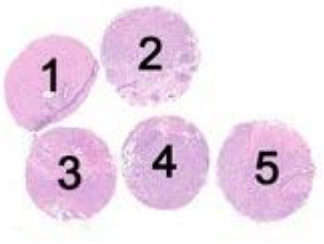
Assessment Run B14 2012

Progesterone Receptor (PR)

Material

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	0 %	Negative
3.	Breast ductal carcinoma	40 - 60 %	Weak to moderate
4.	Breast ductal carcinoma	30 - 50 %	Weak to moderate
5.	Breast ductal 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 a PR staining as optimal were:

- A moderate to strong, distinct nuclear staining reaction of both the columnar and basal squamous epithelial cells and most of the stromal cells (with the exception of endothelial cells and lymphoid cells) in the uterine cervix.
- An at least weak to moderate distinct nuclear staining reaction in the appropriate proportion of the neoplastic cells in the breast ductal carcinomas no. 3, 4 & 5.
- No nuclear staining reaction of the neoplastic cells in the breast carcinoma no. 2 and no more than a weak cytoplasmic staining reaction in cells with a strong nuclear staining reaction - for the mAb clone PgR636 a moderate to strong cytoplasmic staining reaction in the columnar epithelial cells of the uterine cervix was accepted.

258 laboratories participated in this assessment. 87 % achieved a sufficient mark. Table 1 summarizes antibodies (Abs) used and marks.

Table 1. **Abs and assessment marks for PR, run B14**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **16**	25 1 1	Leica/Novocastra Biocare Vector	21	6	0	0	100 %	100 %
mAb clone cocktail **16 + SAN27**	3	Leica/Novocastra	1	2	0	0	-	-
mAb clone **1A6**	1 1	DBS Leica/Novocastra	1	0	1	0	-	-
mAb clone **PgR 636**	48	Dako	37	10	1	0	98 %	100 %
mAb clone **PgR 1294**	3	Dako	2	1	0	0	-	-
rmAb **EP2**	1	Epitomics	1	0	0	0	-	-
rmAb **SP2**	3	Thermo/NeoMarkers	2	1	0	0	-	-
rmAb **SP42**	1	Zytomed	0	1	0	0	-	-
rmAb **Y85**	3	Cell Marque	2	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **16 PA0312**	5	Leica/Novocastra	5	0	0	0	100 %	100 %
mAb clone **16 RTU-PGR-312**	1	Leica/Novocastra	1	0	0	0	-	-

mAb clone **1A6 RTU-PGR**	1	Leica/Novocastra	1	0	0	0	-	-
mAb clone **Pgr 636 IS/IR068**	37	Dako	29	5	1	2	92 %	97 %
mAb clone **PgR 1294 K4071/SK310**	4	Dako	3	1	0	0	-	-
mAb clone **PR88 MU328/AX328-YCD**	2	BioGenex	0	1	1	0	-	-
rmAb clone **1E2 790-2223/4296**	115	Ventana	76	12	4	23	77 %	77 %
rmAb clone **SP42 323R-37**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **Y85 MAD-000302QC**	1	Master Diagnostica	0	0	1	0	-	-
Total	258		183	41	9	25	-	
Proportion			71 %	16 %	3 %	10 %	87 %	

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

Following protocol parameters were central to obtain an optimal staining result:

Concentrated antibodies
mAb clone **16**: Protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (5/5)*, TRS pH 9 (Dako) (2/2), TRS low pH 6.1 (Dako) (1/1), Cell Conditioning 1 (CC1; Ventana) (2/2), Bond Epitope Retrieval Solution 2 (BERS 2; Leica) (5/6), BERS 1 (Leica) (2/3), Tris-EDTA/EGTA pH 9 (1/3) or Citrate pH 6 (3/4) as the retrieval buffer.
The mAb was typically diluted in the range of 1:75-1:800. Using these protocol settings 25 of 25 (100 %) laboratories produced an optimal staining result.
* (number of optimal results/number of laboratories using this reagent)

mAb clone cocktail **16 + SAN27**: The protocol giving an optimal result was based on HIER using CC1 (Ventana) (1/1) as the retrieval buffer. The dilution was 1:200.

mAb clone**1A6**: The protocol giving an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (1/1) as the retrieval buffer. The dilution of the primary Ab was 1:50.

mAb clone **PgR 636**: Protocols giving an optimal result were all based on HIER using either TRS pH 9 (3-in-1) (Dako) (12/12), TRS pH 9 (Dako) (7/10), TRS low pH 6.1 (Dako) (1/1), CC1 (Ventana) (1/2), BERS 2 (Leica) (1/3), BERS 1 (Leica) (1/1), Tris-EDTA/EGTA pH 9 (10/13) or Citrate pH 6 (4/5) as the 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 45 of 45 (100 %) laboratories produced a sufficient staining result (optimal or good).

mAb clone **PgR 1294**: Protocols giving an optimal result were based on HIER using Tris-EDTA/EGTA pH 9 (1/1) or Citrate pH 6 (1/1) as the retrieval buffer.
The mAb was diluted in the range of 1:1.250-1:5.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 of 2 (100 %) laboratories produced an optimal staining.

rmAb clone **EP2**: The protocol giving an optimal result was based on HIER using Citrate pH 6 as the retrieval buffer. The rmAb was diluted 1:100.

rmAb clone **SP2**: Protocols giving an optimal result were based on HIER using either CC1 (Ventana) (1/1) or Citrate pH 6 (1/1) as the retrieval buffer.
The rmAb was diluted in the range of 1:100-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 of 2 (100 %) laboratories produced an optimal staining.

rmAb clone **Y85**: Protocols giving an optimal result were based on HIER using Citrate pH 6 (2/3) as the retrieval buffer. The rmAb was diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 of 3 (100 %) laboratories produced a sufficient staining result (optimal or good).

Ready-To-Use antibodies
mAb clone **16** (prod. no. PA0312, Leica/Novocastra): Protocols giving an optimal result were based on HIER using BERS 2 (Leica), 15 min incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 5 of 5 (100 %) laboratories produced an optimal staining result (optimal or good).

mAb clone **PgR 636** (prod. no. IR/IS068, Dako): Protocols giving 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 the detection system. Using these protocol settings 32 of 33 (97 %) laboratories produced a sufficient staining result.

mAb clone **PgR 1294** (prod. no SK310/K4071, Dako): Protocols giving an optimal result were based on HIER in a Pressure Cooker using Citrate pH 6, 20-30 min incubation of the primary Ab and EnVision (SK310/K4071) as detection system. Using these protocol settings 3 of 3 (100 %) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **1E2** (prod.no 790-2223/4296, Ventana): Protocols giving an optimal result were typically based on HIER using mild or standard CC1 8-60 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 88 out of 114 (77 %) laboratories produced a sufficient staining result.

The most frequent causes of insufficient stains were:

- Less successful primary antibody
- Low sensitivity of the staining protocol
- False positive staining reaction with rmAb clone 1E2 applied as RTU (Ventana) with prolonged incubation.

In this assessment the prominent feature of an insufficient staining was characterized by a false positive staining reaction in > 10 % of the neoplastic cells of the breast carcinoma no. 2, which by the reference laboratories and the vast majority of the participating laboratories was classified as being PR negative. A false positive result was seen in 24 of the 34 insufficient staining results (69 %) and in the remaining insufficient results a false negative or a general too weak staining reaction was seen.

The false positive staining was only seen when the rmAb clone 1E2 applied as RTU (Ventana) was used, whereas as all other Abs and RTU systems consistently gave a negative staining result in this tumour. It might be considered if the breast carcinoma no. 2 expressed low levels of PR and that the positive staining was revealed by using a very sensitive protocol. However, as the majority of protocols based on clone 1E2 and all other Abs (with equal or stronger staining in the remaining cores), gave a completely negative staining in breast carcinoma no. 2, the positive staining with clone 1E2 as RTU (Ventana) was evaluated as false positive. NordiQC has subsequently been in contact with Ventana. According to their investigation and data reported to NordiQC for the observation, there is no evidence to show that there are any quality concerns with a specific PR (1E2) antibody lot, detection, or any other assay components. The vast majority of the participants that failed this assessment due to false positive staining used a protocol with prolonged incubation time in the primary antibody as compared to the package insert protocol. Hence, it is emphasized that using clone 1E2 applied as RTU (Ventana), the package insert protocol must be followed strictly.

The remaining insufficient staining results were primarily caused by a too low sensitivity of the protocol, e.g. too low concentration of the primary Ab or a protocol based on a RTU format applied to a staining system/platform for which the product was not calibrated.

The mAb clones 16 and PgR 636 from Leica/Novocastra and Dako respectively gave a very high pass rate both as a concentrate or as a Ready-To-Use system and provided the expected PR positivity in all the included tissues. For the mAb clone PgR 636 an intracytoplasmic staining reaction in the columnar epithelial cells of the uterine cervix was accepted, as this did not complicate the interpretation in the breast carcinomas.

Controls

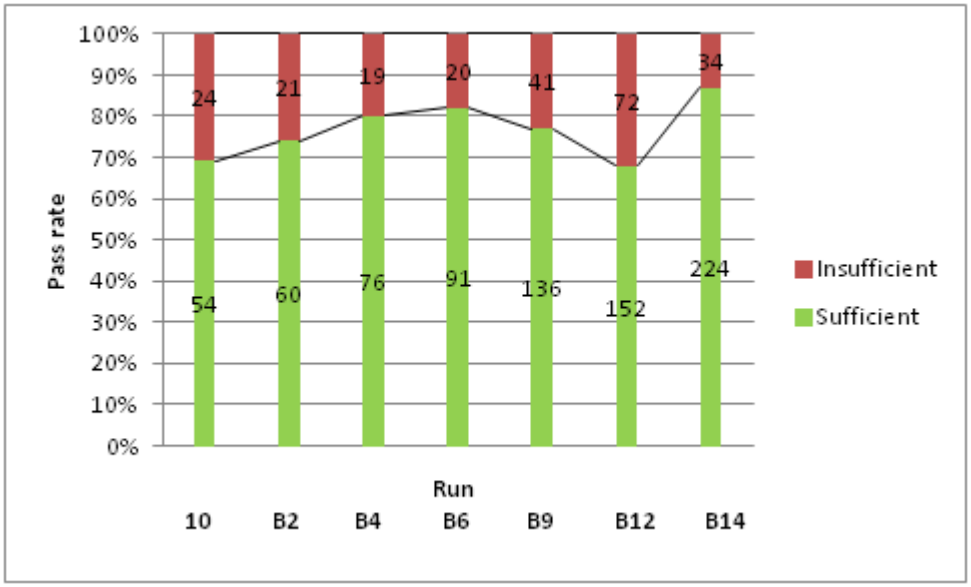
As observed in the previous assessments of PR, uterine cervix is an appropriate positive control for evaluation of the sensitivity of PR staining: With an optimal protocol almost all columnar epithelial cells,

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. As negative control it is recommendable to include tonsillar tissue, in which no nuclear staining should be seen.

Effect of external assessment

This was the 7th assessment of PR in NordiQC. An increase in the proportion of sufficient results was seen compared to the two latest runs for PR as shown in figure 1.

Figure 1 – pass rate in the NordiQC assessments for PR



The improvement has been obtained despite many laboratories participated for the first time and the improvement most likely is influenced by many factors inclusive the access to and extended use of optimized antibodies both as Ready-To-Use systems and as concentrates for the demonstration of 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.

Conclusion

The mAb clones 16 and PgR 636 were in this assessment the most robust Abs for PR. Both had a high pass rate and could be applied either as concentrate or as a Ready-To-Use system.

In this assessment a false positive staining reaction of the rmAb clone 1E2 was the prominent feature of an insufficient staining result. Uterine cervix is an appropriate positive control. Most of the columnar epithelial cells, basal squamous epithelial cells and most of the stromal cells must show a strong and distinct nuclear staining reaction. Lymphocytes and endothelial cells must be negative.

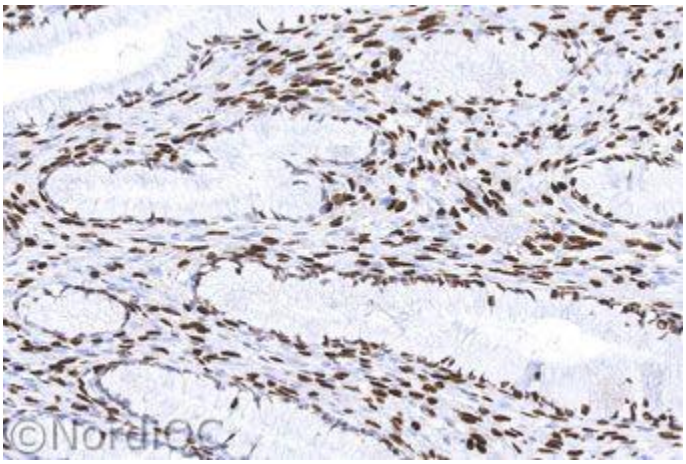


Fig. 1a
Optimal staining for PR of the uterine cervix using the mAb clone 16 optimally calibrated and with efficient HIER in an alkaline buffer. Virtually all the columnar epithelial cells and the majority of the stromal cells show a moderate to strong nuclear staining reaction.

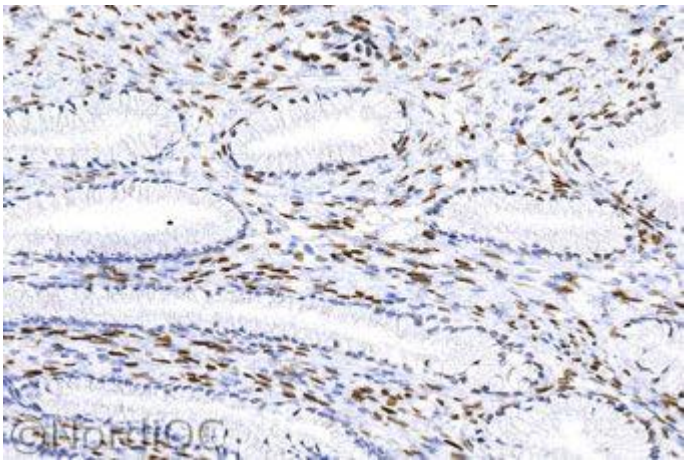


Fig. 1b
Insufficient staining for PR of the uterine cervix, using the mAb clone PR88 with protocol settings giving a too low sensitivity - same field as in Fig. 1a. The stromal cells are demonstrated, but the columnar epithelial cells are virtually negative. Also compare with Fig. 2b – same protocol.

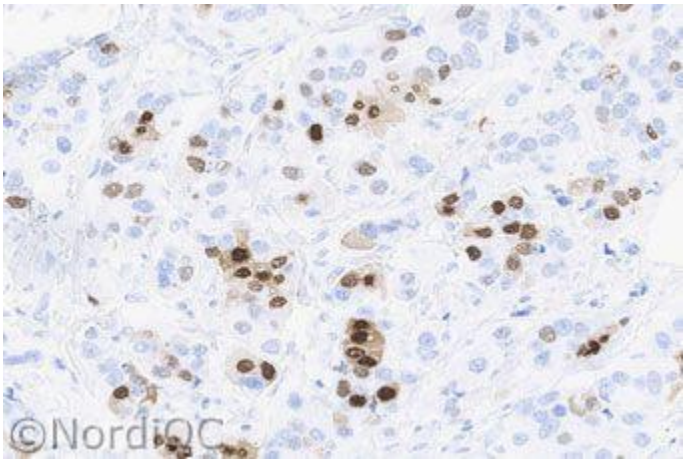


Fig. 2a
Optimal staining for PR of the breast ductal carcinoma no. 3 with 10 - 30 % cells positive using same protocol as in Fig. 1a. The PR positive cells are easily recognized and no background reaction is seen.

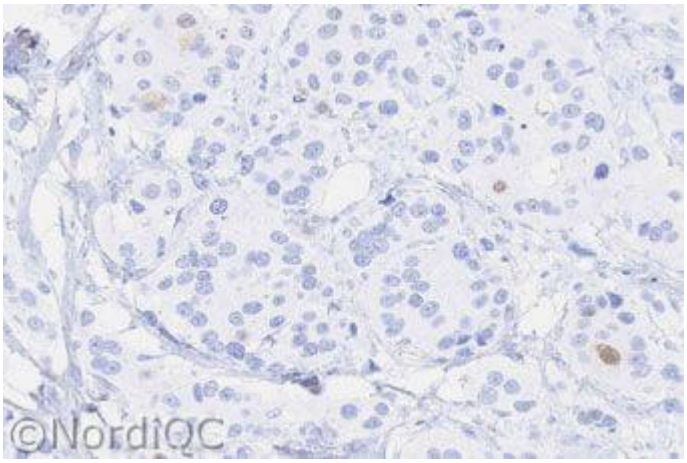


Fig. 2b
Insufficient staining for PR of the breast ductal carcinoma no. 3 with 10 - 30 % cells positive using same protocol as in Fig. 1b. - same field as in Fig. 2a.
Virtually no nuclear staining reaction is seen.

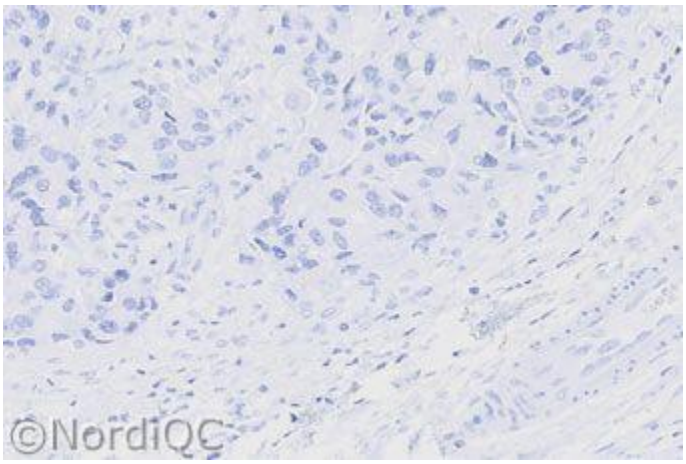


Fig. 3a
Optimal staining of the PR negative breast ductal carcinoma no. 2 using same protocol as in Figs. 1a - 2a.
No nuclear staining reaction is seen in the neoplastic cells. The PR positivity was tested and confirmed by different Abs and protocol settings in the NordiQC reference laboratories.

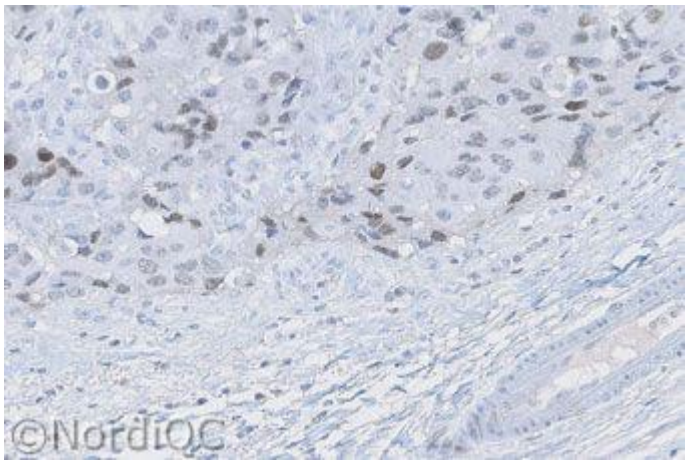


Fig. 3b
Insufficient staining of the PR negative breast ductal carcinoma no. 2 using the rmAb clone 1E2.
The majority of the neoplastic cells show a weak to moderate and aberrant false positive nuclear staining reaction. No single parameter could be identified to give this staining pattern.
Also compare with Fig. 4b – same protocol.

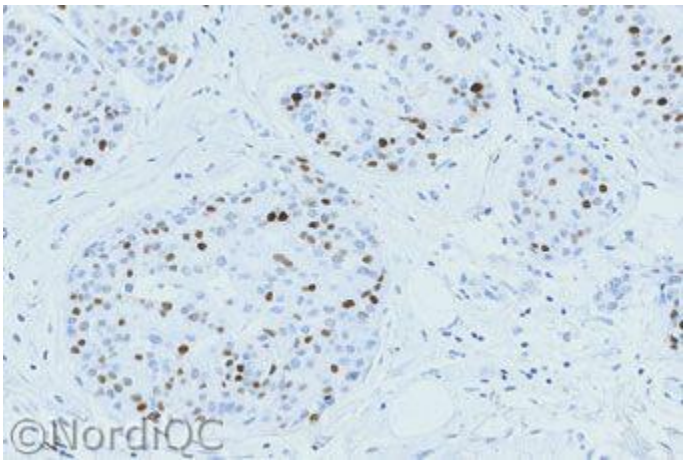


Fig. 4a
Optimal staining for PR of the breast ductal carcinoma no. 4 with 40 – 60 % cells positive using same protocol as in Figs. 1a – 3a.
The PR positive cells are easily recognized and the appropriate proportion of cells is demonstrated.

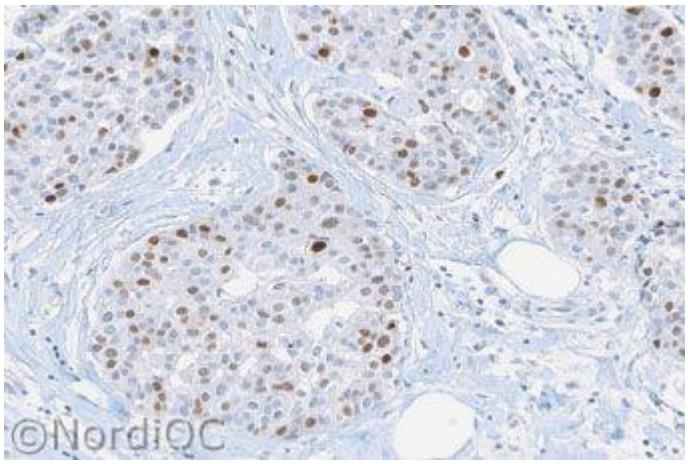


Fig. 4b
Staining for PR of the breast ductal carcinoma no. 4 with 40 – 60 % cells positive using same protocol as in Fig. 3b. – same field as in Fig. 4a.
In this tumour the appropriate proportion of cells is demonstrated. The aberrant positive staining in the breast carcinoma no. 2 thus was not related to the application of the rmAb clone 1E2 by protocol settings give a general too high sensitivity.

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