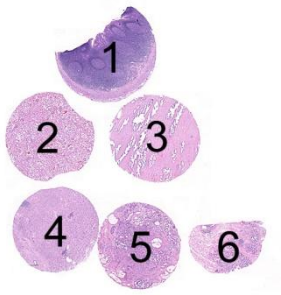
Assessment Run 27 2009

Prostate-specific antigen (PSA)

The slide to be stained for PSA comprised:
1. Appendix, 2. Kidney, 3. Prostate hyperplasia, 4 – 6. Prostate adenocarcinoma. (same tissue block as used for Prostein)
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a PSA staining as optimal included:

- A strong and distinct cytoplasmic staining of virtually all the epithelial cells of the hyperplastic prostate glands.
- A moderate to strong and distinct cytoplasmic staining of virtually all the neoplastic cells of the three prostate adenocarcinomas.
- No reaction of the epithelial cells in the kidney and appendix.
- No more than a weak to moderate background reaction in the vicinity of the positive prostate epithelial cells (antigen diffusion).

126 laboratories participated in this assessment. 76 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for PSA, run 27**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **ER-PR8**	33	Dako	14	11	7	1	76 %	86 %
mAb clone **35H9**	8	Novocastra	7	1	0	0	100 %	100 %
mAb clone cocktail **ER-PR8+PA05**	4 1	NeoMarkers Master Diagnostica	2	1	2	0	60 %	67 %
mAb clone cocktail **ER-PR8+A67-B/E3**	1	Biocare	0	1	0	0	-	-
pAb **A0562**	44	Dako	19	14	9	2	75 %	82 %
pAb **RB-084**	1	NeoMarkers	0	0	1	0	-	-
Ready-To-Use Abs								
mAb clone **ER-PR8, 760-4271**	2 1	Ventana Cell Marque	1	0	2	0	-	-
mAb clone **ER-PR8, N1550**	1	Dako	0	0	0	1	-	-
mAb clone **35H9, PA0431**	2	Novocastra	2	0	0	0	-	-
mAb clone cocktail **ER-PR8+A67-B/E3**	1	Biocare	0	0	1	0	-	-
pAb **IS514/IR514**	13	Dako	9	3	1	0	92 %	100 %
pAb **N1517**	2	Dako	1	1	0	0	-	-
pAb **760-2506**	12	Ventana	1	8	3	0	75 %	100 %
Total	126		56	40	26	4	-	-
Proportion			44 %	32 %	21 %	3 %	76 %	-

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

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **ER-PR8**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (7/13)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (4/6), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1), EDTA/EGTA pH 8 (1/3) or Citrate pH 6 (1/4) as the retrieval buffer. The mAb was typically diluted in the range of 1:50– 1:200 depending on the total

sensitivity of the protocol employed. Using these protocol settings 19 out of 22 (86 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **35H9**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (3/3), Cell Conditioning 1 (BenchMark, Ventana) (2/2), Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/1) or Citrate pH 6 (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:75– 1:600 depending on the total sensitivity of the protocol employed. Using these protocol settings 7 out of 7 (100 %) laboratories produced a sufficient staining.

mAb clone cocktail **ER-PR8+PA05**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (1/1) or EDTA/EGTA pH 8 (1/2) as the retrieval buffer. The mAb was typically diluted in the range of 1:100– 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 out of 3 (67 %) laboratories produced a sufficient staining.

pAb **A0562**: the protocols giving an optimal result were based either or on heat induced epitope retrieval (HIER) or with omission of retrieval. Using HIER a total of 11 out of 28 protocols resulted in an optimal result using either Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (8/15), Cell Conditioning 1 (BenchMark, Ventana) (1/3) or Citrate pH 6 (2/3) as the retrieval buffer. With omission of epitope retrieval 8 out of 14 protocols gave an optimal result. The pAb was typically diluted in the range of 1:1.000 – 1:6.000 without HIER and 1:3.000 – 15.000 when HIER was applied. Using these protocol settings 28 out of 34 (82 %) laboratories produced a sufficient staining.

Ready-To-Use Abs
mAb clone **ER-PR8** (prod. no. 760-4271, Ventana/Cell Marque): The protocol giving an optimal result was based on HIER using standard Cell Conditioning 1 (BenchMark, Ventana), an incubation time of 60 min in the primary Ab and Ultra View (760-500) with amplification kit as the detection system.

mAb clone **35H9** (prod.no. PA0431, Novocastra): The protocols giving an optimal result were all based on HIER using Bond Epitope Retrieval Solution 1 (Bond, Leica), an incubation time of 25 or 30 min in the primary Ab and BOND Polymer Refine Detection (DS9800) as the detection system. Using these protocol settings both laboratories produced an optimal staining.

pAb **IS514/IR514** (Dako): The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings 9 out of 9 (100 %) laboratories produced a sufficient staining.

pAb **N1517** (Dako): The protocol giving an optimal result were based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, 20min), an incubation time of 20 min in the primary Ab (the RTU Ab was diluted 1:8) and EnVision Flex (K8000) as the detection system. Using these protocol settings 1 out of 1 laboratory produced a sufficient staining.

pAb p **760-2506** (Ventana): The protocol giving an optimal result was based on HIER using mild Cell Conditioning 1 (BenchMark, Ventana), an incubation time of 32 min in the primary Ab and Ultra View (760-500) as the detection system. Using this protocol setting 2 out of 2 laboratories produced a sufficient staining.

The most frequent causes of insufficient stains were:
- Too low concentration of the primary antibody
- Insufficient heat induced epitope retrieval
- Too high concentration of the primary Ab – especially the pAb A0562, Dako
- Use of a biotin based detection system (giving a false positive reaction).

In this assessment the prevalent features of an insufficient staining were either a weak/false negative reaction or a false positive reaction in the tested specimens. A weak/false negative reaction was seen in 60 % of the insufficient results and was mainly caused by a too low concentration of the primary Ab and/or insufficient HIER. In 40 % a false positive reaction was observed. The false positive reaction was typically characterized by a distinct reaction in the epithelial cells in the kidney but also in lymphocytes in e.g., the appendix. The pattern was most frequently seen, when the pAb A0562, Dako, was used relatively concentrated and with HIER. However it has to be stressed that similar protocol settings and using same primary Ab also resulted in optimal results – suggesting a lot-to-lot variation of the pAb. It was also observed that HIER actually reduced the background reaction in the prostate specimens compared to omission of HIER for the pAb A0452, Dako.
Also the combination of HIER and a biotin based detection system frequently gave a false positive reaction of

endogenous biotin primarily seen in the epithelial cells of the kidney but also in the epithelial cells of the appendix.
In this assessment the mAb clone 34H9, Novocastra was the most robust marker for PSA, as all 10/10 protocols using this clone obtained a sufficient result.
Prostate and kidney was in this assessment found to be recommendable controls for PSA: The epithelial cells of the prostate glands must show an as strong as positive cytoplasmic reaction, while the epithelial cells of the kidney must be negative. A weak to moderate background reaction in the vicinity of the positive epithelial cells in the prostate is expected and acceptable. In this assessment it was seen, that a reduction of the sensitivity of the applied protocols in order to eliminate this background reaction frequently gave a too weak reaction in the carcinomas.

This was the second assessment of PSA in NordiQC, and the proportion of sufficient results declined from 90 % in run 12, 2004 to 76 % in the current run – see table 2. The lower pass rate is probably due to more challenging tissue material circulated and many laboratories participating for the first time.

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

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

Conclusion

The mAbs clones 35H9, ER-PR-8, ER-PR8+PA05 and the pAbs A0562 Dako, IR514/IS514 Dako and 760-2506 Ventana could be used to obtain an optimal staining. The epitope retrieval and protocol settings have to be specifically tailored to each of the clones/cocktails. Prostate hyperplasia is a recommended positive control provided that the epithelial cells show an as strong as positive cytoplasmic reaction (a weak to moderate background reaction is acceptable). Kidney is a recommended negative control, as no staining reaction must be seen in the epithelial cells.

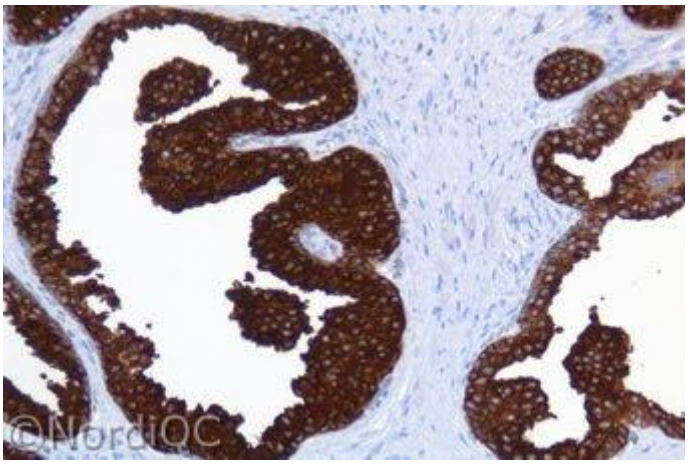


Fig. 1a
Optimal PSA staining of the prostate hyperplasia using the mAb ER-PR8 carefully calibrated and with HIER in an alkaline buffer. All the epithelial cells of the prostatic glands show a strong cytoplasmic staining. Also compare with Figs. 2a left & right – same protocol.

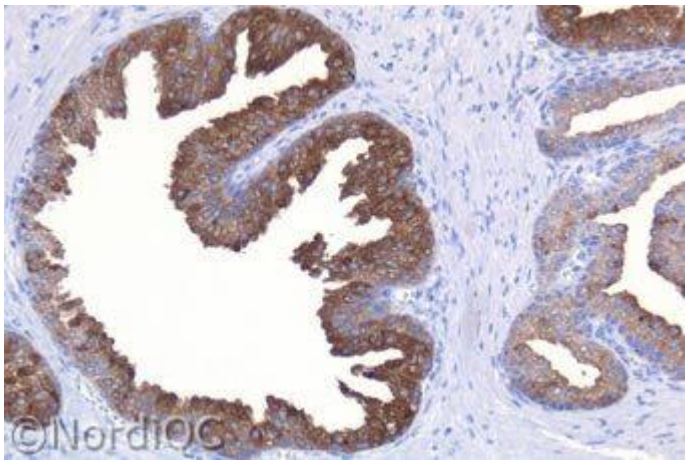


Fig. 1b
Insufficient PSA staining of the prostate hyperplasia using the mAb ER-PR8 too diluted. The majority of the epithelial cells of the prostatic glands are demonstrated. However, the intensity is significantly reduced compared to the staining in Fig. 1a – same field. Also compare with Figs. 2b left & right – same protocol.

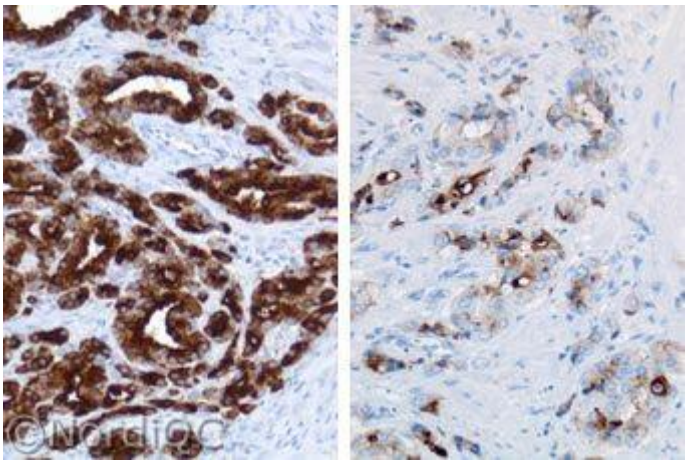


Fig. 2a
Optimal PSA staining using same protocol as in Fig. 1a.
Left: Prostate carcinoma, tissue no. 6: Virtually all the neoplastic cells show a moderate to strong and distinct cytoplasmic staining.
Right: Prostate carcinoma, tissue no. 4: The majority of the neoplastic cells show a moderate but distinct cytoplasmic staining.

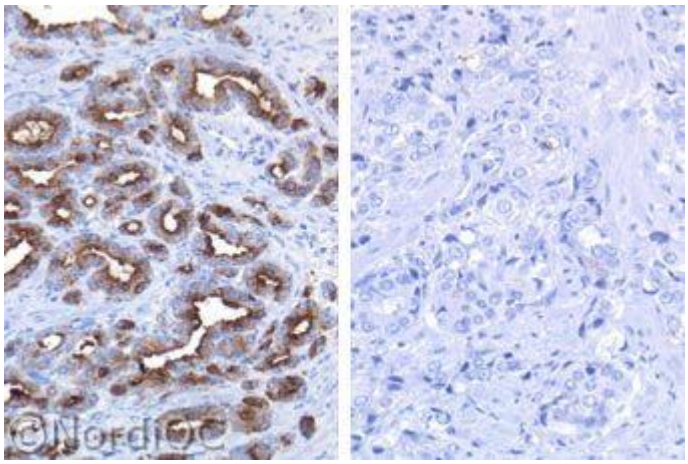


Fig. 2b
Insufficient PSA staining using same protocol as in Fig. 1b.- same fields as in Figs. 2a.
Left: Prostate carcinoma, tissue no. 6: The majority of the neoplastic cells show a moderate cytoplasmic staining.
Right: Prostate carcinoma, tissue no. 4: Only scattered neoplastic cells show a weak, equivocal staining.

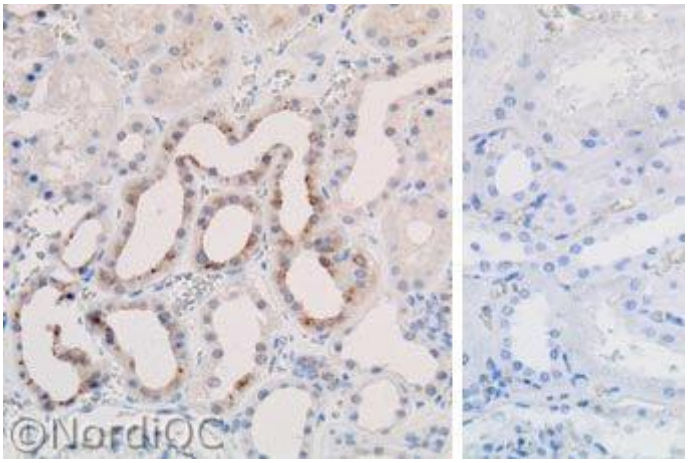


Fig. 3a
Left: Insufficient PSA staining of the kidney using the pAb A0562 diluted 1:3.000 with HIER in Tris-EDTA pH 9 and visualized with a 2-step polymer based detection system. The epithelial cells of the tubules show a weak to moderate and granular staining. This pattern was typically seen when the pAb was used relatively concentrated and with efficient HIER. Also compare with Fig. 3b – same protocol.
Right: Optimal staining for PSA of the kidney. No staining reaction is seen in the epithelial cells.

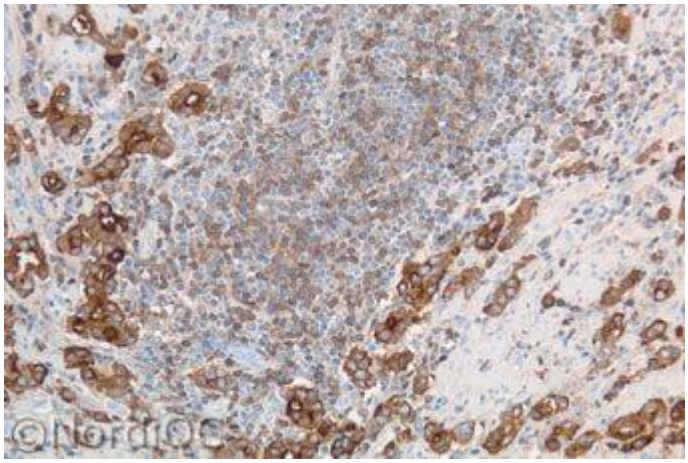


Fig. 3b
Insufficient PSA staining of the prostate carcinoma, tissue no. 4, using same protocol as in Fig. 3a left. The neoplastic cells are demonstrated, but also a distinct false positive staining is seen in the lymphocytes.

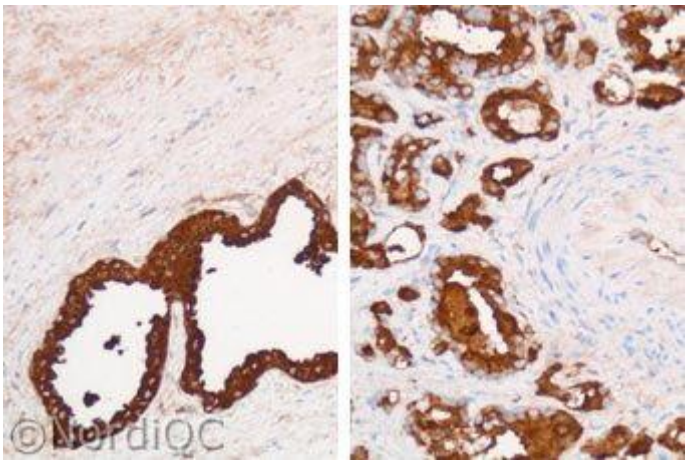


Fig. 4a
Optimal PSA staining using the pAb A0562 diluted 1:8.000 after HIER in Tris-EDTA pH 9 and visualized with a 2-step polymer based detection system.
Left: Prostate hyperplasia
Right: Prostate carcinoma, tissue no. 6. When HIER was applied the signal-to-noise ratio typically was improved. The specific staining in both the normal and neoplastic prostatic cells was intensified and the background reaction was reduced – also compare with Figs. 4b - same fields and same pAb but without HIER.

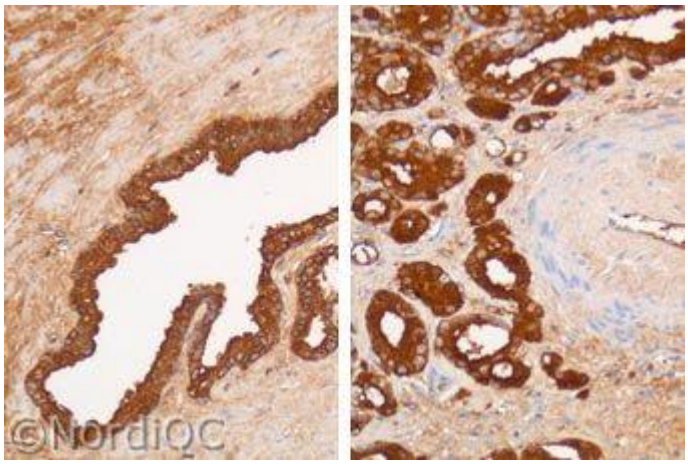


Fig. 4b
PSA staining using the pAb A0562 diluted 1:1.000 without HIER and visualized with a 2-step polymer based detection system.
Left: Prostate hyperplasia
Right: Prostate carcinoma, tissue no. 6. When HIER was omitted and using the pAb, the background reaction typically was amplified complicating the interpretation, compare with Fig. 4a - same fields.

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