13/16), TRS High pH (Dako/Agilent) (3/4), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (6/11), BERS1 (Leica Biosystems) (1/1), TRIS-EDTA pH 9 (1/1) or Citrate buffer pH 6 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50 – 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 28 out of 29 (97%) laboratories produced a sufficient staining.

rmAb **EP109**: Two protocols produced an optimal result. They were based on HIER using either an alkaline buffer as TRS High pH (Dako/Agilent) (1/1) or BERS1 (Leica Biosystems) (1/1) as retrieval buffer. The mAb was diluted in the range of 1:50 – 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 out of 2 laboratories produced a sufficient staining.

pAb **0562**: The protocols giving an optimal result were all based on HIER using an alkaline buffer as CC1 (Ventana/Roche) (8/16) or TRS High pH (Dako/Agilent) (4/6) as retrieval buffer. The pAb was typically diluted in the range of 1:800 – 1:12.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 18 out of 21 (86 %) laboratories produced a sufficient staining.

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

Concentrated antibody	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark GX/XT/Ultra**		**Leica Biosystems Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **ER-PR8**	8/20** (40%)	0/1	20/35 (57%)	0/1	7/18 (39%)	-	1/3	0/1
mAb clone **35H9**	-	-	3/4	-	13/16 (81%)	-	6/11 (55%)	1/1
rmAb clone **EP109**	-	-	1/1	-	0/2	-	0/1	1/1
pAb **0562**	0/1	0/1	4/6 (67%)	-	8/16 (50%)	-	0/2	0/2

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer).

Ready-To-Use antibodies and corresponding systems
pAb, product no. **760-2506,** Ventana/Roche, Benchmark XT/GX/Ultra:
Protocols with optimal results were based on HIER using CC1 (efficient heating time 8-36 min. at 95-100°C), 2 laboratories used protease 3 for 4-8 min. and 11 laboratories did not use any epitope retrieval. The primary Ab was incubated for 8-32 min. UltraView (760-500) with or without amplification (760-080) or OptiView (760-700) with or without amplification (760-099) were used as detection systems. Using these protocol settings, 111 of 117 (95%) laboratories produced a sufficient staining result.

mAb clone **ER-PR8,** product no. **760-4271,** Ventana/Roche, Benchmark Ultra:
Protocols with optimal results were based on HIER using CC1 (efficient heating time 32-92 min. at 95-100°C), 16-60 min. incubation of the primary Ab and OptiView (760-700) being used as detection system. Using these protocol settings, 2 of 2 laboratories produced a sufficient staining result.

mAb clone **35H9,** product no. **PA0431,** Leica Biosystems:
Protocols with optimal result were based on HIER using BERS1 pH 6 or BERS2 pH 9 (efficient heating time 10-30 min. at 99-100°C), 15-25 min. incubation of the primary Ab and BOND Refine (DS9800) as the detection system. Using these protocol settings, 27 of 28 (96%) laboratories produced a sufficient result.

mAb clone **C2C12**, product no. **CPM-0404**, Celnovte, CNT360:
One protocol with an optimal result was based on HIER using a TRIS-EDTA pH 9 based buffer (efficient heating time 20 min. at 100°C) and 20 min. incubation of the primary Ab. MicroStacker TM PLUS was used as detection systems.

rmAb clone **EP109,** product no. **8244-C010,** Sakura Finetek, Tissue-Tek Genie:
One protocol with an optimal result was based on HIER using Tissue-Tek Genie High pH Antigen Retrieval as buffer (efficient heating time 30 min. at 98°C) and 30 min. incubation of the primary Ab. Tissue-Tek Genie Pro Detection Kit, DAB was used as detection system.

rmAb clone **BP6043**, product no. **I10222E** Biolynx Biotechnology LYNX480
One protocol with an optimal result was based on HIER using Antigen Retrieval 2 (EDTA) based buffer (efficient heating time 20 min. at 100°C) and 30 min. incubation of the primary Ab. BXV Visualization System (I20032C) was used as detection systems.

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 PSA for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT/GX pAb **760-2506**	100% (19/19)	53% (10/19)	93% (108/116)	60% (69/116)
VMS Ultra/XT/GX mAb **ER-PR8 760-4271**	-	-	67% (4/6)	33% (2/6)
BOND MAX/III mAb **35H9 PA0431**	94% (15/16)	31% (5/16)	88% (15/17)	47% (8/17)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments

In this assessment for PSA, the prevalent feature of an insufficient staining was characterized by a too weak or completely false negative staining reaction. This pattern was seen in 95% (53 of 56) of the insufficient results. The remaining 5% of insufficient results were characterized by a false positive and/or excessive background reaction.
Virtually all laboratories were able to demonstrate PSA in high-level PSA expressing cells (normal epithelial cells of hyperplastic prostate glands and neoplastic cells of the prostate adenocarcinoma, tissue core no. 4), whereas low-level PSA expressing cells in the prostate adenocarcinoma, tissue core no. 5 could only be demonstrated using an optimal and carefully calibrated protocol.

Concentrated antibodies were used by 44% (166 of 377) of the laboratories with a general pass-rate of 82%, 49% being optimal. Used as concentrates within a Laboratory Developed (LD) assay, the mAb clone 35H9 (Leica Biosystems) and the rmAb clone EP109 (Biocare Medical or Cell Marque) provided the highest proportion of sufficient and optimal results (see Table 1). However, only a limited number of participants (n=5) used the rmAb clone EP109. Optimal results were achieved on both the Omnis and Bond platform in a dilution range of 1:50 – 1:100.
The mAb clone 35H9 were able to produce optimal results on all 3 of the fully-automated IHC systems from Dako/Agilent, Leica Biosystems and Ventana/Roche. Optimal results were achieved with both 2- and 3-layer detection systems and using both high and low pH buffers for HIER. The titer of the primary Ab should be carefully calibrated (1:100 – 1:400) accordingly to the overall sensitivity of the protocol.
The mAb clone ER-PR8 was the most widely used antibody as a concentrate (n=83) within LD assays and provided optimal results on all four main IHC systems (see Table 2). The titer required to produce optimal results for ER-PR8 was relatively low (1:10 – 1:100) compared to the mAb clone 35H9 (1:100 - 1:400), and the choice of detection system also had an impact, as displayed in Table 4. For both Dako/Agilent IHC platforms, the 3-step detection system EnVision Flex+ provided a pass-rate of 100% compared to 56% and 67% using the 2-step system EnVision FLEX on Autostainer and Omnis, respectively. However, for the mAb clone ER-PR8 being performed on Ventana/Roche IHC platforms, the pass rate was higher for 2-step systems compared to 3-step systems, but for 2-step systems no optimal results were generated. This indicate that for this clone a broader focus must be taken to produce optimal results with special attention to both the titer (1:10-50, if product from Cell Marque) and 1:25-100, if product from Dako/Agilent) in combination with a 3-step detection system.

Table 4. Overview of the assessment marks for mAb clone ER-PR8 on the four main IHC instruments in run 69.

PSA score	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark GX / XT / Ultra		Leica Biosystems Bond III / Max
Detection system	**2-layer**	**3-layer**	**2-layer**	**3-layer**	**2-layer**	**3-layer**	**3-layer**
Optimal	5	3	10	10	-	7	1
Good	4	2	6	3	4	3	2
Borderline	5	-	8	-	-	3	2
Poor	2	-	-	-	-	1	-
Total	16	5	24	13	4	14	5
Sufficient %	56%	100%	67%	100%	100%	71%	60%

The pAb 0562 Dako/Agilent was used by 34 laboratories with a pass-rate of 85%, 35% being optimal. In run 49, 62 laboratories used the pAb but it has now been terminated from vendor and is not available for purchase.
Optimal results were only observed on the Dako Omnis and Ventana BenchMark platforms (Ventana/Roche). Optimal results were achieved on the Dako Omnis by using HIER in TRS High for 30 min., primary Ab incubation for 20-30 min. and a titer of 1:10.000 – 1:15.000 and EnVision Flex as detection system. On the Ventana BenchMark platforms optimal results were also provided by using alkaline epitope retrieval, primary Ab for 12-32 min. and a titer between 1:800 – 1:10.000 dependent on using UltraView or OptiView as detection system.

Ready-To-Use (RTU) antibodies were used by 56% (211 of 377) of the laboratories.
The most used RTU-system was based on the pAb 760-2506 from Ventana/Roche used by a total of 135 laboratories. The pAb provided a pass-rate of 94% (127 of 135) in total, 59% being optimal. In the latest run 40 a pass-rate of 88% (45 of 51) was seen and 67% (34 of 51) were optimal. The vendor recommendations have been updated since the latest run and using the vendor recommended protocol settings with CC1 mild and a primary Ab incubation time for 8-12 min. gave a 100% pass rate (19 of 19). The most commonly used laboratory modified settings was based on a prolonged HIER time and/or primary Ab incubation. Prolonging HIER and Ab incubation could result in a higher background staining of the surrounding tissue to structures/cells with high antigen expression, but as this was accepted as long as no background was produced in PSA non-expression tissues as appendix this did not induce an interpretational problem and a pass-rate of 96% (23 of 24) (see figs. 5a and b) was seen for this modification. Less successful modifications were based on omission of HIER or using enzymatic pre-treatment instead of HIER. This was used by 27 laboratories with a pass-rate of 85%.
Optimal results could be obtained by using both OptiView and UltraView, however OptiView had a higher pass-rate 94% (58 of 62), 71% (44 of 62) being optimal, compared to UltraView with a pass-rate of 87% (69 of 73), 48% (35 of 73) being optimal.

The Ventana/Roche RTU-system based on the mAb clone ER-PR8, 760-4271 was used by 6 laboratories with a pass-rate of 67%, 33% being optimal. None of the laboratories followed the vendor recommendations using mild CC1 and 16 min. primary Ab incubation and use of either UltraView or OptiView. Instead, optimal results were achieved by using only OptiView and prolonging HIER. This supporting the fact that the ER-PR8 clone both as a RTU and concentrated format require a very sensitive protocol setup.

The Leica Biosystems RTU-system based on mAb clone 35H9, PA0431 was in total used by 35 laboratories, with a total pass-rate of 91% (32 of 35), 43% being optimal. 3 laboratories transferred the RTU product to either Dako Omnis or Ventana platforms with optimal results. Following vendor recommendations using HIER in BERS1 pH 6 (10 min.) and protocol F (primary Ab 15 min.) gave a pass-rate of 94% (15 of 16), 31% being optimal. Even though the vendor protocol recommendation suggest HIER by low pH optimal results could also be achieved by using HIER in an alkaline buffer such as BERS2 providing a 100% pass-rate (10 of 10).

The RTU systems for both Dako autostainer (IR/IS514) and Dako Omnis (GA514) has been discontinued and only a few laboratories were still using these products. In the latest run both products displayed a pass-rate of 100% with a total of 61 laboratories using the products. In this run only 7 in total were using the pAb but with a total pass-rate of 43% (3 of 7). The reason for the declined pass-rate is unknown but might be related to use after expiry date of the primary RTU vials.

This was the fifth assessment of PSA in NordiQC and a pass-rate of 85% was achieved, which is a slight decrease since the last run 49 in 2017 (89%). The termination of the previously widely used and successful pAb both as concentrated format and RTU-systems from Dako/Agilent and the need to optimize, validate/verify and implement an alternative IHC test might have impacted the pass rate negatively. In addition, many new laboratories participated in this assessment for the first time. In general, a similar pass-rate was observed for both LD-assays based on concentrated formats and RTU systems.

The choice and use of robust (applicable on most IHC platforms) and high quality (specific and sensitive) primary antibodies e.g. mAb clone 35H9 and rmAb clone EP109 seems to be a key parameter to provide a high proportion of sufficient and optimal results (see Table 1). From a technical point of view, the mAb ER-PR8 both as concentrate and RTU format, seems more challenging and may require that all key parameters are properly calibrated to obtain an optimal protocol. For all primary Abs, efficient HIER (although optimal results also could be seen in protocols omitting this step), carefully calibration of the primary Ab and the use of a sensitive detection system is a prerequisite for optimal performance.

Controls
Prostate hyperplasia and appendix was in this assessment found to be recommendable positive and negative tissue controls for PSA, respectively. The epithelial cells of the prostate glands must show an as strong as possible cytoplasmic staining reaction. Due to leakage of the antigen in vicinity to the prostate glands, the stromal cells display a weak to moderate staining reaction. This staining pattern has to be accepted, otherwise giving to low sensitivity of the assay causing a general weak staining reaction of the carcinomas included in this run. Kidney (as observed in run 49) and appendix can be used as negative tissue controls. No staining reaction should be seen in these tissues. If a positive staining reaction in the epithelial cells and/or a diffuse background staining is seen, the protocol must be recalibrated.

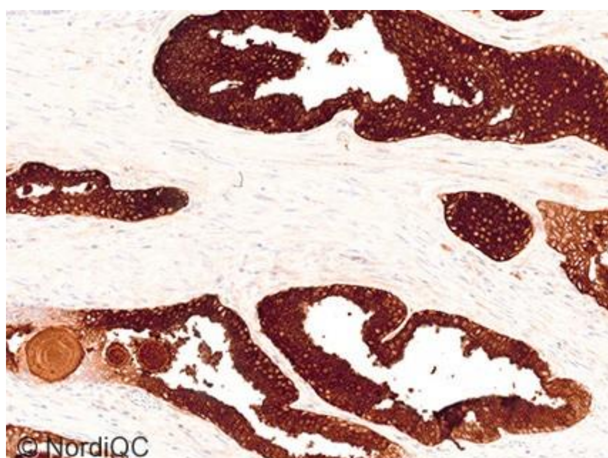


Fig. 1a (x100)
Optimal staining reaction for PSA of the prostate hyperplasia using the mAb ER-PR8 1:50 (conc. format, Dako/Agilent), HIER in an alkaline buffer (TRS High) and a 3-step polymer based detection system (EnVision Flex+) - same protocol settings used in Figs. 2a - 4a.
The epithelial cells of prostate glands show a strong distinct cytoplasmic staining reaction. A weak to moderate stromal reaction is seen (due to leakage of the antigen), which has to be accepted for optimal performance.

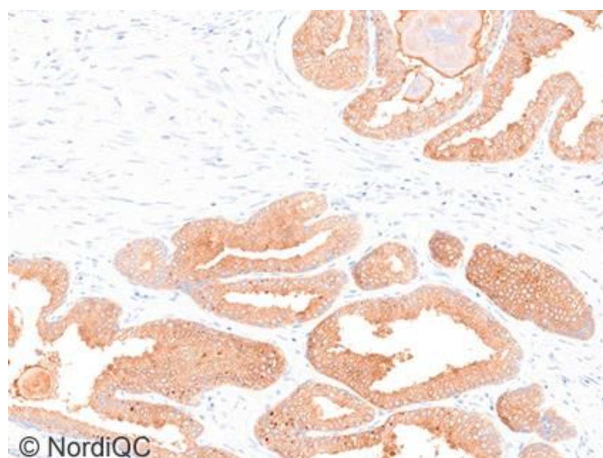


Fig. 1b (x100)
Staining reaction for PSA of the prostate hyperplasia using the mAb ER-PR8 1:100 (conc. format, Dako/Agilent), HIER in an alkaline buffer (TRS High) and a 2-step polymer based detection system (EnVision Flex) - same protocol settings used in Figs. 2b – 4b.
The intensity of the staining reaction is significantly reduced, and stromal reactivity is absent - compare with Fig. 1a (same field).
However, also compare with Figs. 2b and 3b showing an insufficient result.

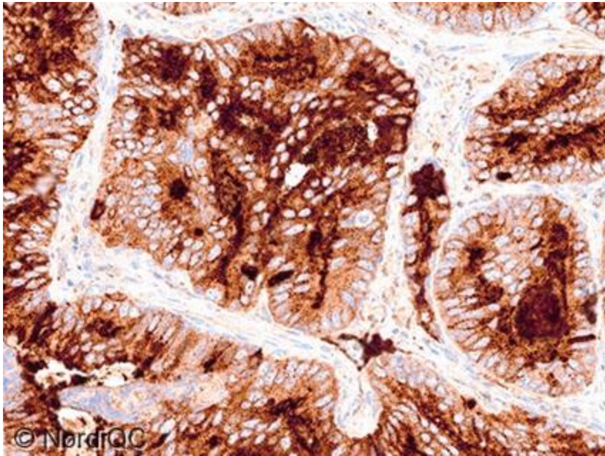


Fig. 2a (x200)
Optimal staining reaction for PSA in the prostate adenocarcinoma, tissue core no 4, using same protocol as in Fig. 1a. All neoplastic cells are strongly stained. Notice all cells are well defined and show a distinct cytoplasmic staining reaction compared to the pattern shown in Fig. 2b.

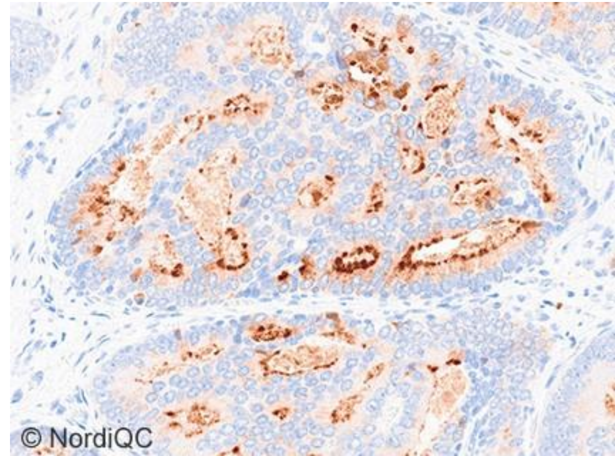


Fig. 2b (x200)
Insufficient staining reaction for PSA in the prostate adenocarcinoma, tissue core no 4, same protocol as in Fig. 1b.
The intensity of the staining reaction in the neoplastic cells is reduced and mainly resides as an apical extracellular staining reaction instead of the expected cytoplasmic pattern.

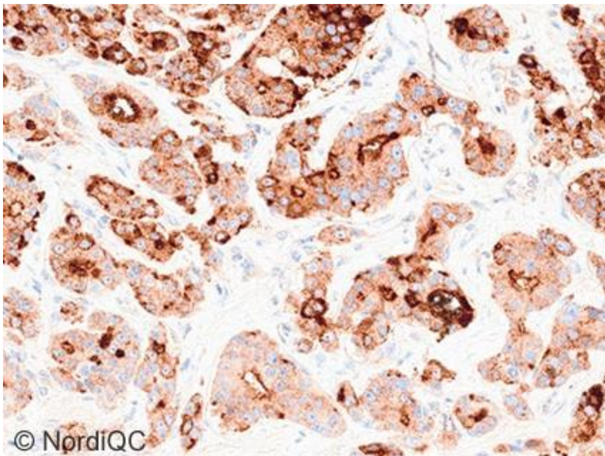


Fig. 3a (x200)
Optimal staining reaction for PSA in the prostate adenocarcinoma, tissue core no 5, using same protocol as in Figs. 1a and 2a.
The majority of the neoplastic shows a weak to moderate but distinct cytoplasmic staining reaction.

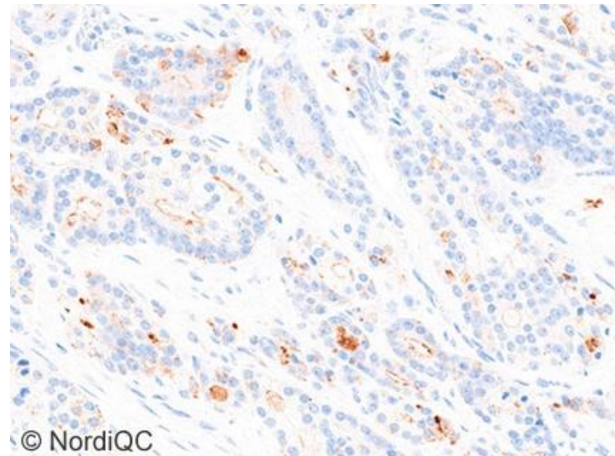


Fig. 3b (x200)
Insufficient staining reaction for PSA in the prostate adenocarcinoma, tissue core no 5, using same protocol as in Figs. 1b and 2b.
The intensity of the neoplastic cells is significantly reduced and some glandular structures completely negative - compare with Fig. 3a.

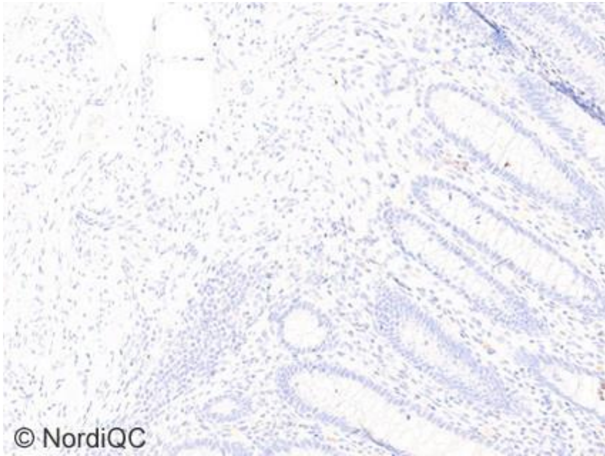


Fig. 4a (x100)
Optimal staining for PSA of the appendix using same protocol as in Figs. 1a - 3a. As expected, no staining reaction is seen in the epithelium, smooth muscle and stromal cells.

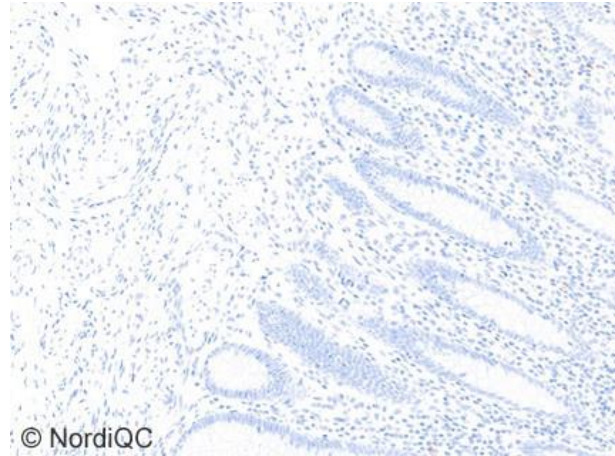


Fig. 4a (x100)
Staining reaction for PSA of the appendix using same protocol as in Figs. 1b – 3b. The epithelium, smooth muscle and stromal cells are negative as expected, but the overall result of the protocol used is insufficient as seen in Figs. 1b - 3b.

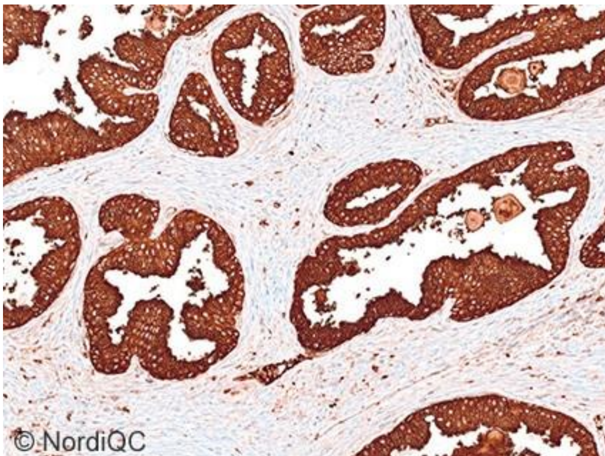


Fig. 5a (x100)
Staining reaction for PSA of the prostate hyperplasia using the pAb 760-2506 (RTU format, Ventana/Roche), HIER in an alkaline buffer (CC1) and a multimer based detection system (OptiView, Ventana) – same protocol used in Fig 5b.
The staining of the prostate glands displays a strong distinct cytoplasmic staining reaction and mimics the optimal staining seen in Fig. 1a. However, comparing with Fig. 5b, the protocol gave an overall result evaluated as insufficient caused by aberrant false positive reaction in appendix.

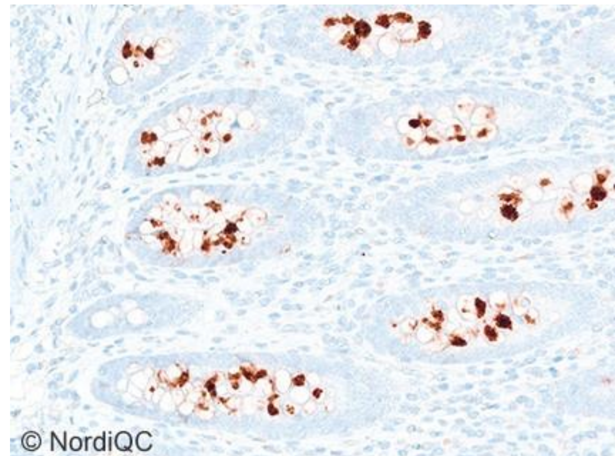


Fig. 5b (x200)
Insufficient staining reaction for PSA of the appendix using same protocol as in Fig. 5a. The epithelial cells and scattered stromal cells are false positive - compare with Fig. 4a.
As prostate epithelial cells display a high PSA antigen expression level and diffusion typically will be seen in surrounding structures, it is of vital importance to verify the specificity and negative result of the PSA IHC assay in tissue not expressing PSA.

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