The slide to be stained for p63 protein comprised:
5. Prostate adenocarcinoma. All specimens were fixed in 10 % NBF.
* An area with prostate adenocarcinoma was revealed as the block was cut for distribution.

Criteria for assessing a p63 staining as optimal included:

- A strong, distinct nuclear staining in almost all squamous epithelial cells in the tonsil and esophagus
- A strong, distinct nuclear staining in the basal cells in the prostate hyperplasia and in areas with prostatic intraepithelial neoplasia (PIN) in the prostate adenocarcinoma specimen
- No staining reaction in the secretory cells of the hyperplastic prostate or the prostate carcinoma
- Only focal nuclear staining reaction in the breast carcinoma
- No or only a week staining reaction of the smooth muscle cells.

68 laboratories submitted stains. At the assessment 42 obtained optimal marks (62 %), 14 good (21 %), 9 borderline (13 %) and 3 poor marks (4 %).

The following Abs were used:
mAb clone 4A4 (Dako, n=47; BioCare, n=4; NeoMarkers, n=3; Diagnostic Biosystems, n=1; Becton Dickinson, n=1; BioGenex, n=1; Biologo, n=1; Santa Cruz, n=1)
mAb clone 4A4 + Y4A3 (NeoMarkers, n=5)
mAb clone 7JUL (Novocastra, n=4)

Optimal staining for p63 in this assessment was obtained with the mAbs clone A4A (38 out of 59) and clones A4A + Y4A3 (4 out of 5).

All optimal protocols were based on Heat Induced Epitope Retrieval (HIER).

Using the clone 4A4, HIER in Tris-EDTA/EGTA pH 9 (33 out of 39), Citrate pH 6 (3 out of 8), EDTA/EGTA pH 8 (1 out of 2), and CC1/Ventana (1 out of 8) gave an optimal staining. The clone 4A4 was typically used in the range of 1:25 – 1:800 depending on the total sensitivity of the protocol employed. One laboratory used 4A4 as a ready-to-use Ab.

Using the clone 4A4 + Y4A3 optimal staining was obtained with HIER in Tris-EDTA/EGTA pH 9 (3 out of 3) and Citrate pH 6 (1 out of 2). The clone 4A4 + Y4A3 was typically used in the range of 1:200– 1:1600 depending on the total sensitivity of the protocol employed.

The most frequent causes of insufficient stains were:
- Too low concentration of the primary antibody
- A less successful primary Ab
- Insufficient or excessive HIER.

In the assessment the prevalent feature of an insufficient staining was a too weak or false negative reaction of the basal cells in the hyperplastic prostate. The majority was capable of detecting p63 in the squamous epithelial cells in the tonsil and esophagus. However, in the insufficient staining the reaction typical was relatively weak with only the basal layer distinctively demonstrated. In general using i.e. tonsil as a positive control, the immunohistochemical reaction for p63 should be calibrated to give a staining as strong as possible without any or with only a weak cytoplasmic reaction. In a few laboratories where the staining reaction was too strong, this was typically accompanied by a cytoplasmic reaction in germinal centre macrophages.

**Conclusion**
- The mAbs clone A4A and A4A+Y4A3 are useful Abs for p63
- HIER is required for an optimal performance of both Abs.
An appropriate **control** for p63 is tonsil, in which all squamous epithelial cells should give a strong nuclear staining reaction (without cytoplasmic reaction).

![Fig. 1a](image1a.png)
**Fig. 1a**
Optimal staining for p63 (mAb clone 4A4) of the tonsil. All the squamous epithelial cells show a strong nuclear staining.

![Fig. 1b](image1b.png)
**Fig. 1b**
Staining for p63 (mAb clone 4A4) of the tonsil using an insufficient protocol (same field as in Fig. 1a.). The squamous cells are weakly stained and a reduced number of cells is demonstrated compared to the staining in Fig 1a.

![Fig. 2a](image2a.png)
**Fig. 2a**
Optimal staining for p63 (mAb clone 4A4) of the prostate hyperplasia. The basal cells show a strong nuclear reaction (same protocol used in Fig. 1a).

![Fig. 2b](image2b.png)
**Fig. 2b**
Insufficient staining for p63 (mAb clone 4A4) of the prostate hyperplasia. (same field as in Fig 2a). The basal cells are only weakly stained or negative (same protocol used in Fig. 1b).

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