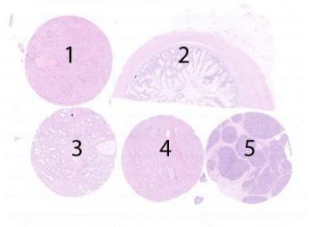
Assessment Run 40 2014

Prostate Specific Antigen (PSA)

Material
The slide to be stained for PSA comprised:



1. Kidney, 2. Appendix, 3. Prostate hyperplasia, 4-5. Prostate adenocarcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing PSA staining as optimal were:

- A moderate to strong predominantly cytoplasmic staining reaction of virtually all epithelial cells of the hyperplastic prostate glands.
- An at least moderate and distinct predominantly cytoplasmic staining reaction of virtually all neoplastic cells of the prostate adenocarcinoma no. 4.
- An at least weak predominantly cytoplasmic staining reaction of the majority of neoplastic cells of the prostate adenocarcinoma no 5.
- No staining reaction of epithelial cells in the kidney and appendix.

Results
237 laboratories participated in this assessment. 175 (74%) achieved a sufficient mark (optimal or good). Antibodies (Abs) used and assessment marks are summarized in table 1 (see page 2).

The most frequent causes of insufficient staining were:

- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- Omission of HIER

Performance history
This was the 3rd NordiQC assessment of PSA. A similar pass rate in the two latest runs has been seen, as shown in table 2.

Table 2. **Proportion of sufficient results for PSA in three NordiQC runs performed**

	Run 12 2004	Run 27 2009	Run 40 2014
Participants, n=	79	126	237
Sufficient results	90%	76%	74%

Conclusion
In this assessment, the concentrated formats of the mAb clone **35H9** and the **pAb A0452** gave a high proportion of sufficient and optimal results. The **pAb A0452** could produce optimal results on all the three main IHC systems (Dako, Ventana and Leica).
The Ready-To-Use systems for PSA from Dako (**IS/IR514**), Leica (**PA0431**) and Ventana (**760-2506**) all gave a high proportion of sufficient results.
Irrespective of the primary antibody applied, protocols based on HIER provided a superior performance compared to omission of HIER. 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.

Table1. **Antibodies and assessment marks for PSA, run 40**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **35H9**	18 1	Leica/Novocastra Monosan	13	4	2	0	89%	100%
mAb clone **ER-PR8**	36 3 2 1 1 1	Dako Cell Marque Immunologic Gentech Zeta Corp Zytomed	7	12	24	1	43%	53%
mAb clone **PSA 28/A4**	3 1	Leica/Novocastra Monosan	0	1	2	1	-	-
mAb clone cocktail **ER-PR8+PA05**	2	Thermo/Neomarkers	1	1	0	0	-	-
mAb clone cocktail **ER-PR8+A67-B/E3**	1	Biocare	0	1	0	0	-	-
rmAb clone **EP1588Y**	1	Biocare	1	0	0	0	-	-
pAb **61-0058**	1	Genemed	0	1	0	0		
pAb **A0562**	61	Dako	29	21	10	1	82%	94%
pAb **PU014**	1	Biogenex	0	0	1	0	-	-
pAb **RP 033**	1	DBS	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **35H9 PA0431**	8	Leica/Novocastra	1	6	1	0	88%	100%
mAb clone **35H9 PDM 087-1**	1	DBS	0	0	1	0	-	-
mAb clone **ER-PR8 AM014**	1	Biogenex	1	0	0	0	-	-
mAb clone **ER-PR8 324M-18**	1	Cell Marque	0	1	0	0	-	-
mAb clone **ER-PR8 MAD-000532QD**	1	Master Diagnostica	0	0	0	1	-	-
mAb clones **ER-PR8 760-4271**	17	Ventana/Cell Marque	3	5	8	1	47%	60%
mAb clone **PSA 28/A4 PSA-28A4-R**	1	Leica/Novocastra	0	0	0	1	-	-
rmAb clone **EP109 ZA-0597**	1	Zhonggshan	1	0	0	0	-	-
rmAb clone **EP1588Y PME390**	1	Biocare	0	1	0	0	-	-
pAb **760-2506**	27	Ventana	17	6	4	0	85%	100%
pAb **GA514**	1	Dako	1	0	0	0	-	-
pAb **N1517**	1	Dako	0	0	1	0	-	-
pAb **IS514/IR514**	41	Dako	34	5	2	0	95%	100%
Total	237		110	65	56	6	-	
Proportion			47%	27%	24%	2%	74%	

1) Proportion of sufficient stains (optimal or good),
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of Prostate Specific Antigen (PSA), Run 40

The following protocol parameters were central to obtain an optimal staining:

Concentrated antibodies
mAb clone **35H9**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (1/1)*, TRS pH 6.1 (Dako) (1/1), Cell Conditioning 1 (CC1; Ventana) (5/5), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (2/5), BERS 1 (Leica) (2/2), Tris-EDTA/EGTA pH 9 (1/2) or Citrate pH 6 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:100-1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings 15 of 15 (100%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **ER-PR8**: Protocols with optimal results were all based on HIER using TRS pH 9, 3-in-1 (Dako) (2/10), CC1 (Ventana) (3/17) or Tris-EDTA/EGTA pH 9 (2/7) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 10 of 19 (53%) laboratories produced a sufficient staining result.

mAb clone cocktail **ER-PR8+PA05**: One protocol with an optimal result, which was based on HIER using CC1 (Ventana) (1/2) as retrieval buffer. The mAb cocktail was diluted 1:100.

rmAb clone **EP1588Y**: One protocol with an optimal result, which was based on HIER using Diva Decloaker pH 6.2 (Biocare) as retrieval buffer. The rmAb was diluted 1:50.

pAb **A0562**: Protocols with optimal results were mostly based on HIER using TRS pH 9, 3-in-1 (Dako) (6/10), TRS pH 9 (Dako) (2/3), TRS pH 6.1 (Dako) (1/1), CC1 (Ventana) (10/13), CC2 (Ventana) (2/3), BERS 2 (Leica) (2/2) or Tris-EDTA/EGTA pH 9 (5/8). The mAb was typically diluted in the range of 1:1,000-1:15,000 depending on the total sensitivity of the protocol employed. Using these protocol settings 32 of 34 (94%) laboratories produced a sufficient staining result.
One laboratory used proteolytic pre-treatment using Protease 1 (Ventana) and the mAb was diluted 1:600.

pAb **RP 033**: One protocol with an optimal result was based on HIER using CC1 (Ventana) as retrieval buffer. The mAb was diluted 1:1,000.

Table 3. **Proportion of optimal results for PSA of the two most frequently used concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	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**	2/8** (25%)	-	3/10 (30%)	-	-	0/1
pAb **A0562**	6/8 (75%)	1/1	9/10 (90%)	2/2	2/2	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)

Ready-To-Use antibodies and corresponding systems
mAb clone **35H9**, product no. PA0431, Leica, Bond-max:
One protocol with an optimal result was based on HIER using BERS 1 pH 6 (efficient heating time 20 min. at 99°C), 20 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 1 of 1 (100%) laboratory produced an optimal staining.

mAb clone **ER-PR8**, product no. 760-4271, Ventana, BenchMark Ultra:
Protocols with optimal results were all based on HIER using mild Cell Conditioning 1, 16-32 min. incubation of the primary Ab and OptiView (760-700) as detection system. Using these protocol settings 3 of 5 (60%) laboratories produced a sufficient staining (optimal or good).

pAb, product no. **760-2506**, Ventana, BenchMark XT/Ultra:
Protocols with optimal results were mostly based on HIER using short, mild or standard Cell Conditioning 1, 8-32 min. incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection systems. Using these protocol settings 13 of 13 (100%) laboratories produced an optimal staining. 4 of 13 laboratories obtained an optimal staining without pre-treatment.

pAb, product no. **GA514**, Dako, Omnis:
One protocol with an optimal result was based on HIER in EnVision™ FLEX, High pH (efficient heating time 30 min. at 97°C), 12.5 min. incubation of the primary Ab and EnVision FLEX (GV800) as detection system.

pAb, product no. **IS/IR514**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 10-20 min. at 95-98°C), 20 min incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 36 of 36 (100%) laboratories produced a sufficient staining.

In this assessment and in concordance with the previous NordiQC runs for PSA, the prevalent feature of an insufficient staining was a too weak or completely false negative staining reaction of the cells expected to be demonstrated. This pattern was seen in 90% of the insufficient results (56 of 62 laboratories). The remaining 10% of insufficient results was characterized by a general poor signal-to-noise ratio and/or false positive staining reaction in e.g. the epithelial cells of the kidney and appendix. 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 no. 4), whereas low-level PSA expressing cells in the prostate adenocarcinoma no. 5 could only be demonstrated using an optimal and carefully calibrated protocol.

Regarding the performance of concentrated Abs, the mAb clone 35H9 (Leica/Novocastra) and the pAb A0562 (Dako) gave the highest proportion of optimal results (table 2) and seemed to be slightly superior compared to the mAb clone ER-PR8 (various vendors).
The pAb A0562 was the most widely used primary Ab. Optimal result could be obtained on all the 3 most widely used IHC systems (Dako, Leica and Ventana), see table 3. The combination of HIER, usage of a non-biotin based detection system and a titre in the range of 1:1,000-15,0000 of the primary Ab A0452 (Dako) were the main protocol prerequisites for an optimal result.
Irrespective of the primary Ab applied, omission of HIER gave an inferior performance compared to protocols based on HIER. This was most apparent for the pAb A0562. If HIER was applied, a pass rate of 95% (38 of 40) was seen of which 68% were assessed as optimal. Omission of HIER gave a pass rate of 60% (12 of 20) and 10% were assessed as optimal.

Corresponding Ready-To-Use (RTU) systems (mainly produced by Dako, Leica and Ventana) provided comparable proportions of sufficient and optimal results. The Dako Autostainer RTU system IS/IR514 gave a pass rate of 100% (39 of 39) and 87% were evaluated as optimal, providing the RTU system was applied according to the protocol settings recommended by the producer. The Ventana RTU system based on the pAb 760-2506 gave a superior performance and pass rate compared to the Ventana RTU system 760-4271 based on the mAb clone ER-PR8 (see table 1). It was observed, that the producer recommended protocol for the Ventana RTU format prod. no. 760-2506 (No epitope retrieval, 16 min incubation of the primary Ab and UltraView as detection system) was less successful compared to modified and laboratory validated protocol settings using HIER in CC1 (mild or standard) and 8-32 min incubation of the primary Ab. Using the laboratory validated protocol settings, a pass rate of 100% was seen (13 of 13) all of which were assessed as optimal. Using the recommended protocol omitting HIER, a pass rate of 69% was seen (9 of 13) and 31% were optimal. The Leica RTU system PA0431, based on the mAb clone 35H9, gave a pass rate of 83% using the RTU system as recommended by the producer.

Controls
Prostate hyperplasia and kidney/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. A weak to moderate stromal staining reaction in the vicinity of the positive epithelial cells in the prostate is expected (due to leakage of the antigen from the glands) and has to be accepted. In this assessment it was observed that reduction of the sensitivity in order to eliminate this background reaction frequently gave a too weak staining reaction of the carcinomas.
Kidney 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.

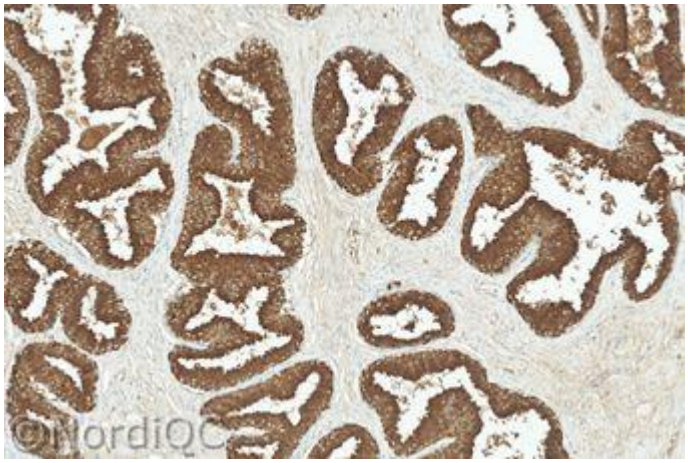


Fig. 1a
Optimal PSA staining of the prostate hyperplasia using the mAb 35H9 carefully calibrated and with HIER in an alkaline buffer (x100). All the epithelial cells of the prostatic glands show a strong cytoplasmic staining reaction. A weak stromal staining reaction is seen, which has to be expected and accepted in prostate tissue. Also compare with Figs. 2a - 5a, same protocol.

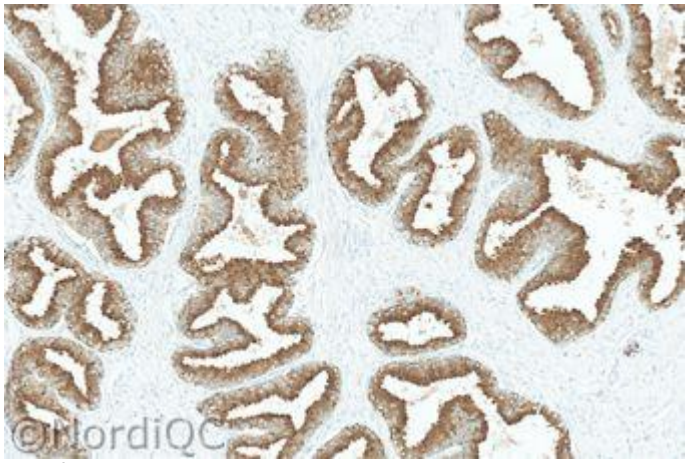


Fig. 1b
Staining for PSA of the prostate hyperplasia using an insufficient protocol based on the pAb A0452 with protocol settings giving a too low sensitivity.Too low concentration of the primary Ab and omission of HIER - same field as in Fig. 1a (x100). The epithelial cells are demonstrated, but a reduced intensity compared to the result seen in Fig. 1a is seen. Also compare with Figs. 2b – 4b, same protocol.

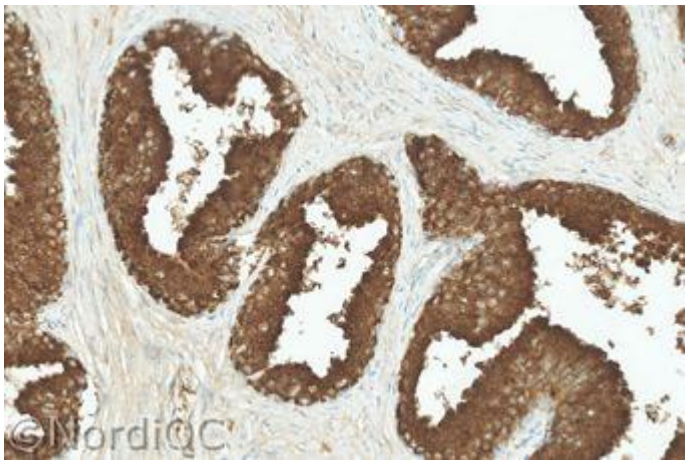


Fig. 2a
High magnification (x200) of the PSA staining of prostate hyperplasia in Fig. 1a. A weak to moderate stromal staining reaction is seen. However no general background staining or poor signal-to-noise ratio is seen, as no staining in the appendix is seen – see Fig. 5a.

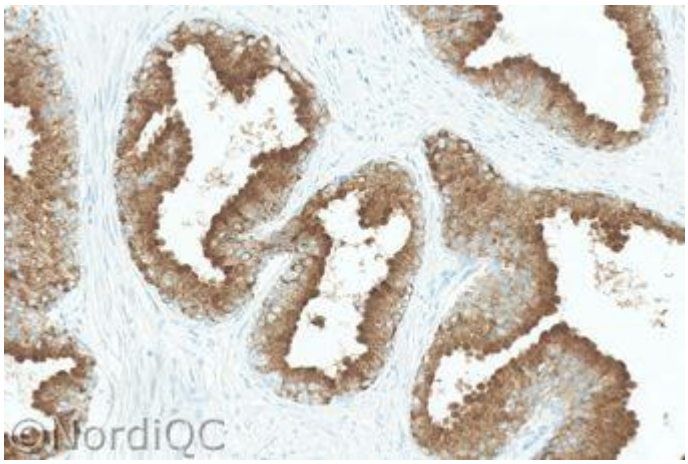


Fig. 2b
High magnification (x200) of the PSA staining of the prostate hyperplasia in Fig. 1b. Also compare with Figs. 2b – 4b, same protocol.

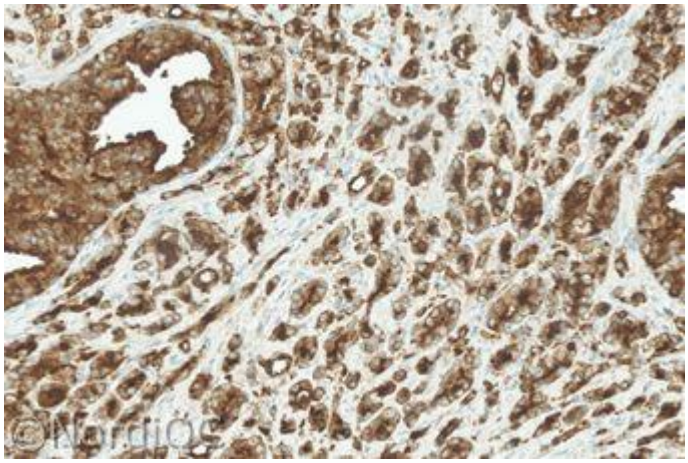


Fig. 3a
Optimal staining for PSA of the prostate adenocarcinoma no. 4 using same protocol as in Figs. 1a and 2a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic staining reaction.

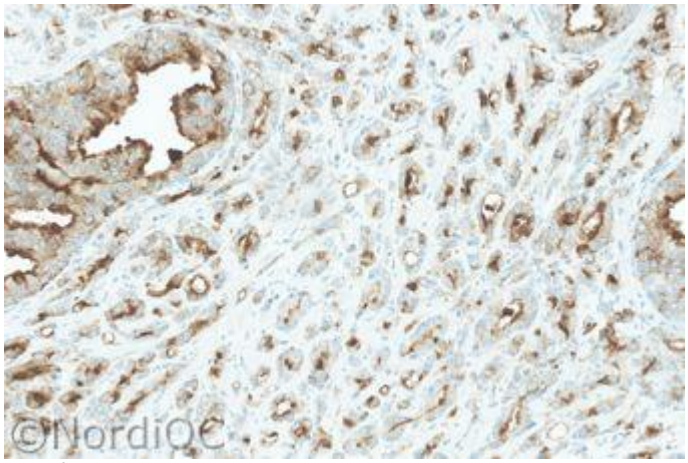


Fig. 3b
Staining for PSA of the prostate adenocarcinoma no. 4 using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a. The majority of the neoplastic cells are demonstrated, but with significantly reduced intensity compared to the level expected.

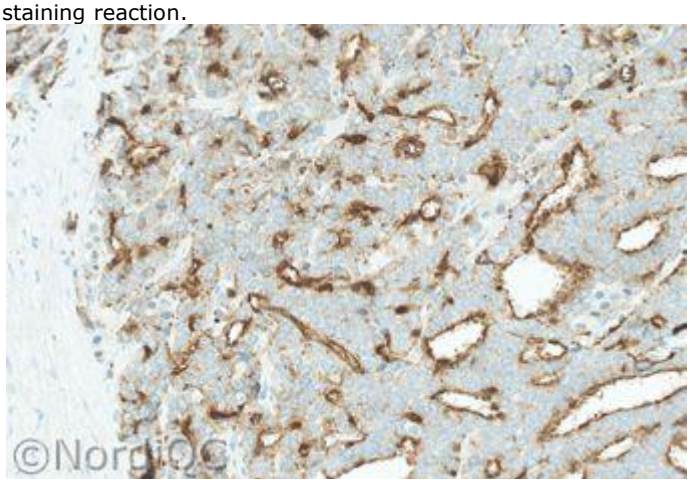


Fig. 4a
Optimal PSA staining of the prostate adenocarcinoma no. 5 using same protocol as in Figs. 1a - 3a. The majority of the neoplastic cells show a weak to moderate cytoplasmic staining.

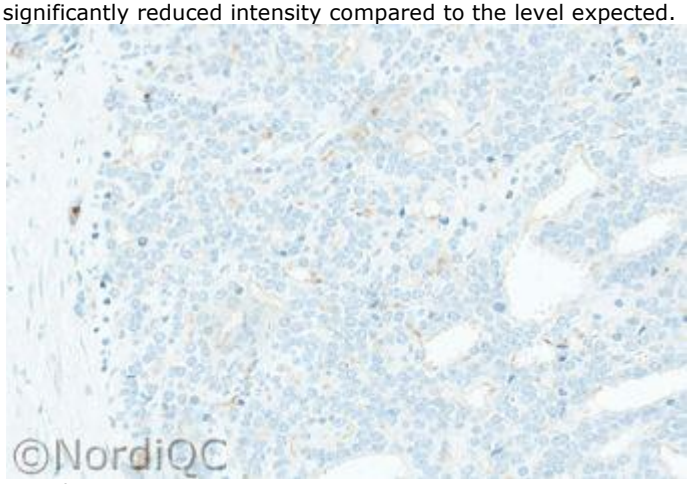


Fig. 4b
Insufficient staining for PSA of the prostate adenocarcinoma no. 5 using same protocol as in Figs. 1b – 3b. Only scattered cells show a faint and dubious staining reaction.

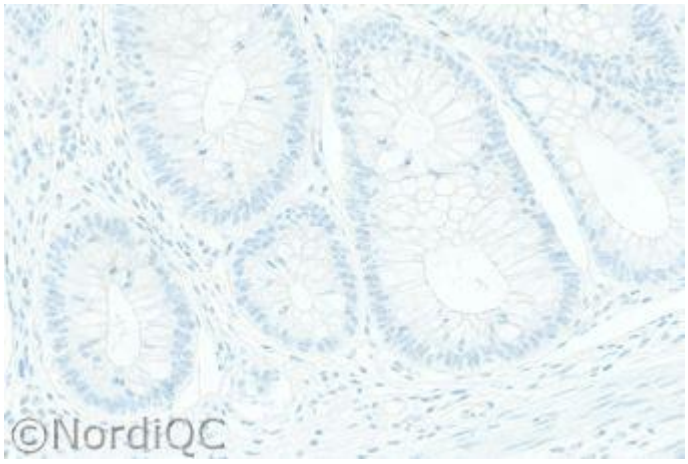


Fig. 5a
Optimal staining for PSA of the appendix using same protocol as in Figs. 1a – 4a. No staining reaction is seen. The weak stromal staining reaction seen in the prostate hyperplasia is related to antigen diffusion and not caused by an inappropriate calibration of the protocol, as seen in Fig. 5b.

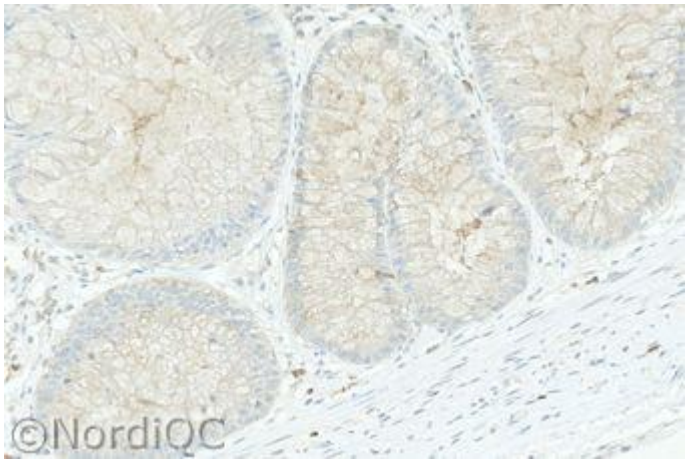


Fig. 5b
Insufficient staining for PSA of the appendix using the pAb A0452 by protocols settings giving a poor-signal-to-noise ratio. A weak to moderate diffuse staining reaction in the epithelial cells is seen. The staining pattern was caused by using the primary Ab too concentrated.

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