Assessment Run B24 2017

Progesterone receptor (PR)

Material

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

No.	Tissue	PR-positivity*	PR-intensity*
1.	Uterine cervix	80-90%	Moderate to strong
2.	Tonsil	0%	Negative
3.	Breast carcinoma	0%	Negative
4.	Breast carcinoma	50-80%	Weak to moderate
5.	Breast carcinoma	40-60%	Weak to moderate
6.	Breast carcinoma	90 - 100%	Moderate to strong

*PR-positivity and intensity as characterized by NordiQC reference laboratories using the mAb clone 16

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 (see table above) of neoplastic cells in the breast ductal carcinomas no. 4, 5 and 6.
- No nuclear staining reaction of neoplastic cells in the breast carcinoma no. 3.
- 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.
- No nuclear staining reaction of cells in the tonsil.

The staining reactions were classified as **good** if ≥ 10% of the neoplastic cells in the breast carcinomas no. 4, 5 and 6 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) ≥ 1 % and < 10% of the neoplastic cells showed a nuclear staining reaction in one or more of the breast carcinomas no. 4, 5 and 6
If a distinct nuclear staining reaction was seen in ≥ 10% of cells in tonsil.

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 B24	395
Number of laboratories returning slides	385 (98%)

Results

385 laboratories participated in this assessment. 99% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Protocols based on rmAb clone 1E2 giving a false positive result (no single reason was identified).

Conclusion

B24 PR preformed the highest pass rate (99%) in the NordiQC PR assessments.
The most widely used Abs clones **16**, **PgR 636, PgR 1294 and 1E2** could all be used to obtain an optimal result.
Irrespective of the clone applied, efficient HIER and careful calibration of the primary antibody were mandatory for optimal performance. In this assessment, Ready-To-Use systems and laboratory developed assays showed a similar 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.
Tonsil is an appropriate negative tissue control – no nuclear staining reaction should be seen.
In concordance with the observations seen in previous PR assessments, it is highly advisable to include both PR negative and PR positive breast tumours and well characterized PR negative tissues as tonsil in the validation/verification 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 B24**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **16**	35 2	Leica/Novocastra Biocare	28	9	0	0	100%	100%
mAb clone cocktail **16 + SAN27**	2	Leica/Novocastra	1	1	0	0	-	-
mAb clone **1A6**	2	Leica/Novocastra	2	0	0	0	-	-
mAb clone **PgR 636**	41	Dako Agilent	29	12	0	0	100%	100%
mAb clone **PgR 1294**	16	Dako Agilent	14	2	0	0	100%	100%
rmAb clone **SP2**	1 1 1	Thermo Scientific BioSystems Spring Biosystems	2	1	0	0	-	-
rmAb clone **SP42**	1 1	Zytomed Cell Marque	1	1	0	0	-	-
rmAb clone **Y85**	1	Cell Marque	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **16 PA0312**	17	Leica/Novocastra	13	4	0	0	100%	100%
mAb clone **16 MAD-000670QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone **16 CPM-0360**	1	Celnovte	1	0	0	0	-	-
mAb **PgR 636 IR/IS068**	43	Dako Agilent	34	9	0	0	100%	100%
mAb **PgR 1294 GA090**	21	Dako Agilent	17	4	0	0	100%	100%
mAb clone **PgR 1294 K4071/SK310**	2	Dako Agilent	2	0	0	0	-	-
rmAb clone **1E2 790-2223/4296**	193	Ventana	146	44	3	0	98%	98%
rmAb clone **SP2 Kit-0013**	1	Maixin	1	0	0	0	-	-
rmAb clone **EP2 AN711-5M**	1	BioGenex	1	0	0	0	-	-
rmAb **SP42 BRB038**	1	Zytomed	1	0	0	0	-	-
Total	385		295	87	3	0		
Proportion			77%	22%	1%	-	99%	

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 B24
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **16**: Protocols with optimal results were based on heat induced epitope retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (4/4)*, TRS pH 6.1 (Dako) (1/1), Cell Conditioning 1 (CC1, Ventana) (4/10), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (10/12), Bond Epitope Retrieval Solution 1 (BERS1, Leica) (6/6), Tris-EDTA pH 9 (TE) (1/1) or Citrate pH 6 (2/2) as retrieval buffer. The

mAb was typically diluted in the range of 1:50-1:800, depending on the total sensitivity of the protocol employed.
Using these protocol settings 33/33 (100%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **1A6**: Protocols with optimal results were based on HIER using CC1, Ventana (1/1) or BERS1 (Leica) (1/1) as retrieval buffer. The mAb was diluted 1:50/100. Using these protocol settings, 2/2 (100%) laboratories produced a sufficient staining result.

mAb clone **PgR 636**: Protocols with optimal results were based on HIER using TRS pH 9 (Dako) (16/18), TRS pH 6.1 (Dako) (3/4), BERS2 (Leica) (4/6), BERS1 (Leica) (1/2), Tris-EDTA/EGTA pH 9 (2/3), or Citrate pH 6 (1/4) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:700, depending on the total sensitivity of the protocol employed.
Using these protocol settings, 39/39 (100%) laboratories produced a sufficient staining result.

mAb clone **PgR 1294**: Protocols with optimal results were based on HIER using TRS high pH (Dako) (6/7), CC1 (Ventana) (5/6) or TRS low pH 6 (Dako) (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:220, depending on the total sensitivity of the protocol employed.
Using these protocol settings, 16/16 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP2**: Protocols with optimal results were based on HIER using Tris-EDTA/EGTA pH 9 (1/2) or DBS Montage EDTA Epitope Retrieval Solution (Diagnostic BioSystems) (1/1) as retrieval buffer. The rmAb was diluted in the range of 1:100-1:600.Using these protocol settings, 3 of 3 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP42**: One protocol with an optimal result was based on HIER using TRS high pH (Dako) as retrieval buffer. The rmAb was diluted 1:200 using a 2-step polymer based detection system.

Table 2. **Optimal results for PR using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	**Dako Agilent Autostainer / Omnis**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **16**	4/4	1/1	4/10 (40%)	-	10/12 (83%)	6/6 (100%)
rmAb clone **1A6**	-	-	1/1	-	-	1/1
rmAb clone **PgR 636**	16/18 (89%)	3/4	-	-	4/6 (67%)	1/2
mAb clone **PgR1294**	6/7 (86%)	1/1	5/6 (83%)	-	-	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms.
** (number of optimal results/number of laboratories using this buffer)

Ready-To-Use antibodies and corresponding systems
mAb clone **16**, product no. **PA0312**, Leica, Bond Max, Bond III: Protocols with an optimal result were typically based on HIER using Bond Epitope Retrieval Solution 1 (BERS1, Leica) or Bond Epitope Retrieval Solution 2 (BERS2, Leica) (efficient heating time 10-30 min. at 100°C), 15-60 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 15 of 15 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **PgR 636**, product no. **IS068/IR068**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using Target Retrieval Solution (TRS) pH 9 (3-in-1) (efficient heating time 10-30 min. at 95-99°C), 15-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Using these protocol settings, 34/34 (100%) laboratories produced a sufficient staining result.
7 laboratories used product no. ISO068/IR068 on Omnis (Dako), BenchMark XT (Ventana), Bond III (Leica) or used for manually staining and data was not used in the description above.

mAb clone **PgR 1294** product no. **GA090**, Dako, Omnis: Protocols with optimal results were based on HIER using TRS pH9 (Dako) (efficient heating time 30 min.), 10-25 min. incubation of the primary Ab and EnVision Flex (GV800) as detection system. Using these protocol settings, 21/21 (100%) laboratories produced a sufficient staining result.

mAb clone **PgR 1294** product no. **SK3109** Dako: Protocols with optimal results were based on HIER in PT-link in ER/PR pharmDx Epitope Retrieval Solution, 30 min. incubation of the primary Ab and ER/PR pharmDX Visualization Reagent as detection system. Using these protocol settings, 2/2 laboratories produced a sufficient staining result.

rmAb clone **1E2** product no. **790-2223/4296,** Ventana, BenchMark GX, XT/Ultra:
Protocols with optimal result were typically based on HIER using Cell Conditioning 1 (CC1, Ventana) (efficient heating time 8-64 min.), 8-64 min. incubation of the primary Ab and IView (760-091), UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings, 190/193 (98%) laboratories produced a sufficient staining result.

rmAb clone **SP2**, product no. **Kit-0013,** Maixin, manual staining:
One protocol with an optimal result was based on HIER using Citrate buffer pH 6 (efficient heating time 20 min. at 100°C), 60 min. incubation of the primary Ab. and KIT-5230 (Maixin) as detection system.

rmAb clone **SP42**, product no. **BRB038,** Zytomed, manual staining:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (efficient heating time 8 min. at 110°C), 45 min. incubation of the primary Ab. and POLHRP100 (Zytomed) as detection system.

Table 3. **Comparison of pass rates for vendor recommended and laboratory modified RTU protocols**

RTU systems	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Leica BOND MAX/ BOND III mAb 16 **PA0312**	4/4	4/4	13/13 (100%)	9/13 (69%)
Dako Autotstainer+/ Autostainer Link mAb PgR 636 **IS068/IR068**	12/12 (100%)	10/12 (83%)	22/22 (100%)	20/22 (91%)
Dako Omnis mAb PgR 1294 **GA090**	17/17 (100%)	13/17 (76%)	4/4	4/4
Ventana BenchMark GX/XT/Ultra rmAb 1E2 **790-2223/790-4296**	52/54 (96%)	42/54 (78%)	138/139 (99%)	104/139 (75%)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit
Only protocols performed on the specified vendor IHC stainer are included.

Comments
In this NordiQC assessment B24 for PR a high overall pass rate of 99% was observed being the highest in the NordiQC PR assessments performed. The features of insufficient staining results (n=3) were a false positive staining reaction or a poor signal-to-noise ratio.

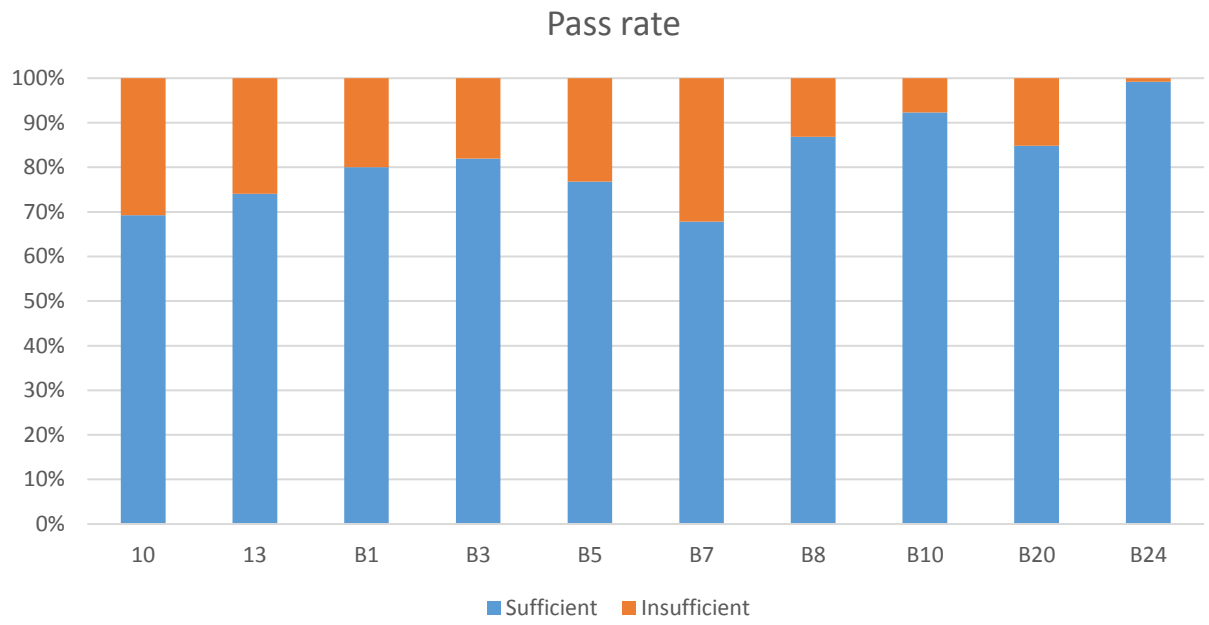
Using a laboratory developed (LD) assay, the three most widely used antibodies, mAb clones 16, PgR 636 and PgR 1294 were all very successful and provided only sufficient and optimal results on the main IHC systems (Dako, Leica and Ventana). Both HIER in alkaline and non-alkaline buffers could be used to obtain a sufficient and optimal result (see Table 2). The main prerequisite for optimal performance seemed to be a careful calibration of the primary Ab and thus adjustment of the titre to the overall level of sensitivity of the IHC system.
The corresponding Ready-To-Use (RTU) systems for mAb clones 16 (Leica), PgR 636 (Dako) and PgR 1294 (Dako) all provided a pass rate of 100% similar to the LD assays based on the same clones – see Table 1. Optimal results could be obtained both by the official recommended protocols provided by the companies but also by laboratory defined modifications of the protocol e.g. adjustment of incubation time of the primary Ab and/or reduced HIER time (see Table 3).

An aberrant and false positive staining reaction was seen in 2 of the insufficient results and was characterized by a distinct nuclear staining reaction of germinal centre B-cells in the tonsil. The false positive staining reaction was only seen for the RTU format of rmAb clone 1E2 (Ventana).
As seen in Table 1, the Ventana RTU system provided a result typically evaluated as either optimal or good.
Optimal results could be obtained both by the vendor recommended protocol settings (16 min. incubation of the primary Ab, HIER in CC1 for 64 min. and UltraView or iView as detection kit) and by laboratory modified protocols adjusting incubation time of the primary Ab, HIER time and detection system as shown in Table 3. However, despite the encouraging results, modified protocols must be meticulously validated

by the end-user. In B20 PR a large number of laboratory modified protocols resulted in an aberrant and false positive staining reaction characterized by a distinct nuclear staining reaction of germinal centre B-cells in the tonsil. The false positive staining reaction was only seen for the RTU format of rmAb clone 1E2 (Ventana) and rmAb clone SP2.
In total only 54 laboratories used the Ventana RTU system based on the rmAb clone 1E2 according to the official recommendations. 96% (n=52) were evaluated as sufficient and 4% insufficient.

Performance history
This was the tenth NordiQC assessment of PR. A significant higher proportion of sufficient results was seen in B24 compared to the previous runs, as shown in Graph 1:

Graph. **Pass rate in the NordiQC assessments for PR**



Controls
As observed in the previous NordiQC assessments of PR, uterine cervix is an appropriate positive tissue 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 thus especially demonstration of PR in squamous epithelial cells can be compromised.
Tonsil is recommendable as negative tissue control, in which no nuclear staining should be seen.

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.

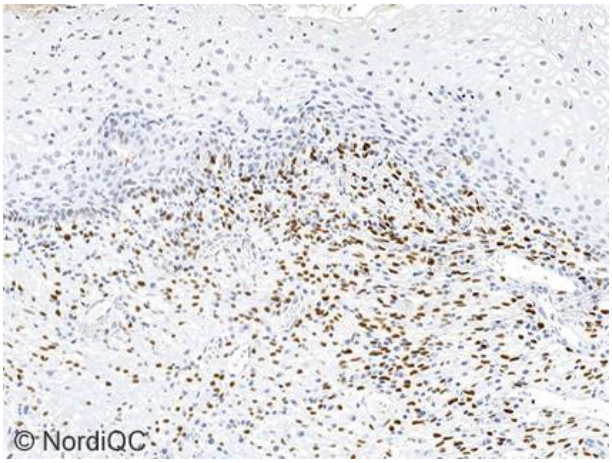


Fig. 1a
Optimal staining for PR of cervix using the mAb PgR 636 as Ready-To-Use format, Dako IR068.
The vast majority of basal squamous epithelial cells show a weak to moderate nuclear staining reaction, whereas the majority of columnar epithelial cells and stromal cells show a moderate to strong nuclear staining reaction.

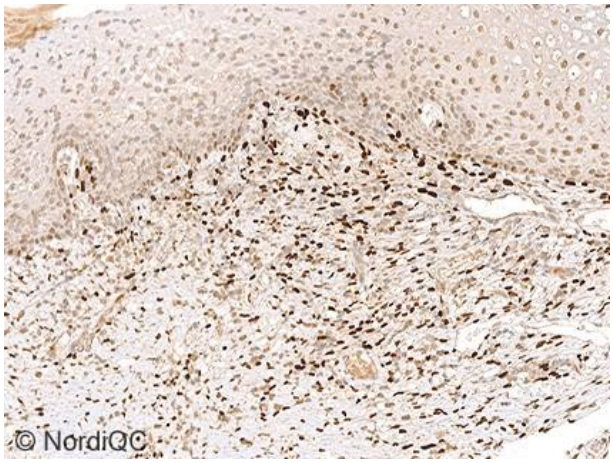


Fig. 1b
Insufficient staining for PR of cervix using rmAb 1E2 as Ready-To-Use format, Ventana 790-4296, with vendor protocol settings – same field as in Fig. 1a.
A weak to moderate cytoplasmic staining reaction compromising the interpretation.

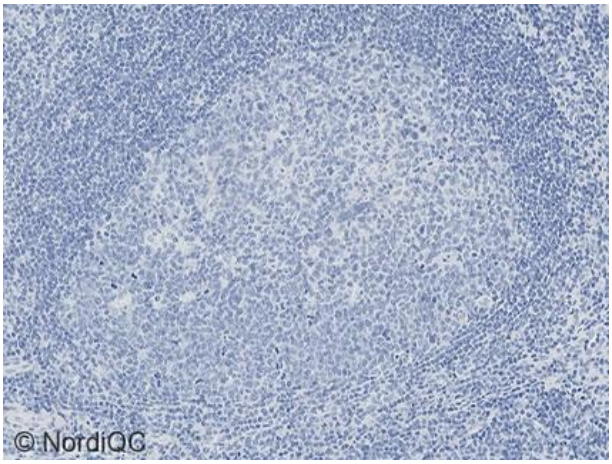


Fig. 2a
Optimal staining for PR of tonsil using same protocol as in Fig. 1a.
No nuclear staining reaction is seen.
This staining pattern was consistently seen for protocols based on mAb clones 1A6, 16, PgR 636 and PgR 1294 irrespective of protocol settings applied.

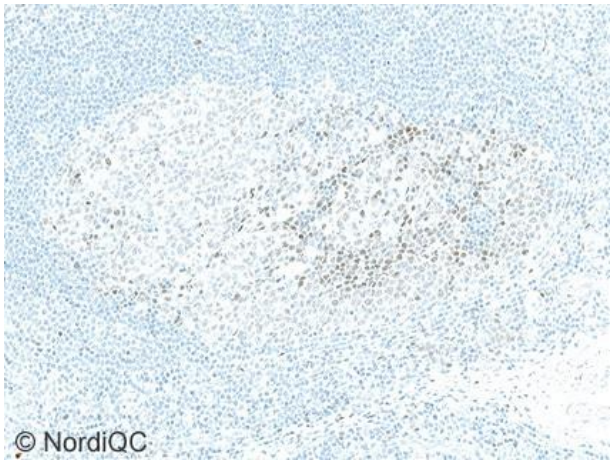


Fig. 2b
Insufficient staining for PR of tonsil using rmAb 1E2 as Ready-To-Use format, Ventana 790-4296, with laboratory modified protocol settings.
The majority of germinal cells show a weak and aberrant false positive nuclear staining reaction.
This aberrant staining reaction was only seen for rmAb clone 1E2 (RTU, Ventana).
For rmAb clone 1E2 prolonged antibody incubation time in combination with a reduced HIER time compared to the recommendations provided by Ventana seemed to enhance the aberrant staining pattern. In case of aberrant positive nuclear staining reaction in tonsil and otherwise an expected staining pattern in the other tissues was seen, the result was evaluated as borderline.

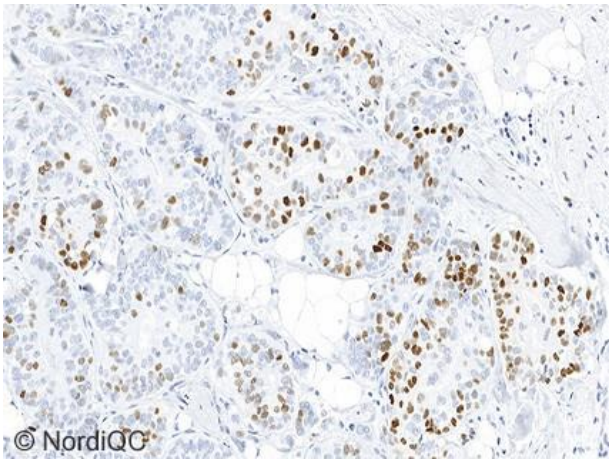


Fig. 3a
Optimal staining for PR of the breast carcinoma no. 4 with 50 - 80% cells positive using same protocol as in Figs. 1a – 2a.
The PR positive cells are easily recognized and the appropriate proportion of cells is demonstrated.

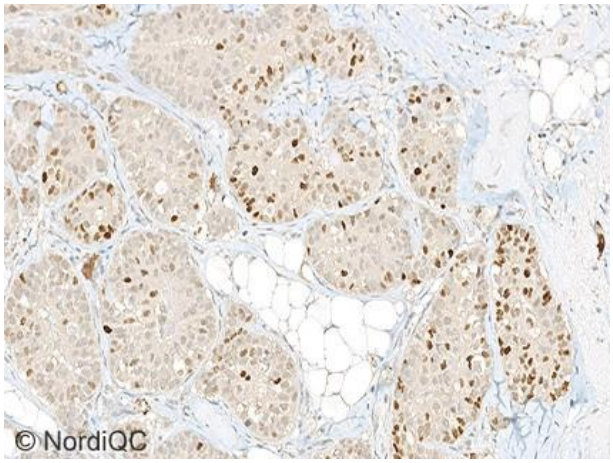


Fig. 3b
Insufficient staining for PR of the breast carcinoma no. 4 with 50 - 80% cells positive using rmAb 1E2 as Ready-To-Use format, Ventana 790-4296, with vendor protocol settings – same field as in Fig. 3a.
A weak to moderate cytoplasmic staining reaction compromising the interpretation.

HLK/LE/RR 13.12.2017