

The slide to be stained for Prostatic Acid Phosphatase (PAP) comprised:

1. Prostate hyperplasia, 2. Kidney, 3. Prostate adenocarcinoma (Gleason score 7),
 4. Prostate adenocarcinoma (Gleason score 9), 5. Prostate adenocarcinoma,
 (Gleason score 7).



Criteria for assessing a PAP staining as optimal included: A moderate to strong distinct cytoplasmic staining of the the hyperplastic prostate and three prostate adenocarcinomas. A focal labelling of the renal collecting tubules and a weak to moderate labelling of the leucocytes was accepted.

24 laboratories submitted stainings. At the assessment 15 achieved optimal staining (63 %), 6 good (25 %) and 3 (12 %) borderline staining.

The following abs were used:

mAb clone PASE/4LJ (DakoCytomation, n=16; Ventana, n=2)
 pAb A0627 (DakoCytomation, n=6)

Optimal staining could be obtained with both these clones. 12 out of 18 using PASE/4LJ (67 %) 3 out of 6 using pAb A0627 (50%) achieved an optimal staining.

Using the PASE/4LJ with HIER (with either Tris-EDTA/EGTA pH 9, Citrate pH 6 or TRS low pH, DakoCytomation) 10 out 13 (77 %) achieved an optimal staining. Without HIER, 2 out of 5 achieved an optimal staining. mAb clone PASE/4LJ was typically used in the range of 1:200 – 4.000.

Using the pAb A0627 both HIER (1 out of 3) and omission of retrieval (2 out of 3) could give an optimal result. The optimal protocol using HIER was based on Tris-EDTA/EGTA pH 9 as the heating buffer and a dilution of the pAb A0627 1:10.000. Without epitope retrieval pAb A0627 was diluted 1:4.000 – 5.000.

Almost all laboratories were able to detect PAP in the hyperplastic prostate and in the two moderately differentiated prostate adenocarcinomas (Gleason's score 7, no. 3 and 5 in the multi-tissueblock), whereas the low differentiated prostate adenocarcinoma (Gleason's score 9, no. 4 in the multi-tissueblock) was only weakly labelled or negative in the insufficient staining.

The most frequent causes of insufficient staining were:

- Too low concentration of the primary Ab
- False positive reaction due to endogenous biotin.

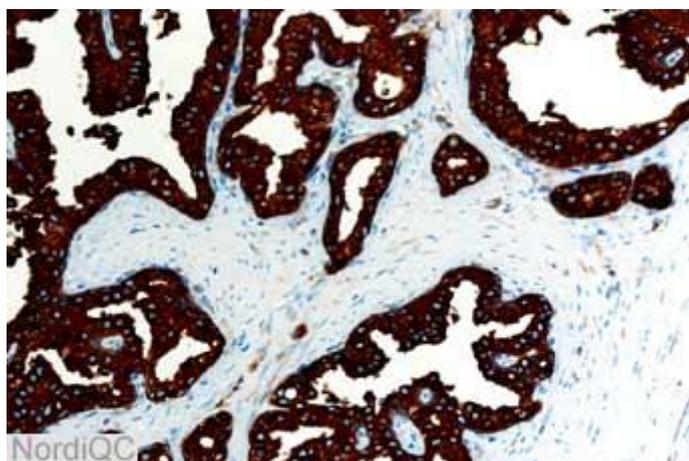


Fig. 1a
 Optimal PAP-staining of hyperplastic prostate. All epithelial cells reveal a strong, distinct cytoplasmic staining.

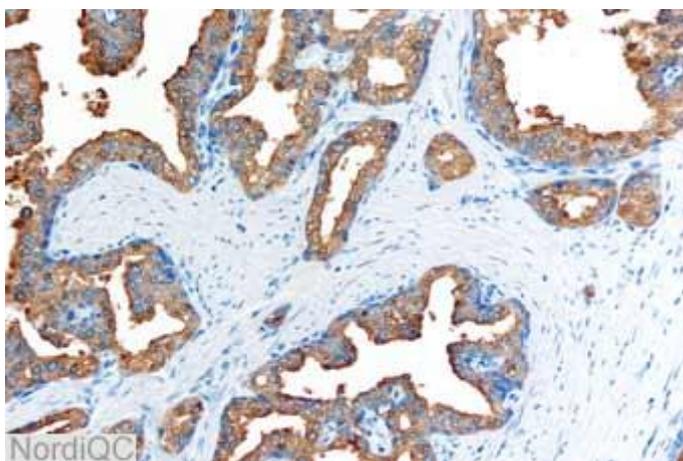


Fig. 1b
 PAP-staining of hyperplastic prostate (same field as in Fig. 1a) using an insufficient protocol. The epithelial cells are moderately stained. However, compare with Fig. 2b. and 2c.

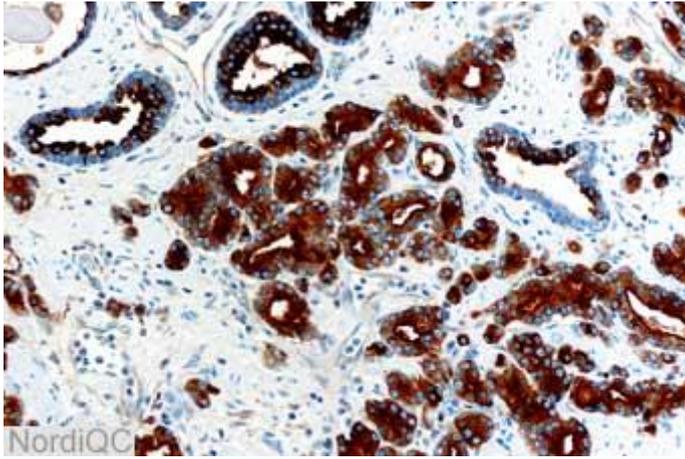


Fig. 2a
Optimal PAP-staining of a prostate adenocarcinoma (Gleason score 7). Most tumour cells reveal a strong, distinct cytoplasmic staining.

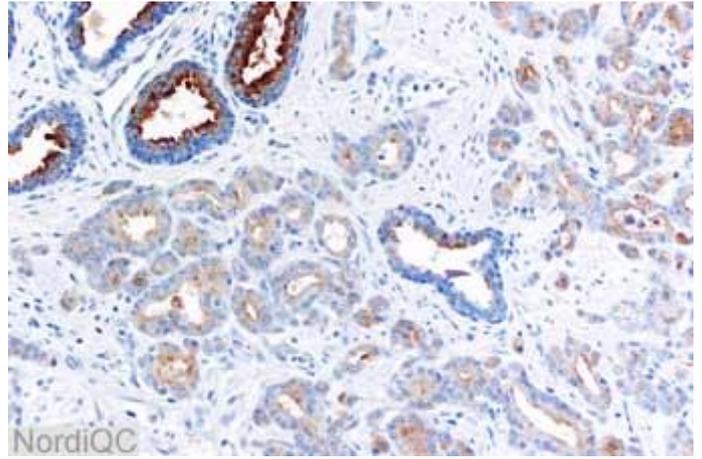


Fig. 2b
Insufficient PAP-staining of a prostate adenocarcinoma (same field as in Fig. 2a). The tumour cells reveal a weak cytoplasmic staining.

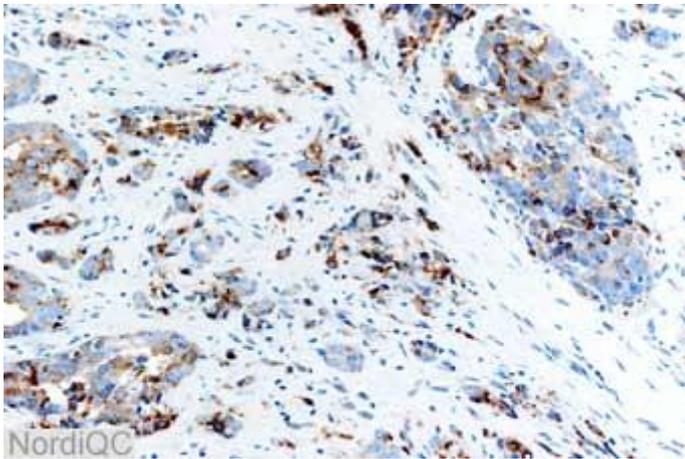


Fig. 3a
Optimal PAP-staining of a prostate adenocarcinoma (Gleason score 9). Most tumour cells reveal a moderate to strong, distinct cytoplasmic staining.

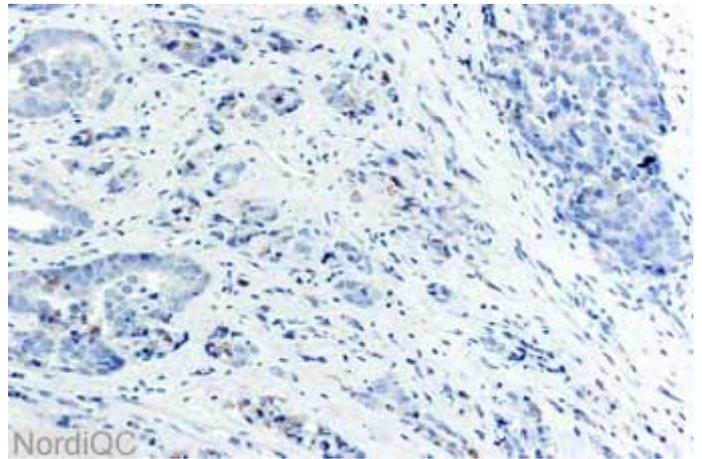


Fig. 3b.
Insufficient PAP-staining of a prostate adenocarcinoma (same field as in Fig. 3a). The tumour cells are virtually negative.

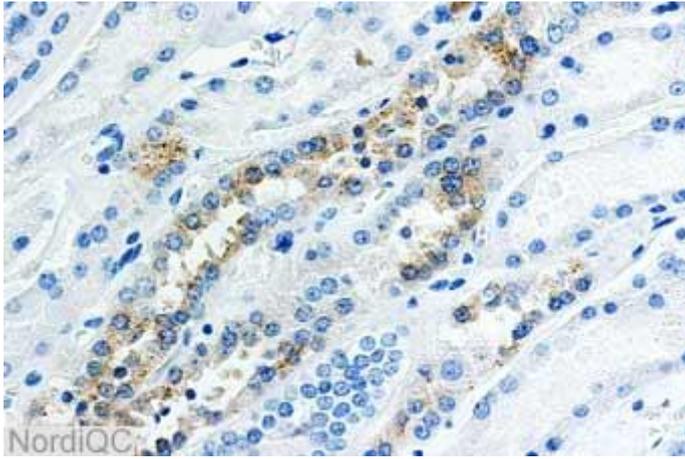


Fig. 4a
PAP-staining of normal kidney revealing a moderate cytoplasmic staining in some collecting tubules.

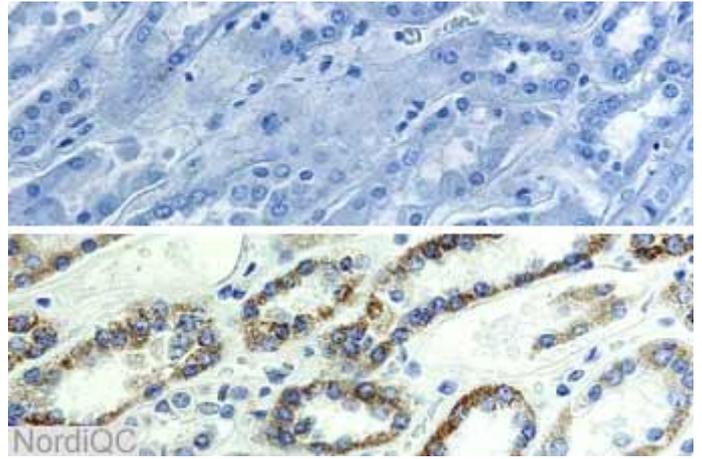


Fig. 4b
PAP-staining of normal kidney revealing insufficient staining: In the upper part, no staining of collecting tubules is seen (compare with Fig. 4a), while in the lower part, the staining results from endogeneous biotin (granular staining also including proximal tubules).

SN/MV/LE 28-11-2004