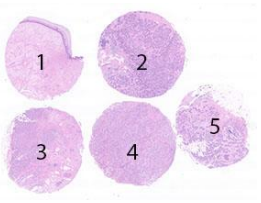
Assessment Run B18 2014

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 carcinoma	Negative	Negative
3.	Breast carcinoma	40 - 60%	Weak to strong
4.	Breast carcinoma	60 - 80%	Weak to strong
5.	Breast carcinoma	80 - 100%	Moderate to strong



*PR-positivity and intensity as characterized by NordiQC reference laboratories using the mAb clone 16 (Leica/Novocastra) and the rmAb clone 1E2 (Ventana).

All tissues were fixed in 10% neutral buffered formalin for 24 – 48 hours and processed according to Yaziji et al. (1).

Criteria for assessing PR staining as **optimal** were:

- A moderate to strong, distinct nuclear staining reaction of both columnar and basal squamous epithelial cells and most of the stromal cells (with the exception of endothelial and lymphoid cells) in the uterine cervix.
- An at least weak to moderate distinct nuclear staining reaction in the appropriate proportion of neoplastic cells in the breast ductal carcinomas no. 3, 4 & 5.
- No nuclear staining reaction of neoplastic cells in the breast carcinoma no. 2
- Not more than a weak cytoplasmic staining reaction in cells with strong nuclear staining reaction - for the mAb clone PgR636, moderate to strong cytoplasmic staining reaction in columnar epithelial cells of the uterine cervix was accepted.

The staining reactions were classified as **good** if ≥ 10% of the neoplastic cells in the breast carcinomas no. 3, 4 and 5 showed an at least weak nuclear staining reaction but less than the range of the reference laboratories.

The staining reactions were classified as **borderline** if ≥ 1% and < 10% of the neoplastic cells showed a nuclear staining reaction in one or more of the breast carcinomas no. 3, 4 & 5.

The staining reactions were classified as **poor** if a false negative or false positive staining reaction was seen in one of the breast carcinomas.

Participation

Number of laboratories registered for PR, run B18	353
Number of laboratories returning slides	338 (96%)

Results

338 laboratories participated in this assessment. 92% achieved a sufficient mark. Table 1 summarizes antibodies (Abs) used and assessment marks.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary Ab
- Insufficient HIER – too short efficient heating time
- Protocols giving a false positive staining reaction (no single reason was identified)

Conclusion

The mAb clones **16**, **PgR 636** and the rmAb clone **1E2** all performed well and are recommendable for demonstration of PR. In general, Ready-To-Use systems for these three Abs showed a slightly superior pass rate compared to in-house developed assays. Efficient HIER (preferably in an alkaline buffer) is mandatory for optimal performance. Uterine cervix is an appropriate positive tissue control - almost all columnar epithelial cells, basal squamous epithelial cells and most of the stromal cells must show a strong and distinct nuclear staining reaction with only a minimal cytoplasmic staining.

Both in this and previous runs for PR, rmAb Abs clones 1E2 and SP2 provided false positive results. For this reason, it is highly advisable to include PR negative breast tumours in the validation process of the PR assay and meticulously monitor the PR expression results and metrics produced in the laboratory.

Table 1. **Antibodies and assessment marks for PR, run B18**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **16**	42 1	Leica/Novocastra Vector	31	8	4	0	91%	91%
mAb clone cocktail **16 + SAN27**	2	Leica/Novocastra	0	2	0	0	-	-
mAb clone **PgR 636**	49	Dako	39	6	4	0	90%	96%
mAb clone **PgR 1294**	5	Dako	5	0	0	0	100%	100%
rmAb **EP2**	1 1	Epitomics BioGenex	2	0	0	0	-	-
rmAb **SP2**	5	Thermo/NeoMarkers	1	1	2	1	40%	-
rmAb **SP42**	1 1	Cell Marque Zytomed	1	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **16 PA0312**	5	Leica/Novocastra	4	1	0	0	100%	100%
mAb clone **16 RTU-PGR-312**	1	Novocastra	0	1	0	0	-	-
mAb clone cocktail **16 + SAN27 RTU-PGR**	1	Novocastra	1	0	0	0	-	-
mAb clone **Pgr 636 IS/IR068**	62	Dako	51	9	2	0	97%	98%
mAb clone **PgR 1294 K4071/SK310**	1	Dako	1	0	0	0	-	-
mAb clone **PR88 AM328-5ME**	1	BioGenex	1	0	0	0	-	-
rmAb clone **1E2 790-2223/4296**	156	Ventana	125	21	6	4	94%	95%
rmAb clone **EP2 RMPD 052**	1	Diagnostic Biosystems	0	0	1	0	-	-
rmAb clone **SP42 RBK020-05**	1	Zytomed	0	1	0	0	-	-
rmAb clone **Y85 RM-2114-RQ**	1	Thermo/NeoMarkers	0	0	0	1	-	-
Total	338		262	50	19	7	-	
Proportion			78%	14%	6%	2%	92%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below

Detailed analysis of PR, run B18
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **16**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using either Bond Epitope Retrieval Solution 2 (BERS 2; Leica) (12/13)*, BERS 1 (Leica) (5/5), Target Retrieval Solution (TRS) pH 9 (Dako) (5/6), Cell Conditioning 1 (CC1; Ventana) (4/12), Citrate pH 6 (3/3), Tris-EDTA/EGTA pH 9 (1/3) or TRS Low pH (Dako) (1/1) as retrieval buffer.
The mAb was typically diluted in the range of 1:50-1:800. Using these protocol settings 38 of 42 (91%) laboratories produced an optimal staining result.
* (number of optimal results/number of laboratories using this reagent)

mAb clone **PgR 636:** Protocols with optimal results were all based on HIER using either TRS pH 9 (Dako) (20/22), BERS 2 (Bond, Leica) (8/8), Citrate pH 6 (6/8) or Tris-EDTA/EGTA pH 9 (5/5) as retrieval buffer. The mAb was typically diluted in the range of 1:100-1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 43 of 45 (96%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **PgR 1294**: Protocols with optimal results were based on HIER using TRS pH 9 (Dako) (2/2), Tris-EDTA/EGTA pH 9 (1/1), CC1 (Ventana) (1/1) or Citrate pH 6 (1/1) as retrieval buffer.
The mAb was diluted in the range of 1:50-1:6,000 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 of 5 laboratories produced an optimal staining result.

rmAb clone **EP2:** Protocols with optimal results were based on HIER using either Tris/EDTA/EGTA pH9 or Citrate pH 6 as retrieval buffer. The rmAb was diluted in the range of 1:50-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 of 2 laboratories produced an optimal staining result.

rmAb clone **SP2**: One protocol with optimal result was based on HIER using Citrate pH 6 (1/1) as retrieval buffer. The rmAb was diluted 1:100 and a 2-step polymer based detection system was used.

rmAb clone **SP42**: One protocol with optimal result was based on HIER using Citrate pH 6 (1/1) as retrieval buffer. The rmAb was diluted 1:200 and a 3-step polymer based detection system was used.

Ready-To-Use antibodies and corresponding systems
mAb clone **16** (prod. no. PA0312, Leica/Novocastra): Protocols with optimal results were based on HIER using BERS 2 (Bond, Leica), 15-20 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 4 of 4 (100%) laboratories produced an optimal staining result (optimal or good).

mAb clone **PgR 636** (prod. no. IR/IS068, Dako): Protocols with an optimal result were typically based on HIER in PT-Link (heating time for 10-20 min. at 95 - 98°C) using TRS pH 9 (3-in-1) (Dako) or TRS pH 9 (Dako) as HIER buffer, 15-30 min. incubation of the primary Ab and EnVision Flex or EnVision Flex+ (K8000/K8002) as detection system. Using these protocol settings 57 of 58 (98%) laboratories produced a sufficient staining result.

mAb clone **PgR 1294** (prod. no SK310/K4071, Dako): One protocol with an optimal result was based on HIER in a Pressure Cooker using Citrate pH 6, 30 min. incubation of the primary Ab and EnVision (SK310/K4071) as detection system.

rmAb clone **1E2** (prod.no 790-2223/4296, Ventana): Protocols with optimal results were typically based on HIER using mild or standard CC1, 8-32 min. incubation of the primary Ab and iView (760-91, Ventana), UltraView (760-500, Ventana) or OptiView (760-700, Ventana) as detection system. Using these protocol settings 107 out of 113 (95%) laboratories produced a sufficient staining result.

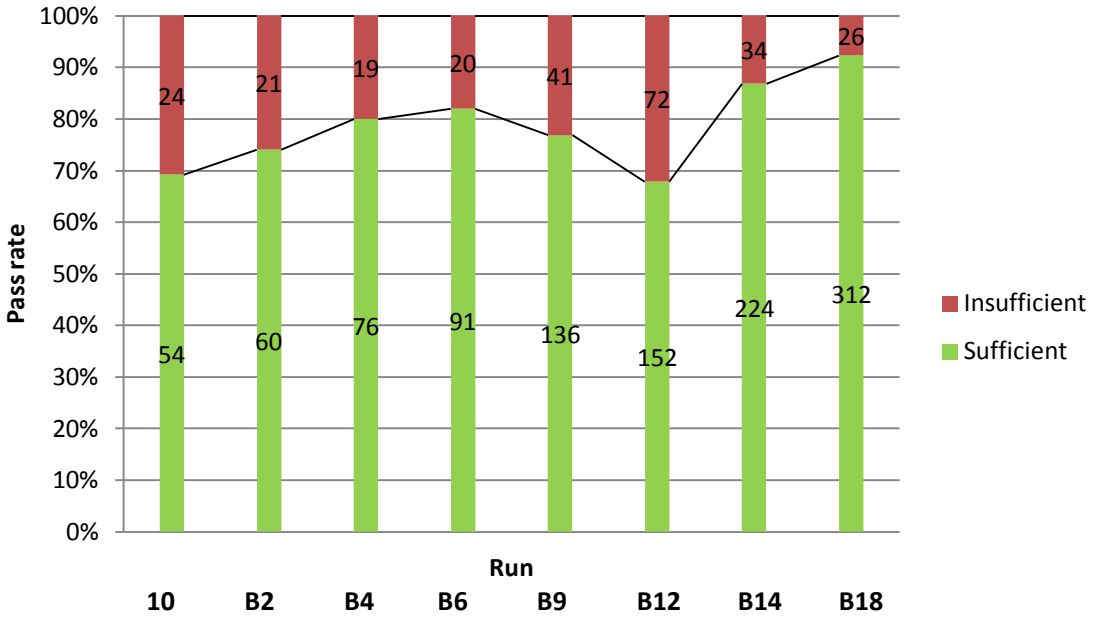
Comments
In this assessment, the prominent features of insufficient staining results were either a generally too weak staining reaction or a false positive staining reaction. Too weak staining reaction was seen in 58% of the insufficient staining results (15 of 26). Virtually all laboratories could demonstrate PR in the ductal breast carcinoma no. 5 with 80-100% positivity and a strong nuclear expression (as established by the NordiQC reference laboratories) whereas the ductal breast carcinoma no. 3 and 4, with 40-80% positivity and a weak to moderate nuclear staining intensity, was more challenging The insufficient staining reactions were typically caused by a too low concentration of the primary Ab and/or insufficient HIER applying too short efficient HIER time. Also, combination of too weak staining and impaired morphology was seen. This pattern was typically seen by use of excessive HIER in combination with a protocol giving a too low sensitivity.
In the remaining insufficient results (42%), a false positive staining reaction in the PR negative ductal carcinoma no. 2 was seen. The false positive result was mainly seen for the Ready-To-Use (RTU) rmAb clone 1E2, Ventana (n=10) but also for the rmAb clone SP2, Thermo/NeoMarkers (n=1), whereas all other Abs and RTU systems consistently gave a negative staining result in this tumour. For the Ventana RTU system it was observed, that all participants that failed this assessment due to false positive staining result used a protocol with reduced HIER time (mild, CC1) and/or prolonged incubation time of the primary antibody. Protocols from package insert suggest HIER in CC1 standard and 16 min. incubation of primary Ab. Hence, it has to be emphasized that package insert protocols must be followed strictly when using the rmAb clone 1E2 applied as RTU (Ventana).

The mAb clones 16 and PgR 636 (Leica/Novocastra and Dako, respectively) gave very high pass rates both as concentrate and as RTU system and provided the expected PR positivity in all the included tissues.
The Leica RTU system provided optimal staining results when the RTU system was used according to the recommendations given in the package insert. Modification, by using HIER in ER1, reduced sensitivity.
Optimal results for the Dako RTU format of the mAb clone PgR 636 were typically obtained by using the official protocol recommendations, but also laboratory modified protocol settings (typically adjusting HIER, incubation time of the primary Ab and/or choice of detection system) could provide sufficient and optimal results.
Intracytoplasmic staining reaction in the columnar epithelial cells of the uterine cervix was accepted for the Dako RTU system based on the mAb clone PgR 636, as this did not complicate interpretation in the breast carcinomas.

Controls
As observed in the previous NordiQC assessments of PR, uterine cervix is an appropriate positive control for evaluation of the sensitivity of PR staining: With an optimal protocol almost all columnar epithelial cells, the majority of basal squamous epithelial cells and most of the stromal cells must show a strong and distinct nuclear staining with only a minimal cytoplasmic reaction. No staining must be seen in endothelial cells and lymphocytes. However, it must be taken into consideration that the PR expression level is reduced in the uterine cervix of post-menopausal women and especially demonstration of PR in squamous epithelial cells can be compromised.Tonsillar tissue is recommendable as negative control, in which no nuclear staining should be seen.

Effect of external assessment
This was the 8th NordiQC assessment of PR. An increase in the proportion of sufficient results was seen compared to the previous runs, as shown in Figure 1:

Figure 1 – pass rate in the NordiQC assessments for PR



The improvement was accomplished despite that many laboratories participated for the first time. The improvement is most likely influenced by many factors, inclusive access to and extended use of optimized antibodies both as RTU systems and as concentrates. In this run, use of one of the RTU systems (from Dako, Leica and Ventana) provided a slightly higher pass rate compared to laboratory developed assays. The three RTU systems grouped together had a pass rate of 95% (211 of 223 laboratories) compared to 89% (101 of 115 laboratories) for laboratory developed assays for PR.

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.

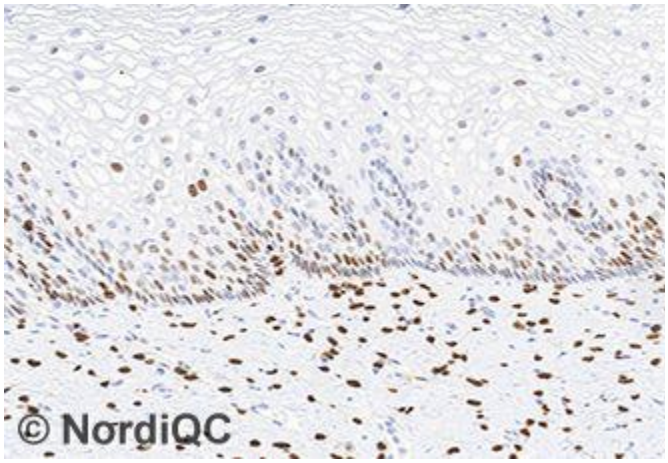


Fig. 1a
Optimal staining for PR of the uterine cervix using the mAb clone 16 optimally calibrated and with efficient HIER in an alkaline buffer. The vast majority of basal squamous epithelial cells show a weak to moderate nuclear staining reaction, whereas the majority of the stromal cells show a moderate to strong nuclear staining reaction.

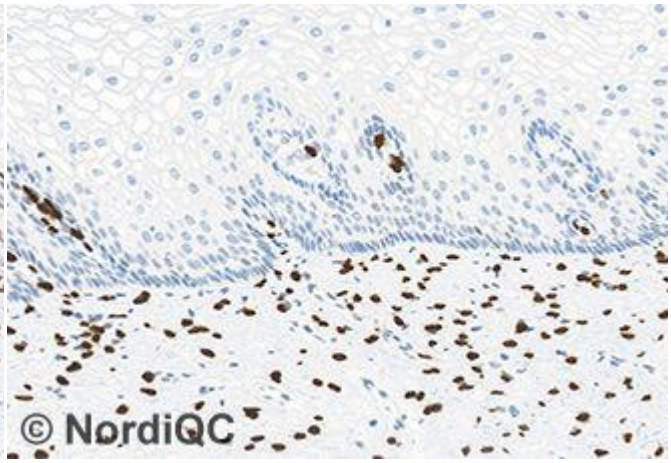


Fig. 1b
Insufficient staining for PR of the uterine cervix, using the mAb clone 16 with protocol settings giving a too low sensitivity - same field as in Fig. 1a. The stromal cells are demonstrated, but the squamous epithelial cells are virtually negative. Also compare with Fig. 2b - 3b – same protocol.

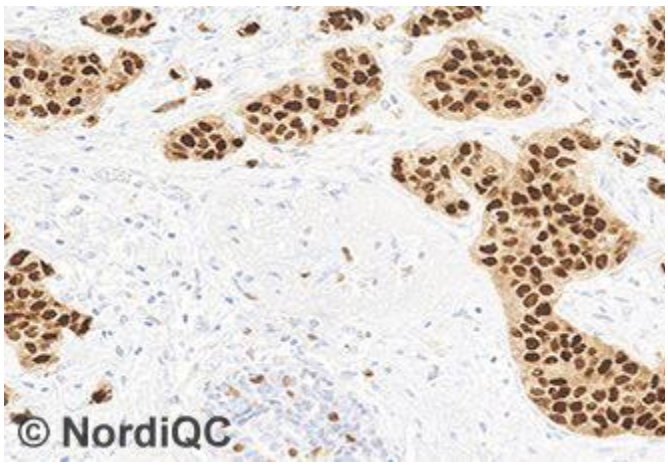


Fig. 2a
Optimal staining for PR of the breast carcinoma no. 5 with 80 - 100% cells positive using same protocol as in Fig. 1a. A strong nuclear staining reaction is seen. A weak cytoplasmic staining reaction in the neoplastic cells is seen, but no background staining.

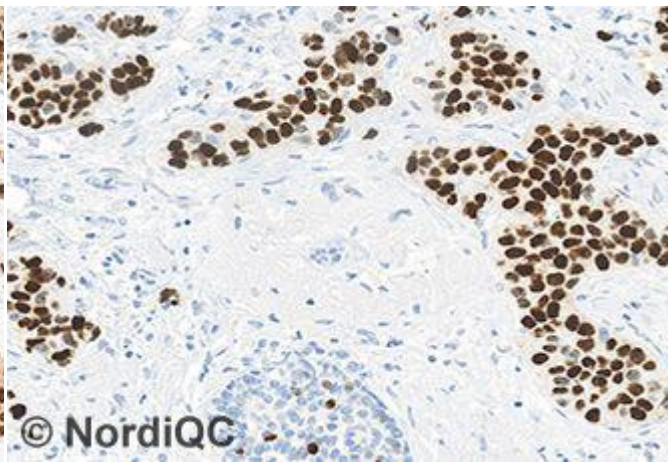


Fig. 2b
Staining for PR of the breast carcinoma no. 5 with 80 - 100% cells positive using same protocol as in Fig. 1b - same field as in Fig. 2a.
A strong and distinct nuclear staining reaction in virtually all the neoplastic cells is seen. However also compare with Fig. 3b – same protocol.

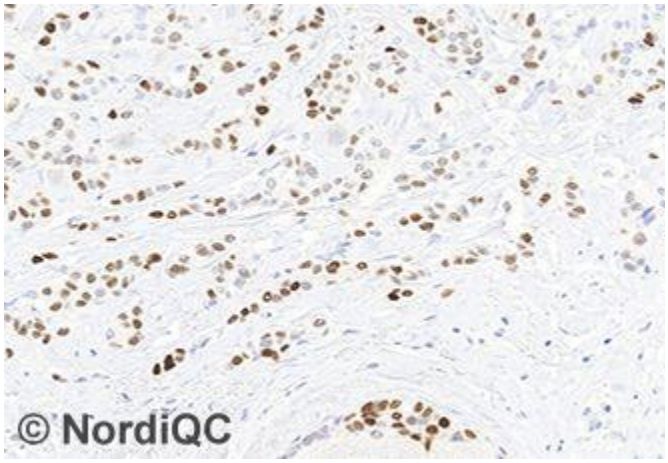


Fig. 3a
Optimal staining for PR of the breast carcinoma no. 3 with 40 - 60 % cells positive using same protocol as in Figs. 1a - 2a. The PR positive cells are easily recognized and the appropriate proportion of cells is demonstrated.

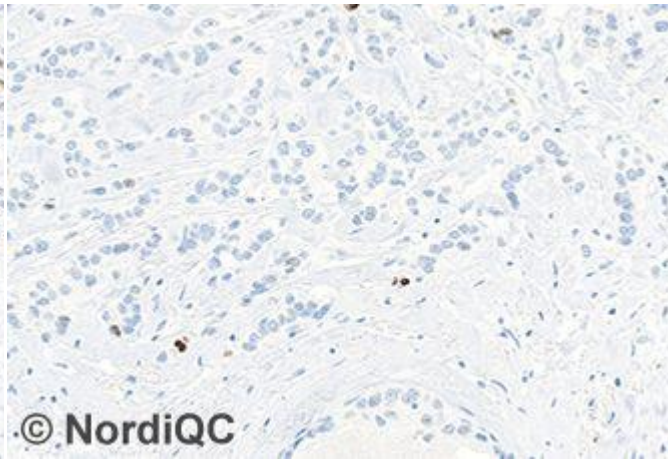


Fig. 3b
Insufficient staining for PR of the the breast carcinoma no. 3 with 40 - 60 % cells positive using same protocol as in Figs. 1b – 2b – same field as in Fig. 3a. Only dispersed cells are demonstrated and a significant reduced proportion of cells are identified compared to the level expected.

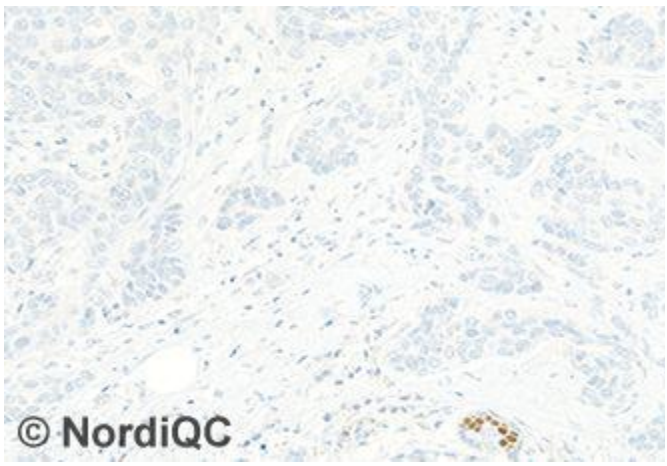


Fig. 4a
Optimal staining for PR of the breast carcinoma no. 2 expected to be negative using same protocol as in Figs. 1a - 3a. No nuclear staining reaction in the neoplastic cells is seen. The PR status was tested and confirmed by different Abs and protocol settings in the NordiQC reference laboratories. The tumour was also ER negative.

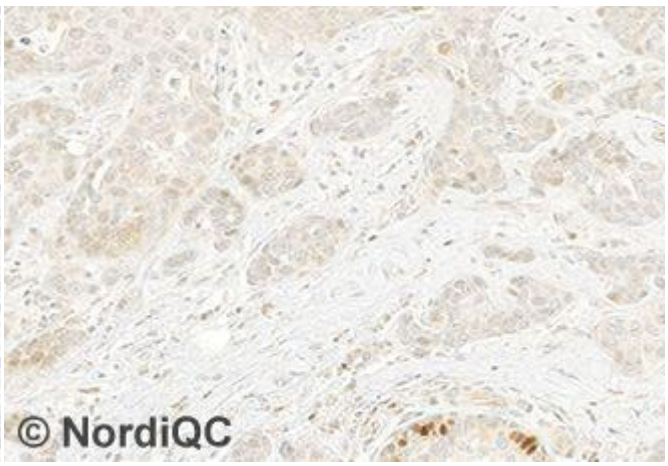


Fig. 4b
Insufficient staining for PR of the breast ductal carcinoma no. 2 expected to be negative – same field as in Fig. 4a. The majority of the neoplastic cells show a weak to moderate and aberrant false positive nuclear staining reaction. The protocol was based on the rmAb clone 1E2, Ventana using HIER in CC1 for 32 min. and an incubation time of 32 min. of the primary Ab.

SN/RR/LE 01-12-2014