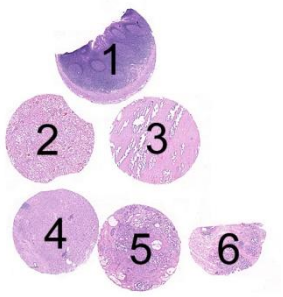
Assessment Run 27 2009
Prostein/P501S

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



Criteria for assessing a P501S staining as optimal included:

- A moderate to strong granular cytoplasmic staining of the epithelial cells of the hyperplastic prostate glands.
- A moderate to strong granular cytoplasmic staining of the majority of the neoplastic cells of the three prostate adenocarcinomas.
- A negative staining in all other cells such as the epithelial cells in the kidney and appendix.

11 laboratories participated in this assessment. 73 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for P501S, run 27**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **10-E3**	10	Dako	3	5	2	0	70 %	100 %
Ready-To-Use Abs								
mAb clone **10-E3, N1610**	1	Dako	0	0	1	0	-	-
Total	11		3	5	3	0	-	-
Proportion			27 %	46 %	27 %	0 %	73 %	-

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 **10-E3**: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (3/5)* as the retrieval buffer. The mAb was typically diluted in the range of 1:10–1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 out of 5 (100 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

The causes of insufficient stains were:
- Too low concentration of the primary Ab
- Too high concentration of the primary Ab (incl the Ready-To-Use Ab).

In this assessment the prevalent feature of an insufficient staining was either a generally too weak staining or a false positive staining. The former was in particular observed in the prostate carcinoma no. 4, while the two other prostate carcinomas were demonstrated by virtually all participants. The false positive staining was seen as a moderate diffuse cytoplasmic staining in e.g. the stromal cells in the prostate specimens but also in the epithelial cells in the kidney and the appendix. For unexplained reasons plasma cells in 2 protocols based on HIER in Target Retrieval Solution, low pH (pH 6.1, Dako) showed a distinct cytoplasmic reaction. These results were assessed as good.

Prostate was found to be an appropriate positive control in which the epithelial cells must show an as strong as possible granular cytoplasmic reaction, while other cells as e.g. the stromal smooth muscle cells must be ne gative.

Conclusion
The mAb clone 10-E3 is a recommendable Ab for P501S. HIER in an alkaline buffer seems mandatory to obtain an optimal staining. Normal prostate is recommended as positive control: The glandular epithelial cells must show an intense granular cytoplasmic reaction, while all other cells should be negative.

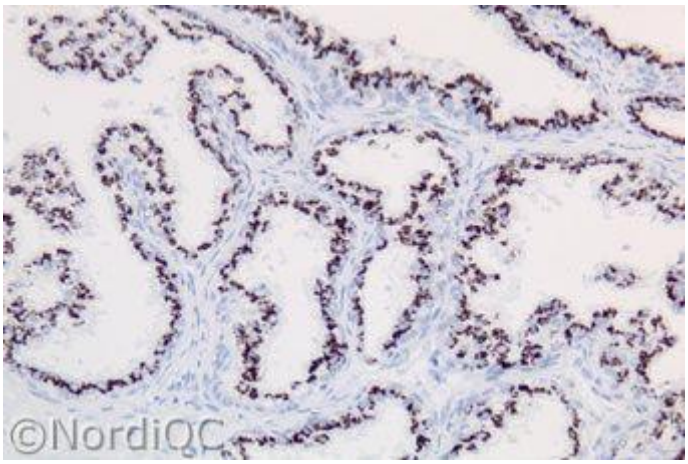


Fig. 1a
Optimal Prostein staining of the prostate hyperplasia using the mAb clone 10E3 carefully calibrated after HIER in Tris-EDTA pH 9.0.
Virtually all the epithelial cells of the prostate glands show a strong granular cytoplasmic staining. No background reaction is seen in the stroma. Also compare with Fig. 1b – same protocol.

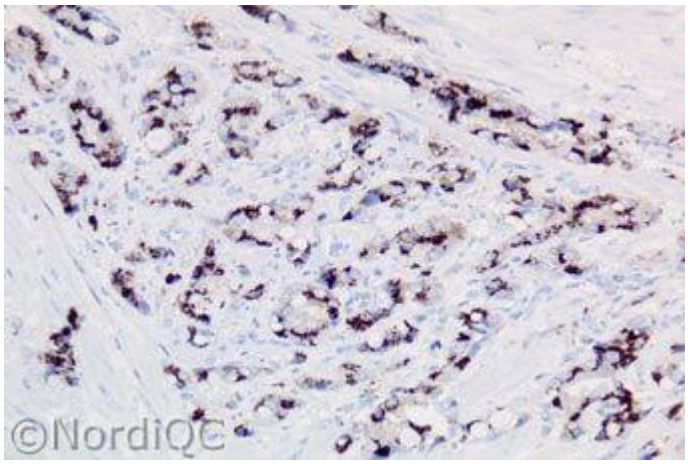


Fig. 1b
Optimal Prostein staining of the prostate carcinoma no. 4 using same protocol as in Fig. 1a.
The majority of the neoplastic show a moderate to strong granular cytoplasmic staining. No background reaction is seen in the stroma.

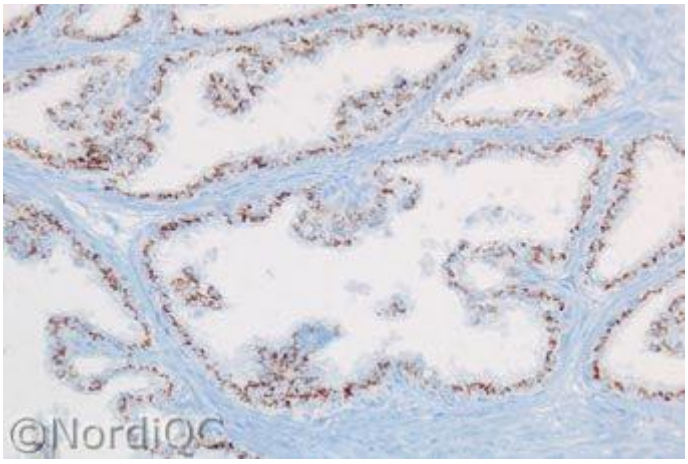


Fig. 2a
Insufficient Prostein staining of the prostate hyperplasia using the mAb clone 10E3 with HIER but the Ab too diluted.
Virtually all the epithelial cells of the prostate glands are demonstrated. However the intensity is significantly reduced compared to the staining result in Fig. 1a.
Also compare with Fig. 2b – same protocol.

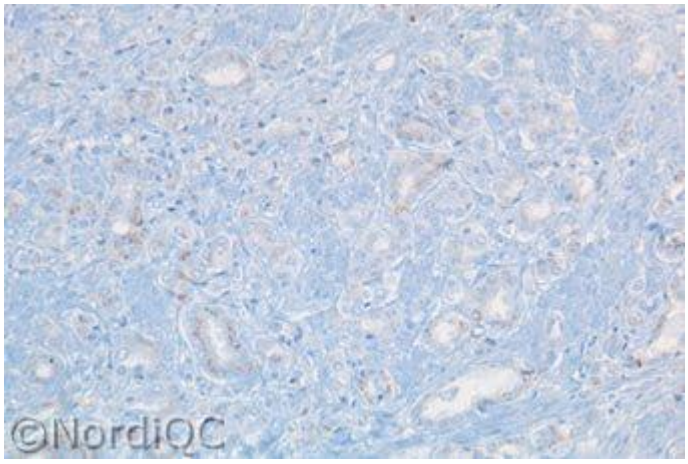


Fig. 2b
Insufficient Prostein staining of the prostate carcinoma no. 4 using same protocol as in Fig. 2a.
Only scattered neoplastic cells show a weak and dubious staining.
Also compare with Fig. 1b – same tumour.

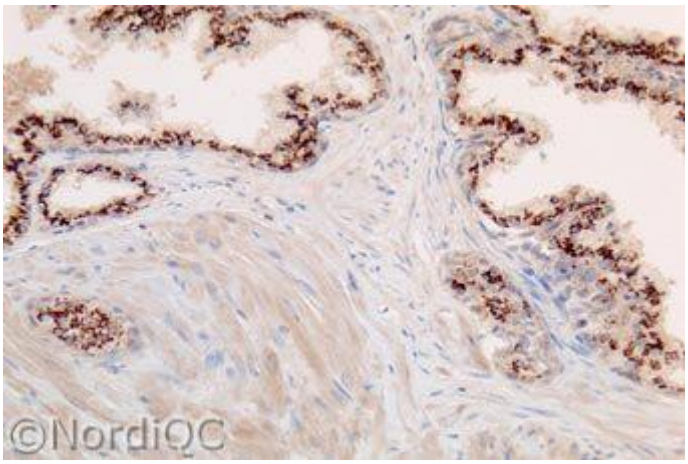


Fig. 3a
Insufficient Prostein staining of the prostate hyperplasia using the mAb clone 10E3 too concentrated. The epithelial cells are distinctively demonstrated, but the stromal cells show a moderate false positive staining.
Also compare with Fig. 3b left – same protocol.

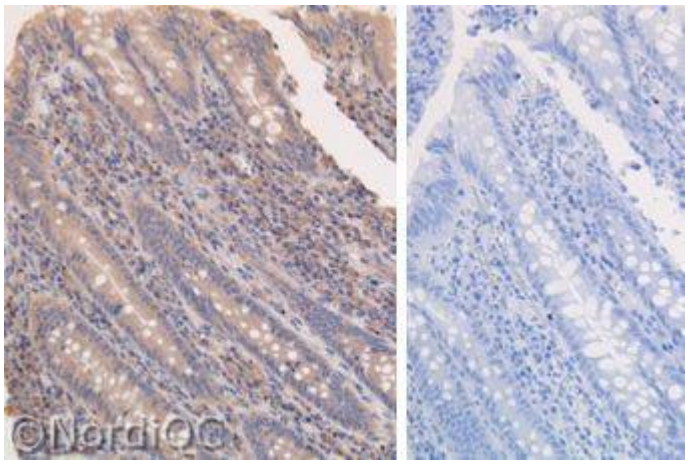


Fig. 3b
Left: Insufficient Prostein staining of the appendix using same protocol as in Fig. 3a. The epithelial cells and lymphocytes show a moderate false positive staining.
Right: Optimal Prostein staining of the appendix using same protocol as in Figs. 1a and 1b. No staining reaction is seen.

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