

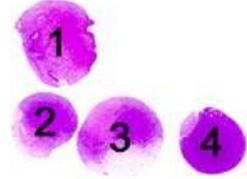
The slide to be stained for progesterone receptor (PR) comprised:

1. Uterine cervix, 2. Ductal breast carcinoma, PR negative, 3. Ductal breast carcinoma, PR 40-60 % positive, 4. Ductal breast carcinoma, PR 80-100 % positive. The positivity of the 3 ductal breast carcinomas was verified in 4 reference IHC laboratories.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a PR staining as optimal included:

- A strong and distinct nuclear staining of the columnar epithelial cells, the basal squamous epithelial cells and the stromal cells in the uterine cervix.
- A moderate to strong and distinct nuclear staining of the ductal breast carcinomas no. 3 and 4 in accordance with the PR status.
- No nuclear staining of the PR negative ductal breast carcinoma no. 2 – only epithelial cells in remnants of normal glands should show a positive reaction.



81 laboratories participated in the assessment. 39 achieved optimal marks (49 %), 21 good (26 %), 19 borderline (23 %) and 2 (2 %) poor marks.

The following Abs were used:

mAb clone **PgR 636** (Dako, n=39)
 mAb clone **16** (Novocastra, n=12; Ventana, n=9)
 mAb clone **1A6** (Novocastra, n=5; Ventana, n=1)
 mAb clone **PR-1** (Immunovision, n=2)
 mAb clone **PgR 1294** (Dako, n=2)
 mAb clone **PR 88** (Biogenex, n=1)
 mAb clone **hPRa 2+hPRa 3** (NeoMarkers, n=1)
 rmAb clone **1E2** (Ventana, n=5)
 rmAb clone **SP2** (NeoMarkers, n=4)

Optimal staining for PR in this assessment was obtained with the mAb clones PgR 636, 16, PR 1294 and the rmAb clones 1E2 and SP2 in the following settings:

PgR 636: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either **Tris-EDTA/EGTA pH 9** (18 out of 29) or **Citrate pH 6,0** (3 out of 7). The clone **PgR 636** was used in the range of 1:20 – 800 depending on the total sensitivity of the protocol employed. Using these protocol settings 28 out of 39 laboratories (72 %) produced a sufficient staining (optimal or good), 21 of them were optimal (54 %).

16: the protocols giving an optimal result were based on HIER using either Cell Conditioning 1 (CC1 Ventana, 5 out of 12), Tris-EDTA/EGTA pH 9 (4 out of 5) or Bond Epitope Retrieval Solution 2 (BERS 2, Vision BioSystems, 1 out of 1) as the HIER buffer. The clone 16 could both be used as a Ready-To-Use product and as a concentrate diluted in the range of 1:100 – 500 depending on the total sensitivity of the protocol employed. Using these protocol settings 14 out of 16 laboratories (88 %) produced a sufficient staining (optimal or good), 10 of them were optimal (63 %).

PgR 1294: the protocols giving an optimal result were performed according to the protocol of the PR-PharmDx kit (Dako) based on HIER (Pressure cooker) in Target Retrieval Solution and a RTU Ab. 2 out of 2 using this kit obtained an optimal mark.

1E2: the protocols giving an optimal result were all based on HIER in Cell Conditioning 1 (CC1, Ventana) and a RTU Ab. Using these protocol settings 4 out of 5 produced a sufficient staining, all 4 assessed as optimal (80 %).

SP2: the protocols giving an optimal result were all based on HIER in either Cell Conditioning 1 (CC1, Ventana 1 out of 1) or Tris-EDTA/EGTA pH 9 (1 out of 3). The clone SP2 was diluted 1:100. Using these protocol settings 2 out of 4 produced a sufficient staining both assessed as optimal (50 %).

The most frequent causes of an insufficient staining were:

- Less successful primary antibody
- Too low or too high concentration of the primary antibody
- Inappropriate epitope retrieval

In this assessment (and in concordance with the observation in the previous PR assessment, run 10, 2004) almost all laboratories were able to demonstrate PR in the ductal breast carcinoma no. 4 with 80-100 % positivity

and a strong staining intensity, whereas the prevalent feature of the insufficient staining was a too weak or false negative staining of the ductal breast carcinoma no. 3 with 40-60 % positivity and only a moderate staining intensity.

A too weak or false negative staining was seen in 90 % of the insufficient results (19 out of 21), while in 9 % (2 out of 21) a too strong staining and false positive PR staining reaction was seen.

The majority of the Abs used in the assessment showed almost the same staining pattern except the mAb clone **1A6**, which did not label the nuclei of the basal squamous epithelium in the cervix, while the other layers showed a positive cytoplasmic staining. The other Abs showed a distinct nuclear reaction in the basal cells and no or only minimal cytoplasmic reaction of the squamous epithelial cells. At the same time the clone **1A6** seemed to have a lower affinity of PR in the breast ductal carcinomas as fewer cells in general were labelled with this clone.

The uterine cervix seemed to be an appropriate control for the evaluation of the sensitivity of the PR staining. In the protocols giving an optimal PR staining of the ductal breast carcinomas the majority of the basal squamous epithelial cells of the cervix showed a distinct nuclear reaction. In the protocols giving an insufficient PR staining of the ductal breast carcinomas the basal cell layer only showed a focal or a negative staining.

PR was also assessed in run 10, 2004, where 79 laboratories participated out of which 30 % (24 laboratories) obtained an insufficient mark. Each of these was given a specific recommendation to improve their protocol. 18 of the laboratories submitted a new PR stain in run B2. 14 of them followed the recommendations to change their protocol and 11 improved from insufficient to either good or optimal (79 %). 4 laboratories did not follow the recommendations and none of these obtained a sufficient staining in run B2.

Conclusion

The clones **PgR 636, 16, PgR 1294, 1E** and **SP2** all seem to be robust Abs for the demonstration of PR. HIER is mandatory to obtain an optimal result. The concentration of the primary Ab should be carefully calibrated and the uterine cervix seems to be an appropriate control tissue for this calibration as the basal squamous epithelial cells should show a distinct nuclear reaction with minimal cytoplasmic reaction.

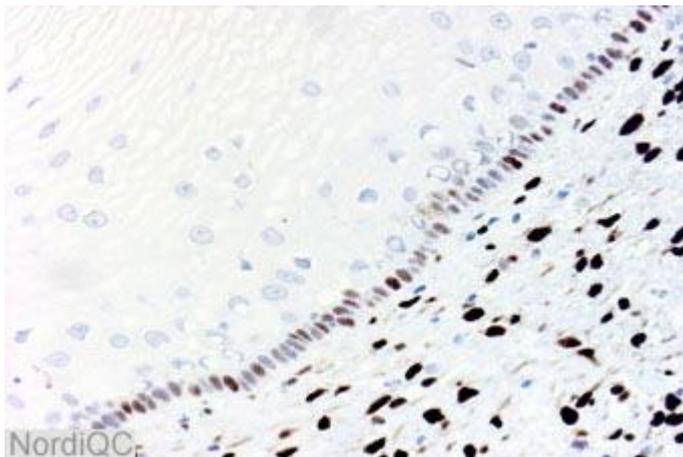


Fig. 1a
Optimal staining for PR of the cervix using the mAb clone PgR 636. The stromal cells show a strong nuclear staining and the basal squamous epithelial cells a moderate, distinct moderate nuclear staining.

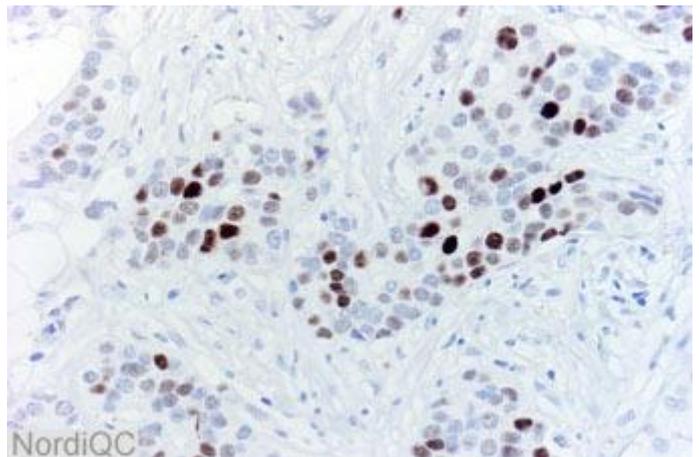


Fig. 1b
Optimal staining for PR of the ductal breast carcinoma no. 3 in which 40-60 % of the neoplastic cells show a nuclear reaction. Same protocol as in Fig.1a.

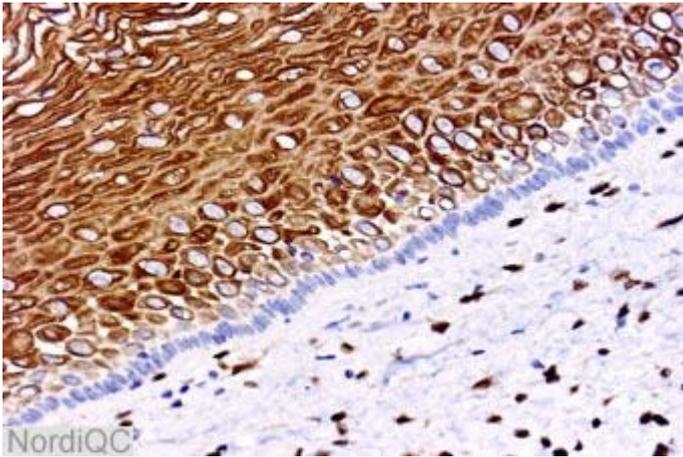


Fig. 2a
Staining for PR assessed as good using mAb clone 1A6, same field as in Fig. 1a. The stromal cells show a distinct nuclear reaction, while the basal squamous epithelial cells are negative and a strong cytoplasmic reaction is seen in the intermediate and superficial squamous epithelial cells.

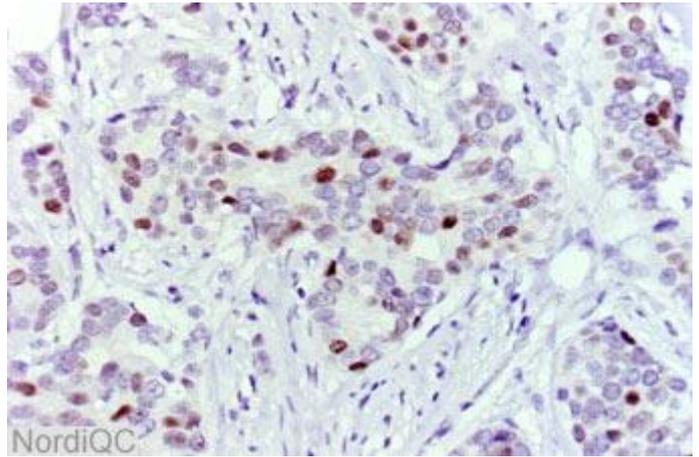


Fig. 2b
Staining for PR assessed as good of the ductal breast carcinoma no. 3 in which 40-60 % of the neoplastic cells show a nuclear reaction, same field as Fig. 1b and same protocol as in Fig. 2a.

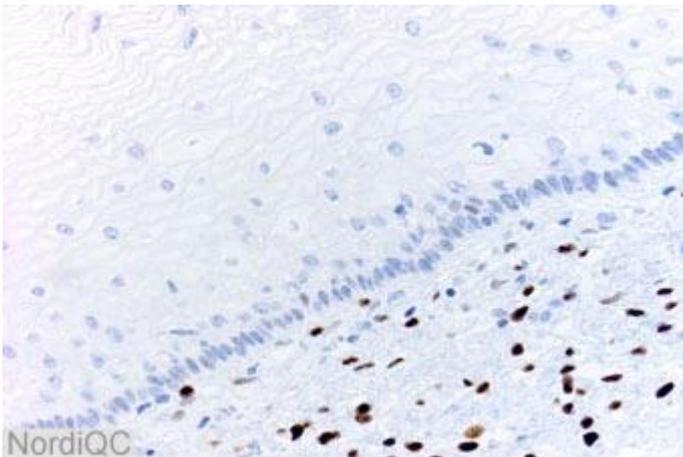


Fig. 3a
Insufficient staining for PR of the cervix using the mAb clone PgR 636, same field as in Fig. 1a. The stromal cells show a strong nuclear staining, but the basal squamous epithelial cells are negative. Compare with Fig. 3b.

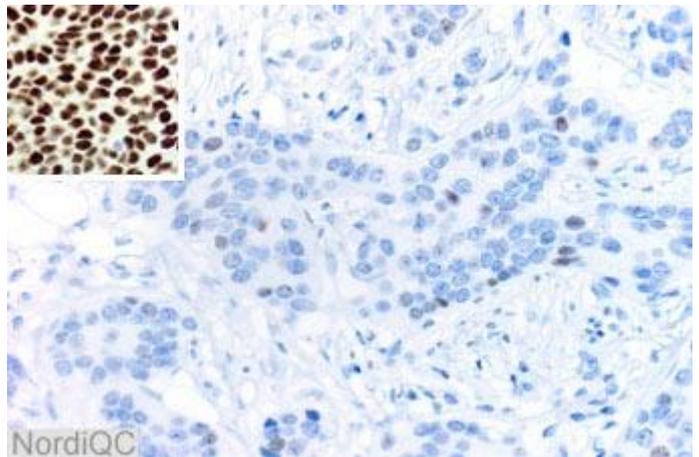


Fig. 3b
Insufficient staining for PR of the ductal breast carcinoma no. 3, same field as Fig. 1b. The proportion of the positive cells is <10 % and thus false negative, due to a too low concentration of the mAb clone PgR 636, same protocol as in Fig. 3a. Insert shows the reaction in the breast carcinoma no. 4, in which almost all cells are positive.

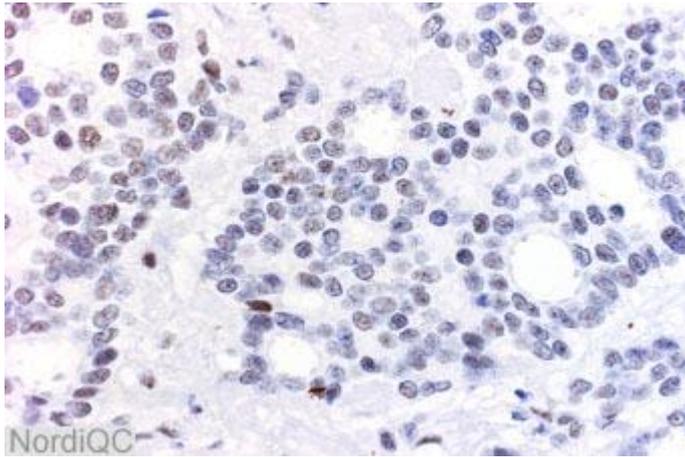


Fig. 4a
Insufficient staining for PR of the PR negative ductal breast carcinoma no. 2 using the rmAb SP2 in a too high concentration. The majority of the neoplastic cells show a false positive nuclear reaction with an accentuation of the nucleoli. Compare with Fig.4b.

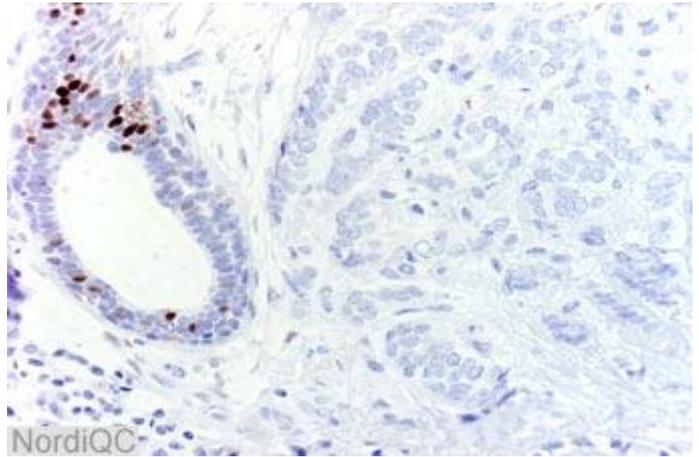


Fig. 4b
Optimal staining for PR of the PR negative ductal breast carcinoma no. 2 using the rmAb SP2 in a correctly calibrated concentration. The neoplastic cells are negative and only the normal epithelial glands show a focal nuclear reaction (left).

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