

The slide to be stained for prostate-specific antigen (PSA) comprised:  
1. Prostate hyperplasia, 2. Kidney, 3 - 5. Prostate adenocarcinoma (Gleason score 7, 9 and 7, respectively).



Criteria for assessing a PSA staining as optimal included: A moderate to strong distinct cytoplasmic staining of the hyperplastic prostate glands and the three prostate adenocarcinomas. A weak to moderate reaction of the prostate stroma was accepted. No reaction should be seen in the kidney.

79 laboratories submitted stainings. At the assessment 38 achieved optimal staining (48 %), 33 good (42 %), 7 borderline (9 %) and 1 poor staining (1 %).

The following Abs were used:

- mAb clone ER-PR8 (DakoCytomation, n=25)
- mAb clone PSA 28/4 (Novocastra, n=5)
- mAb clone 35H9 (Novocastra, n=2)
- mAb clone ER-PR8 + PA05 (NeoMarkers, n=1)
- pAb A0562 (DakoCytomation, n=41)
- pAb 760-2506 from (Ventana, n=5)

In this assessment optimal staining could be obtained with the mAbs clone ER-PR8 (13 out of 25 (52%)), 35H9 (2 out of 2) and ER-PR8 + PA05 (1 out of 1). Optimal staining could also be obtained with both the pAbs A0562 (22 out of 41 (54%)), and 760-2506 (1 out of 5).

To obtain an optimal stain with mAb clone ER-PR8, all used HIER with Tris-EDTA/EGTA pH 9 as the heating buffer. The mAb dilution was 1:50 – 200.

To obtain an optimal stain with mAb clone 35H9, both used HIER with Tris-EDTA/EGTA pH 9 as the heating buffer. The mAb dilution was 1:100 – 500.

To obtain an optimal stain with mAb ER-PR8 + PA05, HIER was used with Citrate pH 6, the antibody dilution was 1:400.

To obtain an optimal stain with pAb A0452 the majority used HIER, either Tris-EDTA/EGTA pH 9 or Citrate pH 6 (20 out 25 (80 %) using one of these buffers). The pAb A0452 was diluted to 1:1.500 – 40.000.

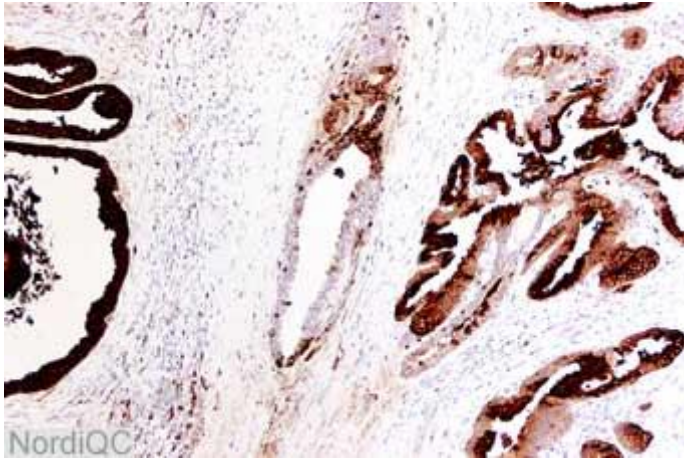
Omission of retrieval gave an optimal result in 2 out of 9 stains (22%).

To obtain an optimal stain with pAb 760-2506 HIER was used with CC1 in the Ventana Benchmark and a Ready-To-Use Ab.

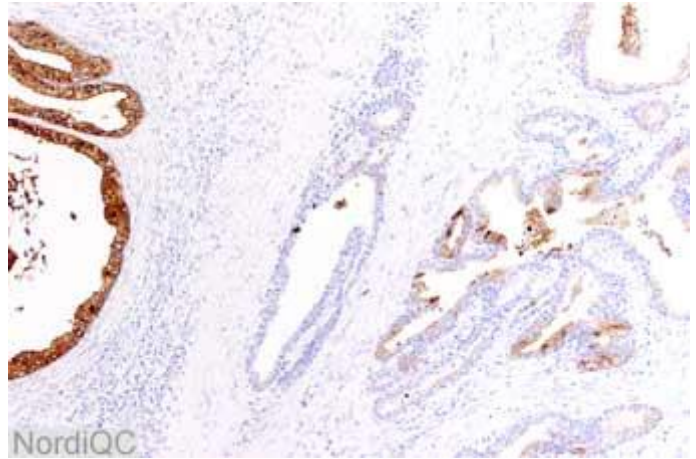
Almost all laboratories were able to detect PSA in the hyperplastic prostate and in two of the carcinomas (both Gleason score 7), whereas the low differentiated prostate carcinoma (Gleason score 9, tissue no. 4) was only weakly labelled or negative in the insufficient staining.

The most frequent causes of insufficient staining were (often in combination):

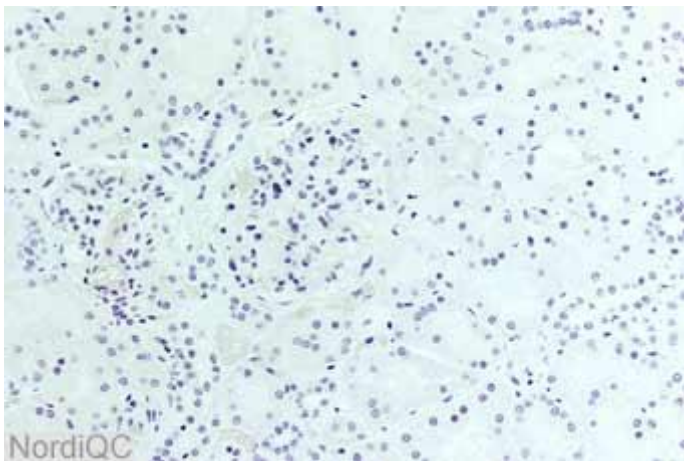
- Too low concentration of the primary Ab
- Inappropriate choice of primary Ab
- Inappropriate epitope retrieval (proteolytic pre-treatment) or no retrieval



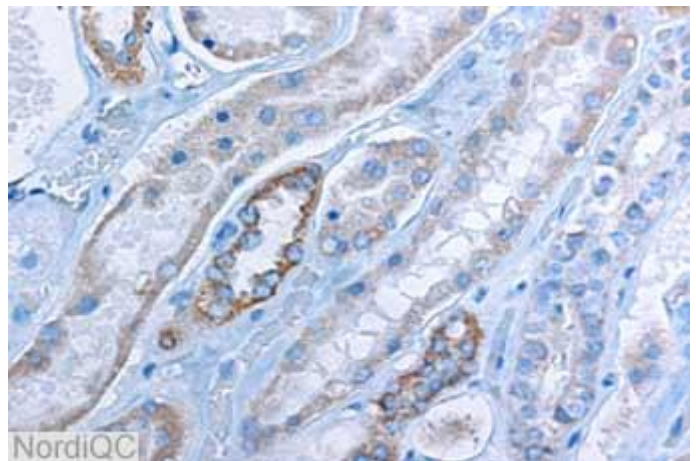
**Fig. 1a**  
Optimal PSA staining of the prostate hyperplasia. A moderate to strong staining is seen in almost all epithelial cells. A weak stromal reaction is unavoidable.



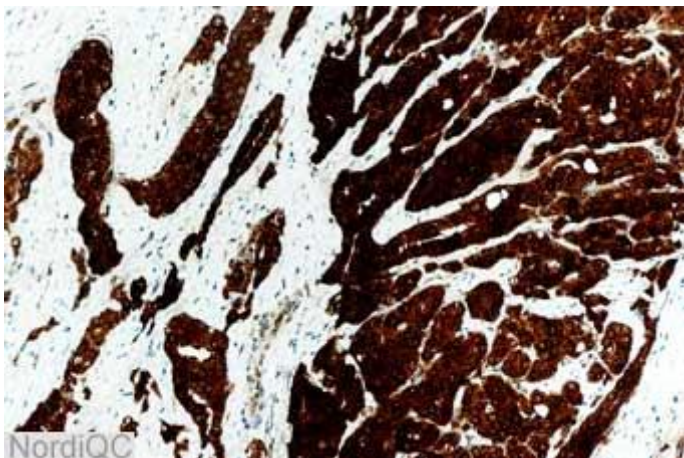
**Fig. 1b**  
Insufficient PSA staining of the prostate hyperplasia. A large proportion of epithelial cells are unstained. Compare with Fig. 4b.



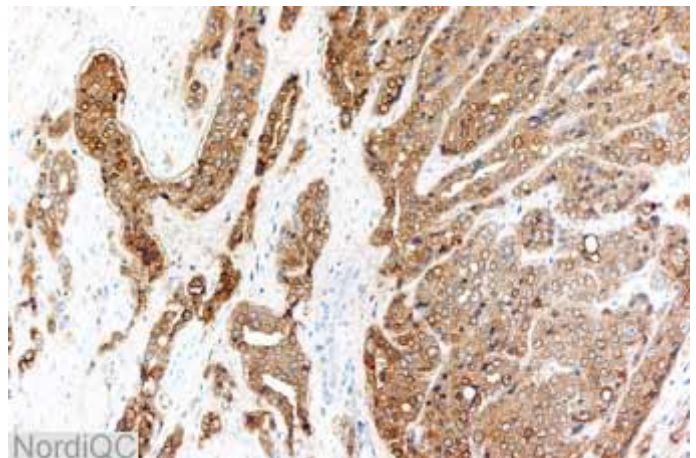
**Fig. 2a**  
Optimal PSA staining of kidney. No staining reaction is seen (same protocol as in Fig. 1a).



**Fig. 2b**  
Insufficient PSA staining of kidney. A false positive staining reaction is seen in the tubules due to endogenous biotin activity.



**Fig. 3a**  
Optimal PSA staining of the prostate adenocarcinoma (Gleason score 7). A strong staining is seen in all the neoplastic cells. A weak reaction is seen in the stroma (same protocol as in Fig. 1a).



**Fig. 3b**  
Insufficient PSA staining of the prostate adenocarcinoma (Gleason score 7). The staining is somewhat weaker than in Fig. 3a (same field). Compare with Fig. 4b. Same protocol as in Fig. 1b.

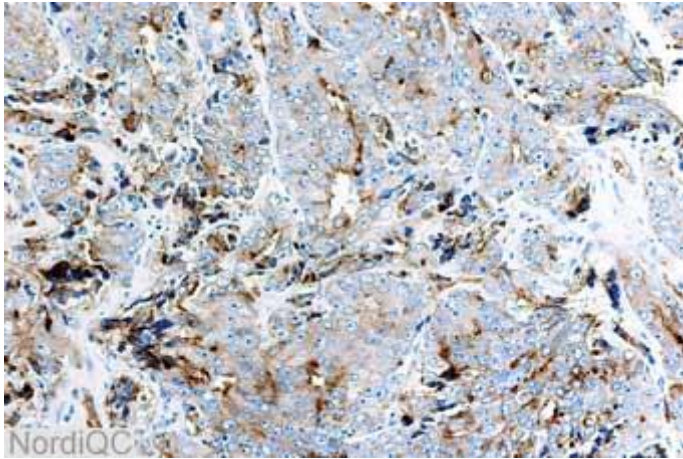


Fig. 4a  
Optimal PSA staining of the prostate adenocarcinoma (Gleason score 9). A weak but distinct staining is seen in the majority of the neoplastic cells (same protocol as in Fig. 1a).

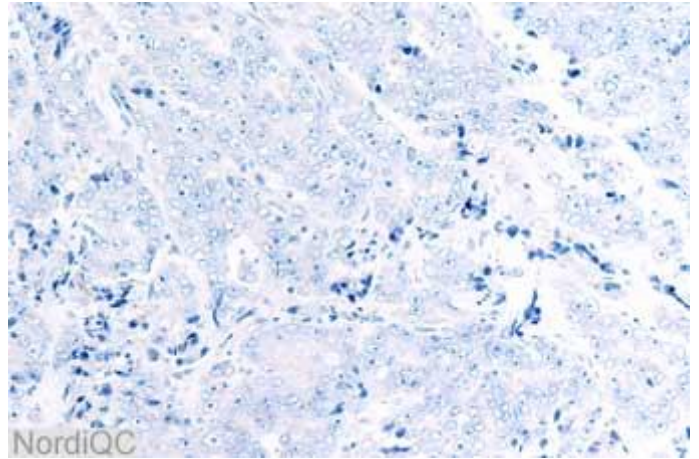


Fig. 4b  
Insufficient PSA staining of the prostate adenocarcinoma (Gleason score 9; same field as in Fig 4a). The tumour is virtually negative (same protocol as in Fig. 1b).

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