Estrogen receptor (ER)

Material

The slide to be stained for ER comprised:

No.	Tissue	ER-positivity*	ER-intensity*
1.	Uterine cervix	80-90%	Moderate to strong
2.	Breast carcinoma	0%	Negative
3.	Breast carcinoma**	40-60%	Weak to moderate
4.	Breast carcinoma	60-90%	Weak to moderate
5.	Breast carcinoma	90-100%	Moderate to strong
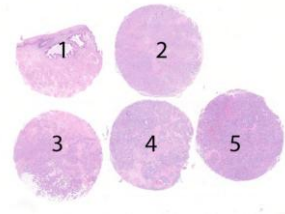
*ER-status and staining pattern as characterized by NordiQC reference laboratories using the mAb clone SP1.
** In some slides the tissue was partially detached and the evaluation was performed on the remaining 4 tissues.

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

Criteria for assessing ER staining result as **optimal** were:

- Moderate to strong, distinct nuclear staining reaction of virtually all columnar epithelial cells, basal squamous epithelial cells and most stromal cells (except endothelial and lymphoid cells) in the uterine cervix.
- At least weak to moderate distinct nuclear staining reaction in the appropriate proportion of the neoplastic cells in the breast carcinomas no. 3, 4 and 5.
- No nuclear staining reaction of neoplastic cells in the breast carcinoma no. 2.
- No more than a weak cytoplasmic staining reaction in cells with strong nuclear staining reaction.

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.

Results

281 laboratories participated in this assessment. 248 (88%) achieved a sufficient mark (optimal or good). Antibodies (Abs) used and assessment marks are summarized in table 1 (see page 2).

Conclusion

The rmAb clones **EP1** and **SP1** were in this assessment the most robust Abs for demonstration of ER. The Ready-To-Use (RTU) format of the rmAb clone **SP1** (Ventana) provided the highest proportion of sufficient and optimal results.
In this assessment, false negative staining reactions were prominent features of insufficient staining results. Uterine cervix is an appropriate positive tissue control for ER. Virtually all stromal, columnar epithelial and squamous epithelial cells must show a moderate to strong and distinct nuclear staining reaction. Lymphocytes and endothelial cells must be negative.

Table 1. **Antibodies and assessment marks for ER, run B17**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1D5**	6 3 1	Dako Immunologic Zytomed	0	6	2	2	60%	-
mAb clone **6F11**	28 2 1	Leica/Novocastra Monosan Vector	11	9	11	0	65%	68%
rmAb clone **EP1**	17 1	Dako Epitomics	3	10	3	2	72%	100%
rmAb clone **SP1**	25 3 2 1	Thermo/NeoMarkers Immunologic Spring Bioscience Cell Marque	21	9	0	1	97%	96%
Ready-To-Use antibodies								
mAb clone **1D5** IS/IR657	12	Dako	2	6	3	1	67%	75%
mAb clone **6F11** PA0151	5	Leica/Novocastra	0	2	3	0	40%	-
mAb clones **1D5+ER-2-123** K4071	1	Dako	0	1	0	0	-	-
rmAb clone **EP1** IS/IR084	42	Dako	12	25	5	0	88%	92%
rmAb clone **SP1** 790-4324/25	128	Ventana	118	10	0	0	100%	100%
rmAb clone **SP1** IR151*	1	Dako	0	1	0	0	-	-
rmAb clone **SP1** RM-9101-R7	1	Thermo/NeoMarkers	1	0	0	0	-	-
rmAb clone **SP1** MAD-000306QD	1	Master Diagnostica	1	0	0	0	-	-
Total	281		169	79	27	6	-	
Proportion			60%	28%	10%	2%	88%	

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

Detailed analysis of ER, Run B17
The following protocol parameters were central to obtain an optimal staining reaction

Concentrated antibodies
mAb clone **6F11**: Protocols with optimal results were all based on HIER using TRS pH 9 (Dako) (1/3), Cell Conditioning 1 (CC1; Ventana) (1/6), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (5/13), BERS 1 (Leica) (2/3), PT module buffer pH 6 (Thermo) (1/1) or Tris-EDTA/EGTA pH 9 (1/3) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 15 of 22 (68%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **EP1**: Protocols with optimal results were all based on HIER using TRS pH 9, 3-in-1 (Dako) (1/10), TRS pH 9 (Dako) (1/5) or BERS 2 (Leica) (1/1) as retrieval buffer. The rmAb was diluted in the range of 1:25-1:30 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 of 3 (100%) laboratories produced an optimal staining result.

rmAb clone **SP1**: Protocols with optimal results were all based on HIER using TRS pH 9, 3-in-1 (Dako) (2/3), CC1 (BenchMark, Ventana) (6/11), BERS 2 (Leica) (6/6), Tris-EDTA/EGTA pH 9 (5/6) or Citrate pH 6 (2/3) as retrieval buffer. The rmAb was diluted in the range of 1:30-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 27 of 28 (96%) laboratories produced a sufficient staining result.

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

Concentrated antibodies	Dako Autostainer Link / Classic		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **6F11**	0/2**	-	1/5 (20%)	-	5/8 (63%)	1/2
rmAb clone **EP1**	2/2	-	-	-	1/1	-
rmAb clone **SP1**	2/4	-	6/10 (60%)	-	6/6 (100%)	-

* 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 **1D5**, product no. IS/IR657, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 15-20 min. at 97°C), 20 min incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 3 of 4 laboratories produced a sufficient staining result (optimal or good).

rmAb clone **EP1**, product no. IS/IR084, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 10-20 min. at 95-98°C), 20-30 min incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 34 of 37 (92%) laboratories produced a sufficient staining result.

rmAb clone **SP1**, product no. 790-4324/25, Ventana, BenchMark GX/XT/Ultra:
Protocols with optimal results were all based on HIER using short, mild or standard Cell Conditioning 1, 8-64 min incubation of the primary Ab and Iview (760-091), UltraView (760-500, +/- amplification kit) or OptiView (760-700) as detection system. Using these protocol settings 128 of 128 (100%) laboratories produced a sufficient staining result.

The most frequent causes of insufficient stainings were:

- Insufficient HIER (too short efficient HIER time)
- Too low concentration of the primary Ab.

In this assessment, the prominent feature of an insufficient staining result was a too weak or false negative staining reaction. This pattern was seen in 94% of the insufficient results (31 of 33) and was typically caused by insufficient HIER and/or too low titre of the primary Ab irrespectively of clone applied. Virtually all laboratories were able to demonstrate ER in the high level ER expressing breast carcinoma no. 5 in which 90-100% of the neoplastic cells were expected to show a moderate to strong nuclear staining reaction. Demonstration of ER in the breast carcinomas no. 4 and especially no. 3 in which at least a weak nuclear staining of 40-90 % of the neoplastic cells was expected, were much more challenging and required an optimally calibrated protocol.
The rmAb clones EP1 and SP1 were the most successful Abs, and in particular the Ventana RTU format of the rmAb clone SP1 provided a high proportion of sufficient and optimal results. Optimal result could be obtained both by the official recommended protocol (16 min incubation of the primary Ab, HIER in CC1 for 64 min and UltraView as detection kit) and by laboratory defined modifications of the protocol e.g. prolonged of incubation time of the primary Ab and/or reduced HIER time.
The concentrated format of the rmAb clone SP1 could be used to obtain optimal staining result on the IHC systems from Dako, Leica and Ventana, see table 2.

Performance history
This was the 13th NordiQC assessment of ER. A slight increase in the proportion of sufficient results was seen compared to the latest runs (Figure 1).

Fig. 1. **Participant numbers and Pass rates for ER during 13 runs**
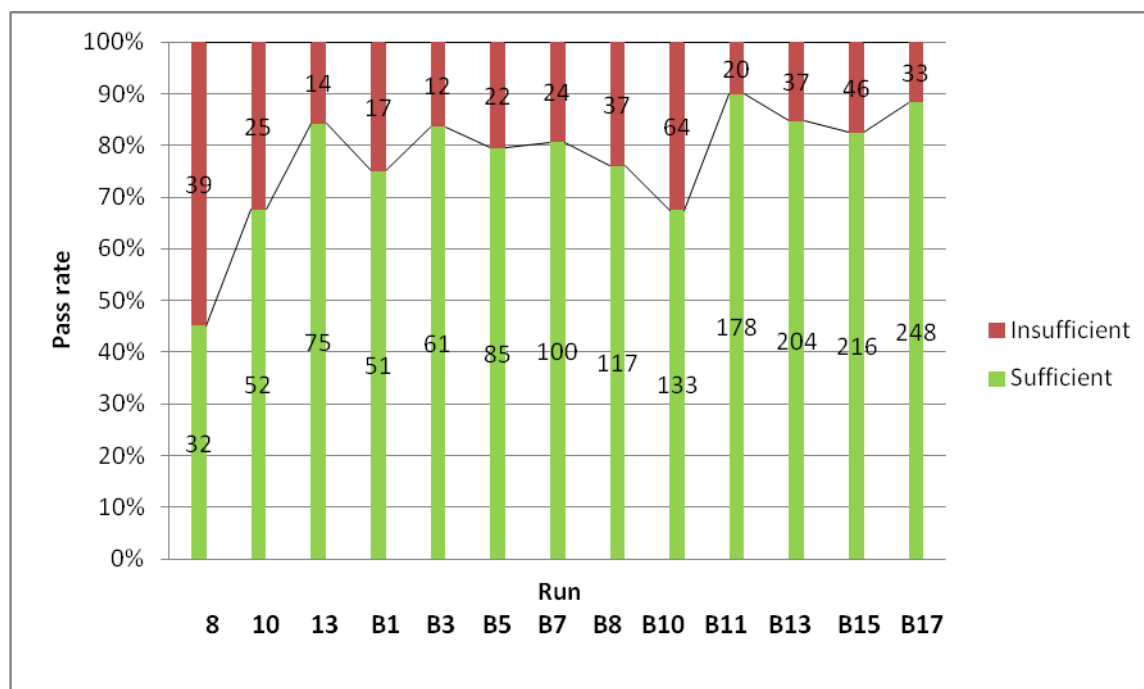
As seen in previous runs for ER, an important factor to obtain and maintain the high pass rates was the impact from the extended use of properly calibrated RTU systems for ER instead of laboratory developed assays. For example, the RTU systems in the current run grouped together provided a pass rate of 94% (179 of 191 laboratories) compared to 77% (69 of 90 laboratories) for laboratory developed assays for ER. In this context it has to be mentioned, that the Ventana RTU system based on the rmAb clone SP1 provided a pass rate of 100% and was used by 128 of the 191 laboratories using RTU systems for ER.

Controls

In concordance with previous NordiQC runs, uterine cervix was found to be an appropriate and recommendable positive tissue control for ER staining: In optimal protocols, virtually all epithelial cells throughout the layers of the squamous epithelium and in the glands showed a moderate to strong and distinct nuclear staining reaction. In the stromal compartment, moderate to strong nuclear staining reaction was seen in most cells except endothelial and lymphatic cells. If the staining intensity in the epithelial cells of the uterine cervix was significantly reduced, a too weak or even false negative staining was seen in the breast carcinomas no. 3 and 4.

In order to validate the specificity of the IHC protocol, ER negative breast carcinoma must be included in which only remnants of normal epithelial and stromal cells must be ER positive serving as internal positive tissue control. Positive staining reaction of the stromal cells breast tissue indicates that a high sensitive protocol is being applied, whereas the sensitivity cannot be evaluated in the normal epithelial cells as they express high levels of ER.
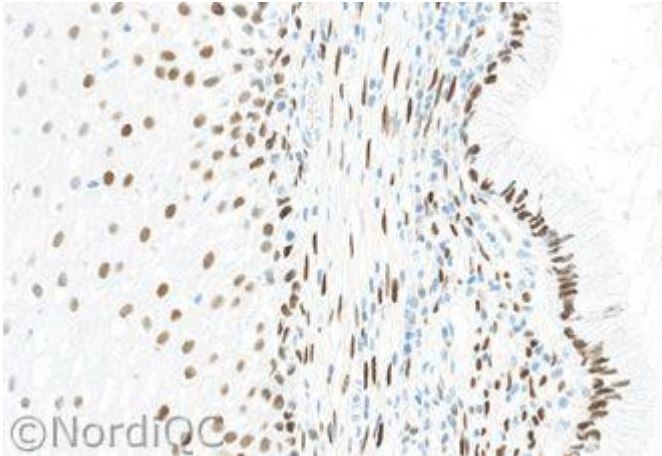
Fig. 1a
Optimal ER staining of the uterine cervix using the rmAb clone SP1 optimally calibrated and with HIER in an alkaline buffer. Virtually all the squamous and columnar epithelial cells show a moderate to strong, distinct nuclear staining reaction. The majority of the stromal cells are demonstrated and only endothelial and lymphoid cells are negative.
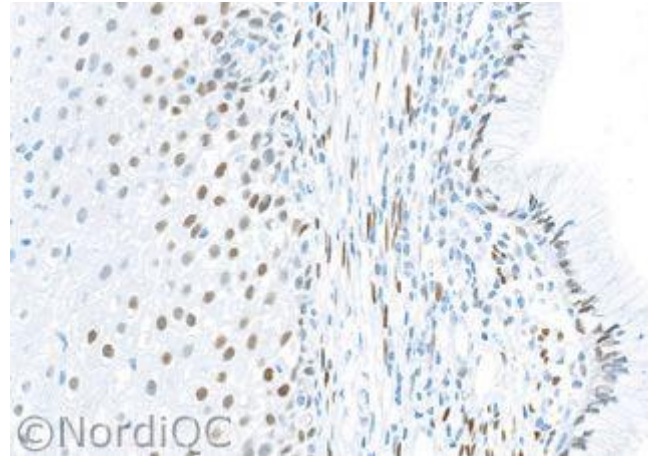

Fig. 1b
Insufficient ER staining of the uterine cervix, same field as in Fig. 1a. The proportion and intensity of the staining reaction in the squamous and especially in columnar epithelial cells are reduced. Also compare with Figs. 2b - 4b – same protocol. The protocol was based on the mAb clone 6F11 applied with protocol settings giving a too low sensitivity – most likely a too dilute titre of the primary Ab.
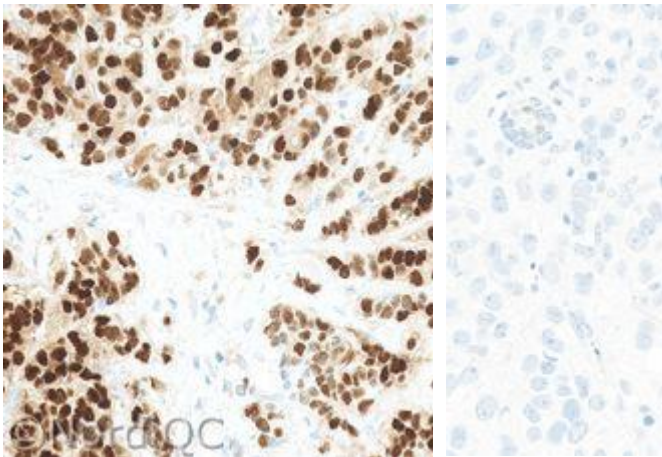
Fig. 2a
Left: Optimal ER staining of the breast ductal carcinoma no. 5 with 80 – 100% cells strongly positive using same protocol as in Fig. 1a.
Virtually all the nuclei of the neoplastic cells show a strong, distinct nuclear staining reaction with only a weak cytoplasmic staining reaction. No background staining is seen.
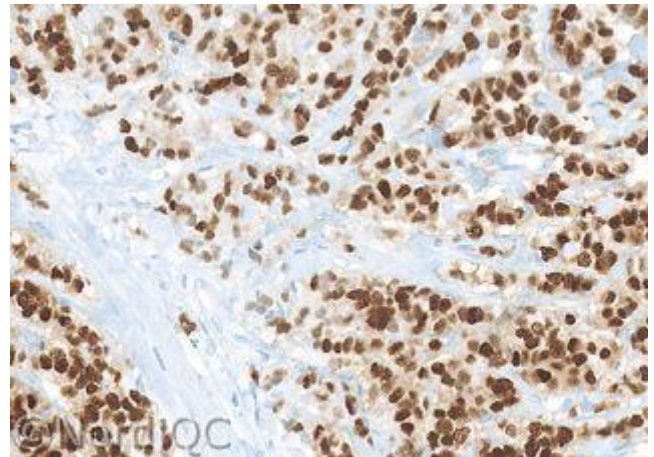Right: No staining reaction in the ER negative breast carcinoma no. 2 is seen.

Fig. 2b
ER staining of the breast ductal carcinoma no. 5 with 80 – 100% cells positive using the same protocol as in Fig. 1b – same field as in Fig. 2a. Virtually all the neoplastic cells are demonstrated, but also compare with Figs. 2b - 4b – same protocol.
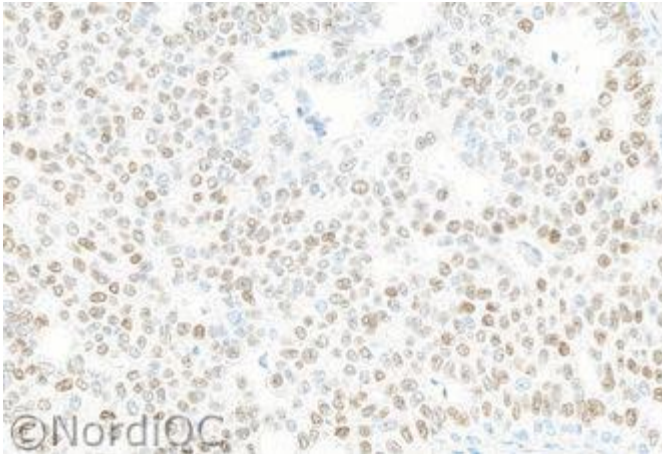
Fig. 3a
Optimal ER staining of the breast ductal carcinoma no. 4 with 60 – 90% cells positive. A weak to moderate, distinct nuclear staining in the appropriate proportion of the neoplastic cells is seen. Same protocol as in Figs. 1a and 2a.
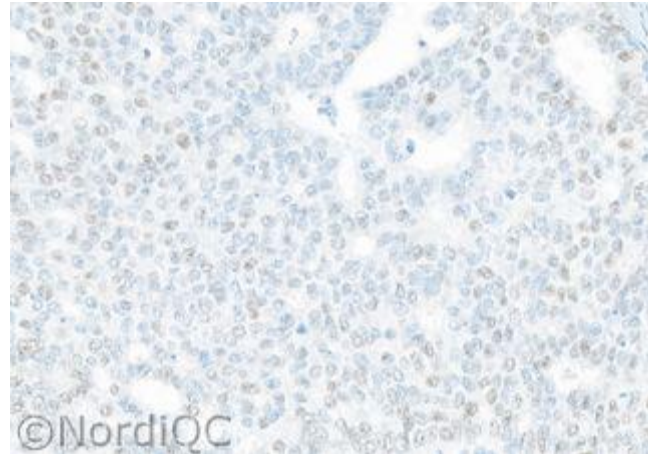

Fig. 3b
Insufficient ER staining of the breast ductal carcinoma no. 4 with 60 – 90% cells positive using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. More than 10% of the neoplastic cells are demonstrated, but the proportion and intensity is significantly reduced compared to level expected and obtained in Fig. 3a. Also compare with Fig. 4b - same protocol.
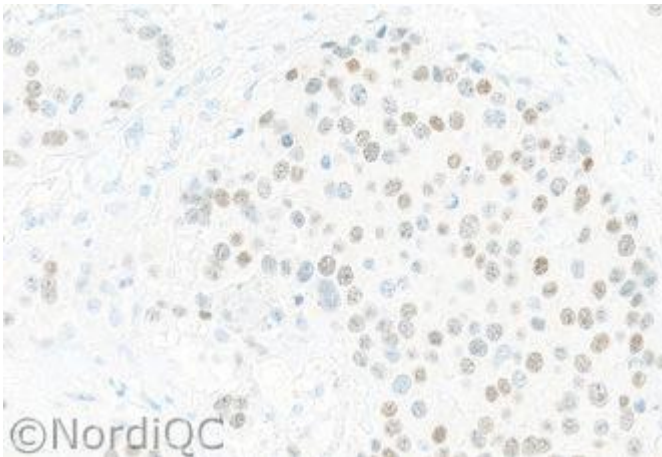

Fig 4a
Optimal ER staining of the breast ductal carcinoma no. 3 with 50 – 80% cells positive. A weak but distinct nuclear staining reaction is seen in the appropriate proportion of the neoplastic cells and no background staining is seen.
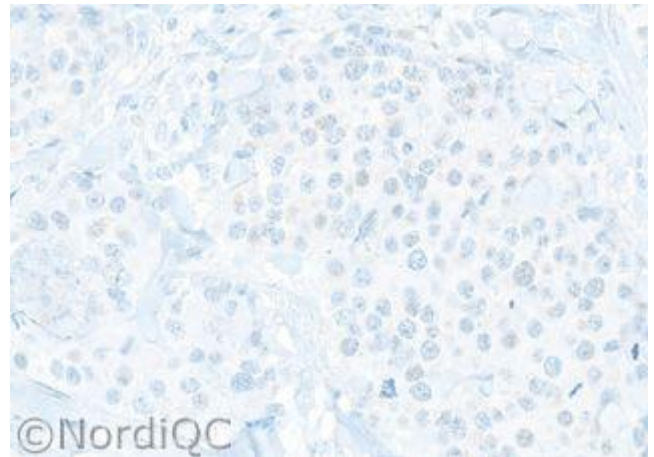Same protocol as in Figs. 1a – 3a

Fig 4b
Insufficient ER staining of the breast ductal carcinoma no. 3 with 50 – 80% cells positive using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a. A false negative staining reaction is seen.
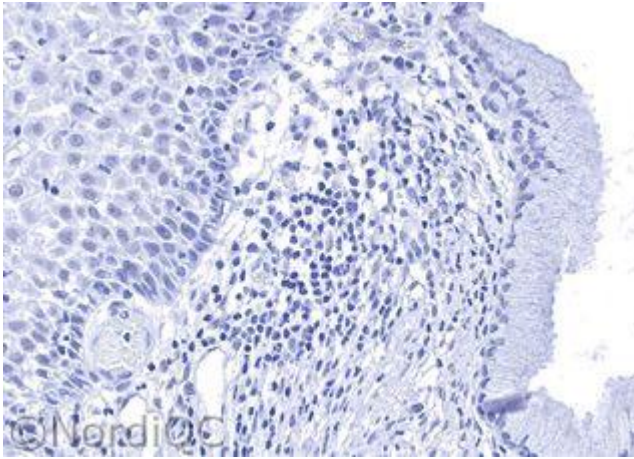
Fig. 5a
Insufficient ER staining of the uterine cervix, same field as in Fig. 1a. Virtually no nuclear staining reaction in the squamous epithelial cells, columnar epithelial cells and stromal cells is seen. Also compare with Figs. 5b left and right, same protocol. A combination of a too low titre of the primary Ab, insufficient HIER and excessive counterstaining provided a too low sensitivity of the protocol.
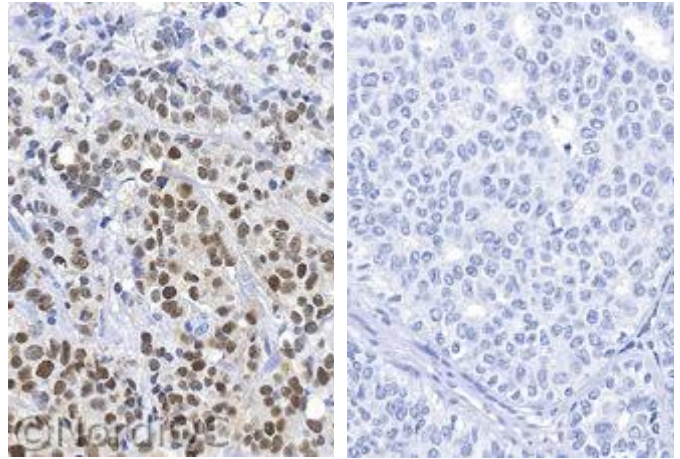
Fig. 5b
Left: ER staining of the breast ductal carcinoma no. 5 with a high level of ER expression. The vast majority of the neoplastic cells are demonstrated as expected.
Right: Insufficient and false negative staining result of the breast ductal carcinoma no. 4 with a moderate level of ER expression (60 – 90% cells positive, weak to moderate intensity). Using the protocol as shown in Figs. 5a and 5b both the breast ductal carcinomas no. 3 and 4 were false negative.

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