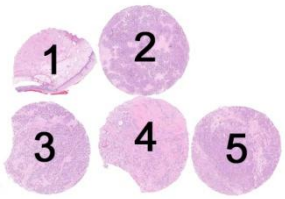
Assessment Run B9 2010

Progesterone Receptor (PR)

The slide to be stained for PR comprised the following five tissues:

No.	Tissue	PR-positivity*	PR-intensity*
1.	Uterine cervix	80-90%	Moderate to strong
2.	Breast ductal carcinoma	Negative	Negative
3.	Breast ductal carcinoma	30-50%	Weak to moderate
4.	Breast ductal carcinoma	50-70%	Moderate to strong
5.	Breast ductal carcinoma	90-100%	Strong



*PR status and staining pattern (using mAb clone PgR 636 and rmAb clone 1E2) as assessed by by two reference laboratories.

All tissues were fixed in 10% neutral buffered formalin for 24 – 48 hours according to consensus recommendations.[1]

Criteria for assessing a PR staining as optimal included:

- A moderate to strong, distinct nuclear staining of the columnar epithelial cells, the basal squamous epithelial cells and most of the stromal cells (with the exception of endothelial cells and lymphoid cells) in the uterine cervix.
- A moderate to strong, distinct nuclear staining of the ductal breast carcinomas no. 3, 4 & 5 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.

177 laboratories participated in this assessment. 77% achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for PR, run B9**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **PgR 636**	61	Dako	32	14	2	13	75%	87%
mAb clone **16**	22 1 1	Novocastra Monosan Vector	18	2	2	2	83%	86%
mAb clone **1A6**	3 1	Novocastra BioGenex	0	2	2	0	-	-
mAb clone **PgR 1294**	2	Dako	0	2	0	0	-	-
mAb clone **PR88**	2	BioGenex	0	1	0	1	-	-
mAb clone **PR-1**	2	Immunovision	0	0	0	2	-	-
mAb clone cocktail **16+SAN27**	2	Novocastra	1	0	0	1	-	-
rmAb clone **SP2**	7	NeoMarkers	2	0	0	5	40%	50%
rmAb clone **Y85**	1	Master Diagnostica	0	0	1	0	-	-
Unknown	1	Unknown	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **PgR 636, IR068**	12	Dako	11	1	0	0	100%	100%
mAb clone **PgR 636, N1630**	2	Dako	0	0	2	0	-	-

mAb clone **PgR 636, PM343**	1	Biocare	0	1	0	0	-	-
mAb clone **PgR 1294, SK310/K1904/K4071**	5	Dako	2	2	0	1	-	-
mAb clone **16, PA0312**	1	Leica	1	0	0	0	-	-
rmAb clone **1E2, 790-2223/790-4296**	47	Ventana/Cell Marque	35	8	2	2	91%	93%
rmAb clone **SP2, RM-9102-R7**	2	NeoMarkers	0	0	0	2	-	-
rmAb clone **SP2, ZA0255**	1	Unknown	0	0	0	1	-	-
Total	177		102	34	11	30	-	-
Proportion			58%	19%	6%	17%	77%	-

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 **PgR 636**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (10/21)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (17/23), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/3), Diva Decloaker pH6 (Biocare)(1/1), PTM buffer pH 6 (Thermo)(1/1) or Citrate pH 6 (2/6) as the retrieval buffer. The mAb was typically diluted in the range of 1:100– 1:600 depending on the total sensitivity of the protocol employed. Using these protocol settings 40 out of 46 (87%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **16**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (5/6), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako; 5/5), Bond Epitope Retrieval Solution 2 (Bond, Leica; 2/3), Cell Conditioning 1 (BenchMark, Ventana; 1/3), Diva Decloaker pH6 (Biocare; 1/1) or Citrate pH 6 (4/5) as the retrieval buffer. The mAb was typically diluted in the range of 1:40– 1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 19 out of 22 (86%) laboratories produced a sufficient staining.

mAb clone cocktail **16+SAN27**: The protocol giving an optimal result was based on HIER using Bond Epitope Retrieval Solution 1 (Bond, Leica) as the retrieval buffer. The mAb was diluted 1:300.

rmAb clone **SP2**: The protocols giving an optimal result were based on HIER using Tris-EDTA/EGTA pH 9 (1/3) or Bond Epitope Retrieval Solution 1 (Bond, Leica; 1/1) as the retrieval buffer. The mAb was diluted in the range of 1:50– 1:1,000 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 out of 4 laboratories produced an optimal staining.

Ready-To-Use Abs
mAb clone **PgR 636** (prod. no IR068, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 15 or 20 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings all of 12 (100%) laboratories produced a sufficient staining (optimal or good).

mAb clone **PgR 1294** (prod. no SK310/K1904/K4071, Dako): The protocols giving an optimal result were based on HIER in a Pressure Cooker using Citrate pH 6, an incubation time of 30 min in the primary Ab and EnVision (K1904/K4071) as the detection system. Using these protocol settings all of 3 laboratories produced a sufficient staining.

mAb clone **16** (prod. no. PA0312, Leica): The protocol giving an optimal result was based on HIER using Bond Epitope Retrieval Solution 2 (Bond, Leica), an incubation time of 8 min in the primary Ab and BOND Polymer Refine Detection (DS9800) as the detection system.

rmAb clone **1E2** (prod. no. 790-2223/790-4296, Ventana), The protocols giving an optimal result were based on HIER using mild or standard Cell Conditioning 1 (1 lab used Citrate pH 6 in a MWO), an incubation time of 16-32 min in the primary Ab and iView (760-091) or UltraView (760-500) as the detection system. Using these protocol

settings 41 out of 44 (93%) laboratories produced a sufficient staining (optimal or good).

The most frequent causes of insufficient stainings were:

- Too low concentration of the primary antibody
- Insufficient epitope retrieval – too short efficient HIER time
- Less successful primary antibody
- Endogenous biotin complicating the interpretation

In this assessment and in concordance with the previous PR assessments in NordiQC, the insufficient results were mainly due to a too weak or completely false negative staining. This pattern was seen in 39 out of the 41 insufficient staining results (95%). Virtually all the laboratories could demonstrate PR in the ductal breast carcinoma no. 5 with 90-100% positivity and strong nuclear staining intensity (as established by the NordiQC reference laboratories) whereas the prevalent feature of the insufficient staining was a too weak (< 10% positivity) or entirely false negative staining of the ductal breast carcinoma no. 3 (with 40-60% positivity and a weak to moderate nuclear staining intensity expected). The insufficient staining reaction was typically caused by a too low concentration of the primary Ab and/or insufficient HIER, but also when using Abs with a presumed low PR affinity. In 10 of the 39 stains with a too weak/false negative staining also a false positive staining due to endogenous biotin was seen, which especially complicated the interpretation in the ductal breast carcinoma no. 3.

In two insufficient stains a weak but distinct false positive nuclear staining in the PR negative ductal carcinoma no. 2 was seen. Both protocols were based on the rmAb clone 1E2. No single parameter causing the false positive reaction could by identified, but the combination of efficient HIER and insufficient buffer washing may be the reason for this pattern. A false positive nuclear reaction was also observed and described in the previous assessment run B6, 2008.

As also observed in previous PR assessments, the uterine cervix seems to be an appropriate control for the evaluation of the sensitivity of the PR staining: With an optimal protocol almost all the columnar epithelial cells, the basal squamous epithelial cells and most of the stromal cells must show a strong and distinct nuclear staining with only a minimal cytoplasmic reaction. Virtually all laboratories obtaining this staining pattern were assessed as sufficient. However, differences regarding the reaction pattern are seen depending on the Ab selected. When using the mAb clone 1A6, the basal squamous epithelial cells are negative and a cytoplasmic reaction is seen in the intermediate and superficial squamous epithelial cells, while the clone PgR 636 gives an intense cytoplasmic reaction in the columnar epithelial cells.
This was the fifth assessment of PR in the NordiQC breast cancer module. A relative constant proportion of sufficient results have been obtained as shown in table 2.

Table 2. **Proportion of sufficient results for PR in the five NordiQC runs performed**

	Run 10 2004	Run B2 2006	Run B4 2007	Run B6 2008	Run B9 2010
Participants, n=	79	81	95	111	177
Sufficient results	69%	75%	78%	82%	77%

The availability of several robust Abs for PR seems to be the main reason for the high pass rate. The three most commonly used clones PgR 636, 16 and 1E2 have shown a pass rate of > 80% in the last four PR assessments as shown in table 3:

Table 3. **Cumulated pass rate for PR in four runs**

	Total B2, B4, B6 & B9	
	Stains submitted	**Sufficient stains**
mAb clone 1A6	21	10 (48%)
mAb clone 16	76	61 (80%)
mAb clone PgR 636	205	163 (80%)
rmAb 1E2	104	95 (91%)
rmAb SP2	26	9 (35%)

Similar results as regards the less successful clones 1A6 and SP2 have also been puplished by UK NEQAS.[2]

Conclusion
The mAb clones PgR 636, 16 and the rmAb clone 1E2 are all well performing and recommendable Abs for PR. HIER is mandatory. A non-biotin based detection system is preferable. The concentration of the Ab must be carefully calibrated using an appropriate control such as the uterine cervix, in which almost all the columnar epithelial cells, the basal squamous epithelial cells and most of the stromal cells must show a strong and distinct nuclear staining with only a minimal cytoplasmic reaction.

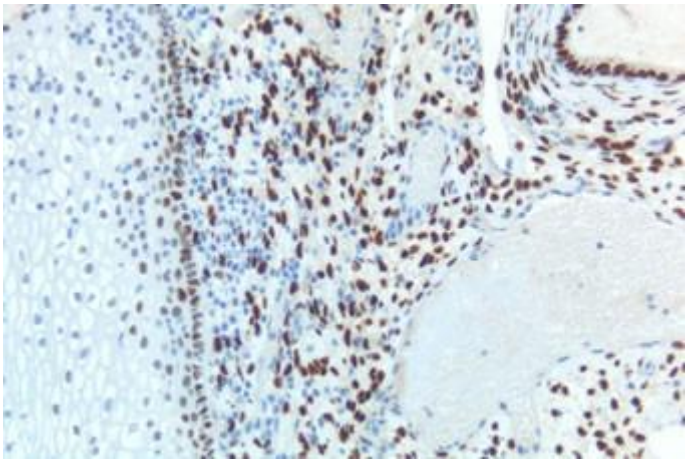


Fig. 1a
Optimal PR staining of the uterine cervix using the mAb clone 16. The columnar epithelial cells and the majority of the stromal cells show a strong nuclear staining and the basal squamous epithelial cells show a moderate to strong nuclear staining.

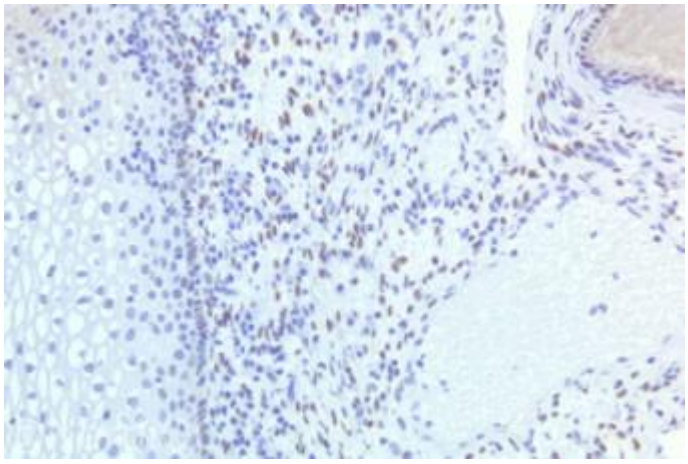


Fig. 1b
Insufficient PR staining of the uterine cervix, using the mAb clone PgR 636 with too short HIER time in Citrate pH 6 - same field as in Fig. 1a. The stromal cells show a weak to moderate nuclear staining, but the basal squamous epithelial cells are virtually negative. Also compare with Figs. 2b & 3b – same protocol.

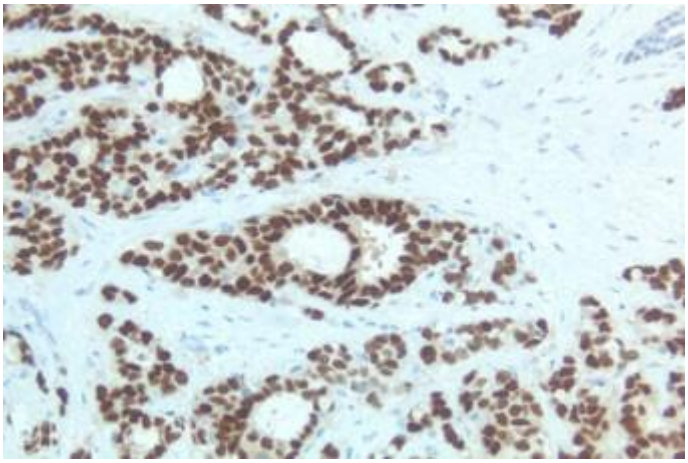


Fig. 2a
Optimal staining for PR of the breast ductal carcinoma no. 5 with 90 - 100% cells positive using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct nuclear staining. No background reaction is seen.

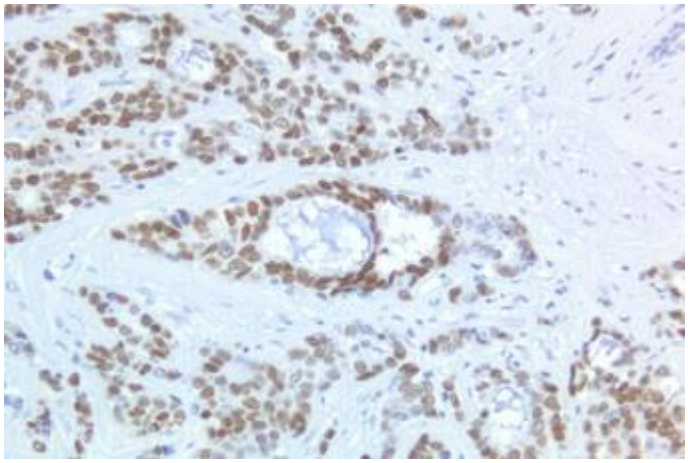


Fig. 2b
Staining for PR of the breast ductal carcinoma no. 5 with 90 - 100% cells positive using same protocol as in Fig. 1b - same field as in Fig. 2a.
The majority of the neoplastic cells show a moderate and distinct nuclear staining. However, compare with Fig. 3b - same protocol

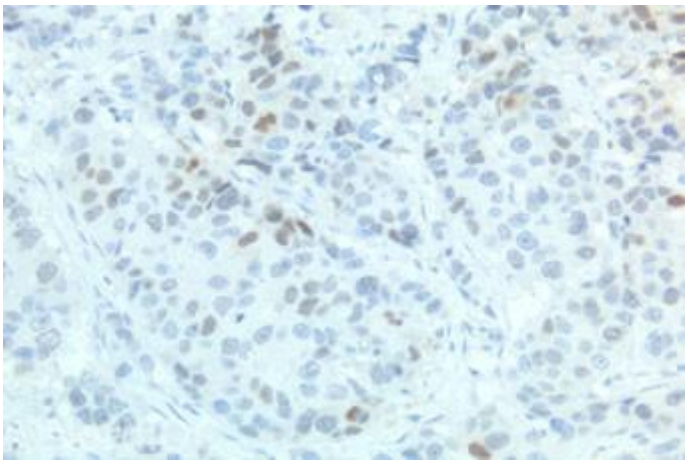


Fig. 3a
Optimal PR staining of the breast ductal carcinoma no. 3 with 30 - 50% positive cells using same protocol as in Fig. 1a & 2a. The PR positive cells are easily recognized, as no cytoplasmic or background reaction is seen.

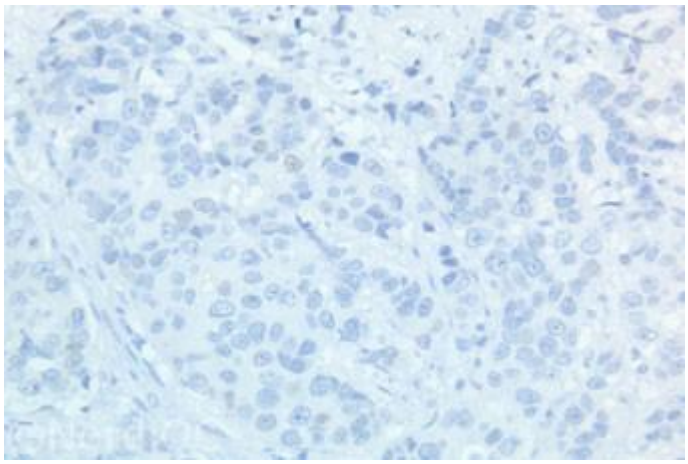


Fig. 3b
Insufficient PR staining of the breast ductal carcinoma no. 3 with 30 - 50% positive cells using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a.
Virtually no nuclear staining reaction is seen.

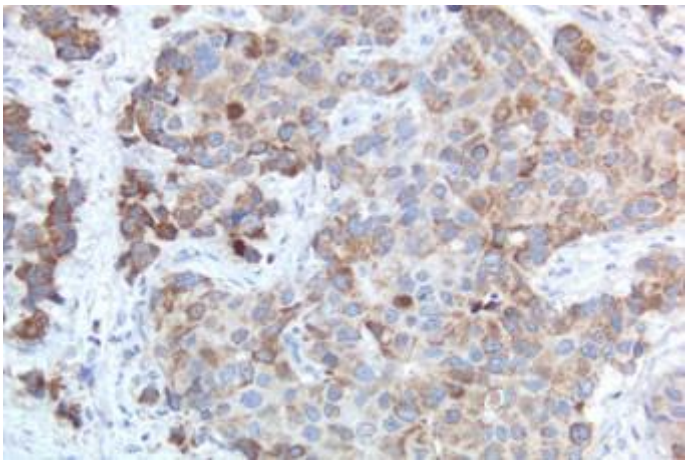


Fig. 4a
Insufficient PR staining of the breast ductal carcinoma no. 3 (with 30 - 50% positive cells) using the mAb clone 16 too diluted and with HIER in an alkaline buffer and a biotin based detection system. A strong cytoplasmic staining due to endogenous biotin complicates the interpretation of a weak specific nuclear staining in scattered cells.

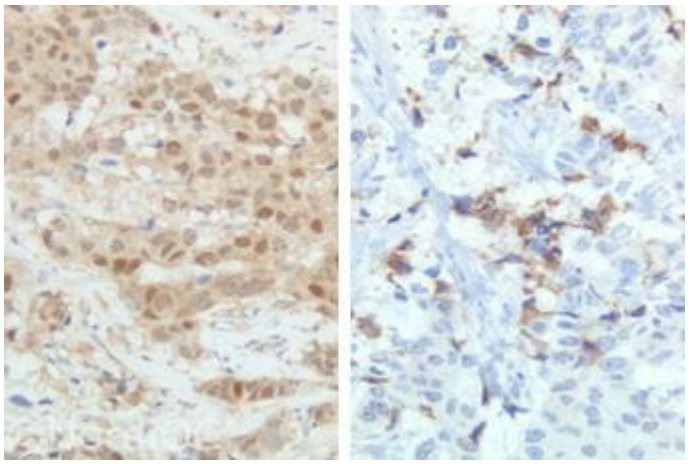


Fig. 4b
Insufficient PR staining of the breast ductal carcinoma no. 3 with 30 - 50% positive cells.
Left: The mAb clone 1A6 used too concentrated giving an excessive cytoplasmic staining and high level of background reaction complicating the interpretation.
Right: The mAb clone PR-1 used with HIER in an alkaline buffer and a 3-step labelled polymer system. Despite a highly sensitive protocol was applied, no nuclear staining reaction is seen, only an aberrant cytoplasmic reaction is seen in the stromal cells.

References

1. Yaziji H, Taylor CR, Goldstein NS, Dabbs DJ, Hammond EH, Hewlett B, Floyd AD, Barry TS, Martin AW, Badve S, Baehner F, Cartun RW, Eisen RN, Swanson PE, Hewitt SM, Vyberg M, Hicks DG; Members of the Standardization Ad-Hoc Consensus Committee. Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry. Appl Immunohistochem Mol Morphol. 2008 Dec;16(6):513-20. PubMed PMID: 18931614.

2. Ibrahim M, Dodson A, Barnett S, Fish D, Jasani B, Miller K. Potential for false-positive staining with a rabbit monoclonal antibody to progesterone receptor (SP2): findings of the UK National External Quality Assessment Scheme for Immunocytochemistry and FISH highlight the need for correct validation of antibodies on introduction to the laboratory. Am J Clin Pathol. 2008 Mar;129(3):398-409. PubMed PMID: 18285262.

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