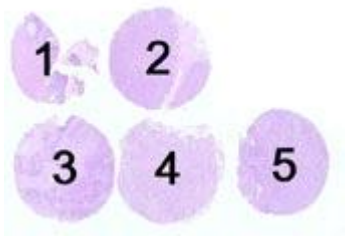
Assessment Run B11 2011
ER

Material

The slide to be stained for ER comprised the following 5 tissues:

No.	Tissue	ER positivity*	ER intensity*
1.	Uterine cervix	80 - 90 %	Moderate to strong
2.	Breast ductal carcinoma	Negative	Negative
3.	Breast ductal carcinoma	40 - 60 %	Weak to moderate
4.	Breast ductal carcinoma	40 - 60 %	Weak to moderate
5.	Breast ductal carcinoma	90 - 100 %	Strong



*ER-status and staining pattern as characterized by NordiQC reference laboratories using the mAb clone 6F11 and the rmAb clone SP1.

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

Criteria for assessing an ER staining as optimal included:

- A moderate to strong, distinct nuclear staining of most columnar and squamous epithelial cells as well as most stromal cells (with the exception of endothelial and lymphoid cells) in the uterine cervix.
- At least a weak to moderate distinct nuclear staining of the proportion of the neoplastic cells in the breast ductal carcinomas no. 3 & 4 as indicated above.
- A strong distinct nuclear staining of the proportion of the neoplastic cells in the breast ductal carcinoma no. 5 as indicated above.
- No nuclear staining in the neoplastic cells in the breast carcinoma no. 2 and no more than a weak cytoplasmic reaction in cells with a strong nuclear staining.

A cytoplasmic reaction in the breast ductal carcinoma no. 2 was accepted when using the mAb clone 1D5, provided this did not influence the interpretation.

A staining was classified as good if ≥ 10 % of the neoplastic cells in the breast ductal carcinomas no. 3, 4 & 5 showed an at least weak nuclear staining but less than the reference ranges.

A staining was assessed as borderline if ≥ 1 % and < 10 % of the neoplastic cells showed a nuclear staining in one or more of the breast ductal carcinomas no. 3, 4 & 5.

A staining was assessed as poor if < 1% of the neoplastic cells showed a nuclear staining in one or more of the breast ductal carcinomas no. 3, 4 and 5 or a false positive staining was seen in the breast ductal carcinoma no. 2.

198 laboratories participated in this assessment. 90 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for ER, run B11**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone **SP1**	22 8 1 1 1	NeoMarkers Dako Biocare Master Diagnostica Spring	20	8	1	4	85 %	88 %
mAb clone **6F11**	24 3 1	Leica/Novocastra Vector Monosan	22	5	0	1	96 %	96 %
mAb clone **1D5**	14	Dako	6	6	2	4	67 %	69 %

	2 2	Immunologic Zytomed						
mAb clones **1D5+6F11**	3	NeoMarkers	1	1	1	0	-	-
Unknown	1	Unknown	1	0	0	0	-	-
Ready-To-Use Abs								
rmAb clone **SP1, 790-4324/25**	78	Ventana	76	2	0	0	100 %	100 %
rmAb, clone **SP1, IS/IR151**	22	Dako	12	4	3	3	73 %	76 %
rmAb, clone **SP1, RM-9101-R7**	2	NeoMarkers	1	0	1	0	-	-
rmAb, clone **SP1, ZA-01002**	1	Zhongshan Jinqiao	0	1	0	0	-	-
mAb/rmAb, clones **6F11+SP1, PM308**	1	Biocare	1	0	0	0	-	-
mAb, clone **6F11, RTU-ER-6F11**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **1D5, IR654**	5	Dako	2	3	0	0	100 %	100 %
mAb clones **1D5+ER-2-123, SK310/K4071**	5	Dako	3	2	0	0	100 %	100 %
Total	198		145	33	8	12	-	
Proportion			73 %	17 %	4 %	6 %	90 %	

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

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
rmAb clone **SP1**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (3/7)*, Target Retrieval Solution (TRS) pH 9 (Dako) (1/2), TRS pH 9 (3-in-1, Dako) (4/9), Cell Conditioning 1 (CC1) (BenchMark, Ventana) (2/3), Bond Epitope Retrieval Solution 2 (BERS 2) (Bond, Leica) (3/3) Bond Epitope Retrieval Solution 1 (BERS 1) (Bond, Leica) (1/1), DIVA Decloaker (Biocare) (1/1), BORG Decloaker (Biocare) (1/1) EDTA/EGTA pH 8 (2/4) or Citrate pH 6 (2/2) as the retrieval buffer. The mAb was typically diluted in the range of 1:25– 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 28 out of 32 (88 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

mAb clone **6F11**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (5/5), TRS pH 9 (Dako) (3/4), TRS pH 9 3-in-1 (Dako) (3/3), TRS pH low (3-in-1, Dako) (1/1), BERS 2 (Bond, Leica) (7/9), BERS 1 (Bond, Leica) (1/2), CC1 (BenchMark, Ventana) (1/2) or EDTA/EGTA pH8 (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:25– 1:250 depending on the total sensitivity of the protocol employed. Using these protocol settings 26 out of 27 (96 %) laboratories produced a sufficient staining.

mAb clone **1D5**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (1/3), TRS pH 9 (Dako) (1/6), TRS pH 9 3-in-1 (Dako) (1/4), EDTA/EGTA pH8 (1/1) or Citrate pH 6 (2/3) as the retrieval buffer. The mAb was diluted in the range of 1:35– 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 11 out of 16 (69 %) laboratories produced a sufficient staining.

mAb clones **1D5+6F11**: The protocol giving an optimal result was based HIER using BERS 2 (Bond, Leica) (1/1) as the retrieval buffer. The mAb was diluted 1:50.

Ready-To-Use Abs
rmAb clone **SP1** (prod. no. 790-4324/25, Ventana): The protocols giving an optimal result were typically based on HIER using mild or standard CC1, an incubation time of 16-32 min in the primary Ab and iView (760-091) or UltraView (760-500) as the detection system. Some labs used the amplification kit. Using these protocol settings 78 out of 78 (100 %) laboratories produced a sufficient staining.

rmAb clone **SP1** (prod. no. IS/IR151, Dako): The protocols giving an optimal result were typically based on HIER in PT-Link using TRS pH 9 or TRS pH 9 (3-in-1) for 10-20 min and an incubation time of 20-30 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings 16 out of 21 (76 %) laboratories produced a sufficient staining.

rmAb clone **SP1** (prod. no. RM-9101-R7, NeoMarkers): The protocol giving an optimal result was based on HIER in mild CC1 (Benchmark, Ventana) and an incubation time of 32 min in the primary Ab and iView (760-091) as the detection system.

mAb/rmAb clones **6F11+SP1** (prod. no. PM308, BioCare): The protocol giving an optimal result was based on HIER using DIVA Decloaker (Biocare) in a pressure cooker, an incubation time of 45 min in the primary Ab and MACH4 Universal HRP Polymer kit (M4U534, Biocare) as the detection system.

mAb clone **1D5** (prod. no. IR654, Dako). The protocols giving an optimal result were based on HIER in PT-Link using TRS pH 9 or TRS pH 9 3-in-1 for 20 min and an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings 3 out of 3 (100 %) laboratories produced a sufficient staining.

mAb clones **1D5 + ER-2-123** (prod. no K4071/SK310, Dako (pharmDx™ kit). The protocols giving an optimal result were based on HIER using Epitope Retrieval Solution (K4071/SK310) in a pressure cooker, an incubation time of 20-30 min in the primary Ab and K4071/SK310 (pharmDx™ kit) as the detection system. Using these protocol settings 5 out of 5 (100 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient stains were:

- Too low concentration of the primary antibody
- Insufficient HIER (too short efficient heating time)

In this assessment the prevalent feature of an insufficient staining was a generally too weak or false negative reaction, especially seen in the ductal carcinomas no. 3 & 4, in which an at least a weak nuclear staining of 40-60% of the neoplastic cells was expected. This pattern was seen in 19 out the 20 insufficient results (95 %). Insufficient HIER and/or a too low concentration of the primary Ab were the most common causes for the insufficient results.
As observed in the previous assessment for ER, all the 3 most widely used Abs for ER, the mAb clones 1D5 and 6F11 and the rmAb clone SP1 could be used to obtain an optimal staining. In the protocols based on the mAb clone 1D5 giving an otherwise optimal staining an aberrant cytoplasmic staining was seen in the ER negative breast ductal carcinoma. This was accepted as long as this staining pattern did not compromise the interpretation.
In concordance to the observations generated in these runs, both the mAb clone 6F11 and the rmAb clone SP1 (both as concentrates and Ready-To-Use formats) gave a higher proportion of sufficient and optimal results than the mAb clone 1D5, see table 2.
In table 2 the overall performance of the three most widely used Abs for ER in the NordiQC assessments is listed.

Table 2. **Results for the three most widely used Abs in eight NordiQC ER tests**

	All ER assessments* All protocol settings			**All ER assessments* Optimal protocol settings****		
	Protocols	**Sufficient**	**Optimal**	**Protocols**	**Sufficient**	**Optimal**
mAb clone 1D5	299	175 (59 %)	57 (19 %)	164	113 (69 %)	57 (35 %)
mAb 6F11	303	226 (75 %)	125 (41 %)	235	200 (85 %)	125 (53 %)
rmAb SP1	508	439 (86 %)	344 (68 %)	472	431 (91 %)	344 (73 %)

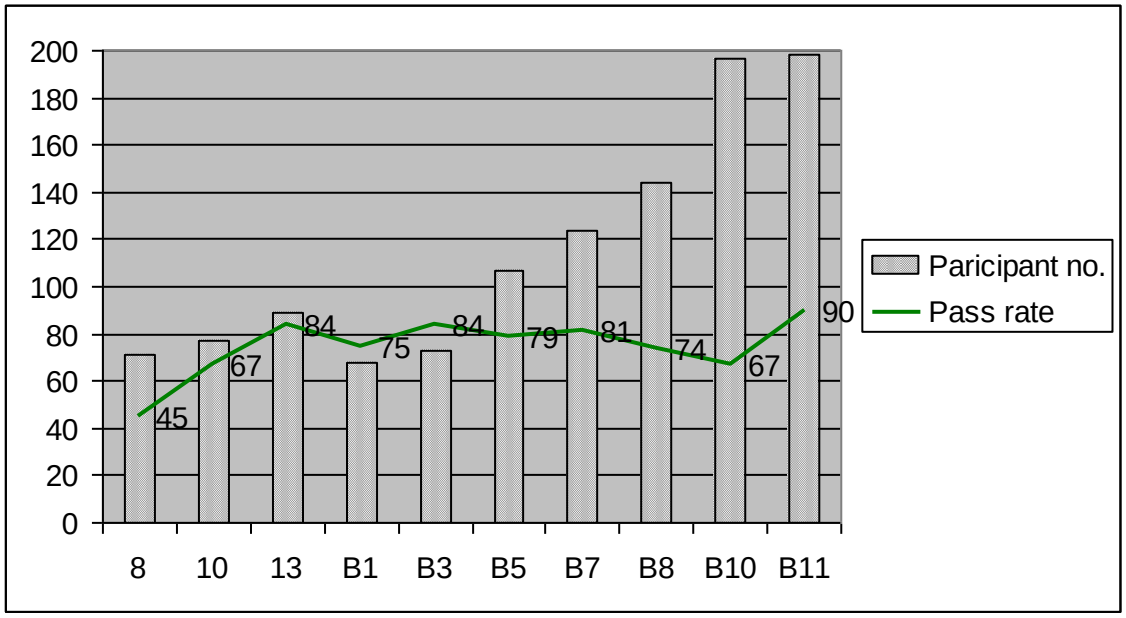
*Runs 8, 10, 13, B1, B3, B5, B7, B8, B10 & B11.
** HIER settings and dilution range of the Ab in all assessments giving an optimal result.

As shown in the previous runs, uterine cervix was an appropriate control for ER staining: In the optimal protocols virtually all the epithelial cells throughout the layers of the squamous epithelium and in the glands showed a moderate to strong and distinct nuclear reaction. In the stromal compartment a moderate to strong nuclear staining was seen in most cells and only endothelial and lymphatic cells were negative.

This was the 10th assessment of ER in NordiQC and a significant increase of the proportion of sufficient results was seen compared to the previous run B10 as shown in table 3:

Table 3. **Proportion of sufficient results for ER in the earlier NordiQC runs performed**

	Run 8 2003	Run 10 2004	Run 13 2005	Run B1 2006	Run B3 2007	Run B5 2008	Run B7 2009	Run B8 2009	Run B10 2010	Run B11 2011
Participants, n	71	77	89	68	73	107	124	144	197	198
Sufficient results, %	45 %	67 %	84 %	75 %	84 %	79 %	81 %	74 %	67 %	90 %



The improved pass rate may be due to many factors. In this run, 3 breast ductal carcinomas with a range from 40-100 % positivity were included, whereas the breast carcinomas in the previous run B10 showed a range from 10-80 % positivity. Although the tumours in both runs comprised a range from weak to strong regarding the intensity of ER, the material used for current run may be slightly less challenging than that of the previous run as no tumours with 10-30 % positivity were included as in run B10.
Another important factor was the impact from the extended use by the laboratories of properly calibrated and commercially available RTU systems for ER instead of in-house validated assays. E.g. in this run the RTU systems based on the rmAb clone SP1 and the mAb clone 1D5 from Ventana and Dako were used by 110 out of the 198 participating laboratories (56 %) and as a group a pass rate of 95 % was obtained (104 out of 110 laboratories). Using the same clones as a concentrate and applied within an in-house validated assay the pass rate was only 78 % (40 out of 51 laboratories). In run B10, only 83 out of the 197 participating laboratories (42 %) used the two clones in an RTU system from Ventana or Dako.

Conclusion
The mAb clone 6F11 and the rmAb SP1 were the most robust Abs for ER. In this assessment the increasingly used RTU systems for ER based on the rmAb clone SP1 (Ventana and Dako) and the mAb clone 1D5 (Dako) gave a higher pass rate for the demonstration of ER than the in-house validated assays.
HIER is mandatory, preferable in an alkaline buffer.
Uterine cervix is an appropriate control for ER: Virtually all the epithelial cells and most stromal cells must show a strong distinct nuclear staining reaction with a minimal cytoplasmic reaction.

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.

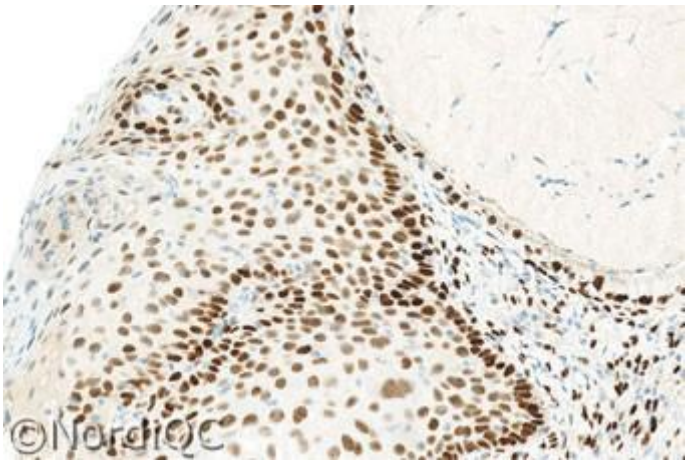


Fig. 1a
Optimal ER staining of the uterine cervix using the rmAb clone SP1 optimally calibrated and with HIER in an alkaline buffer. Virtually all the squamous and columnar epithelial cells show a distinct nuclear staining. The majority of the stromal cells are demonstrated and only endothelial and lymphoid cells are negative.

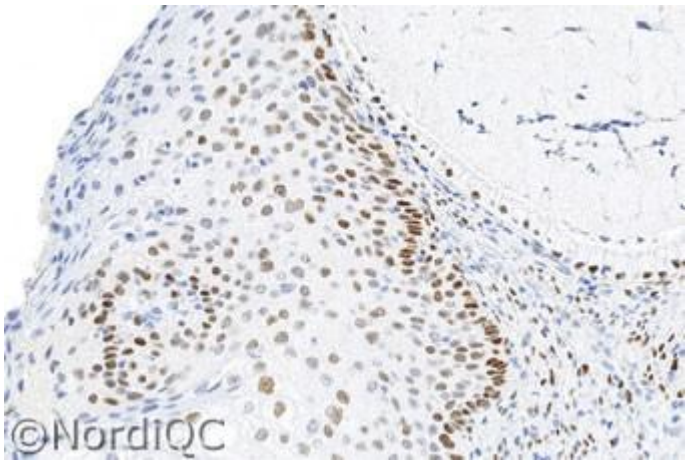


Fig. 1b
Insufficient ER staining of the uterine cervix, same field as in Fig. 1a. The proportion and intensity of the positive epithelial cells and stromal cells are reduced. Also compare with Figs. 2b and 3b, same protocol. The protocol was based on the rmAb clone SP1 applied with protocol settings giving a too low sensitivity – too diluted Ab and/or insufficient HIER.

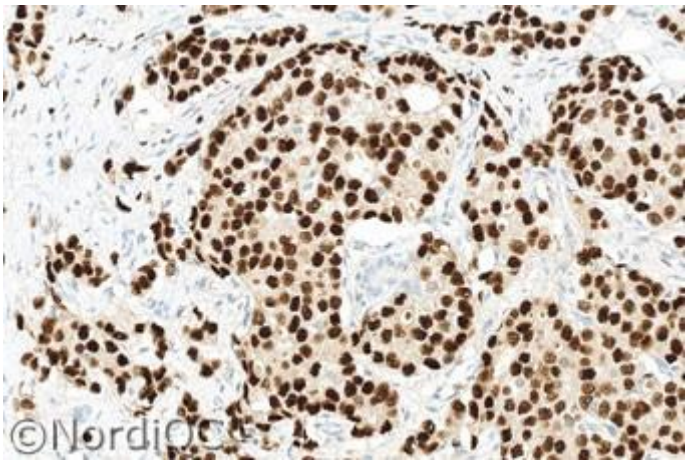


Fig. 2a
Optimal ER staining of the breast ductal carcinoma no. 5 with 90 – 100 % cells positive. Virtually all the nuclei of the neoplastic cells show a strong nuclear staining and a weak cytoplasmic staining. No background staining is seen. Same protocol as in Fig. 1a.

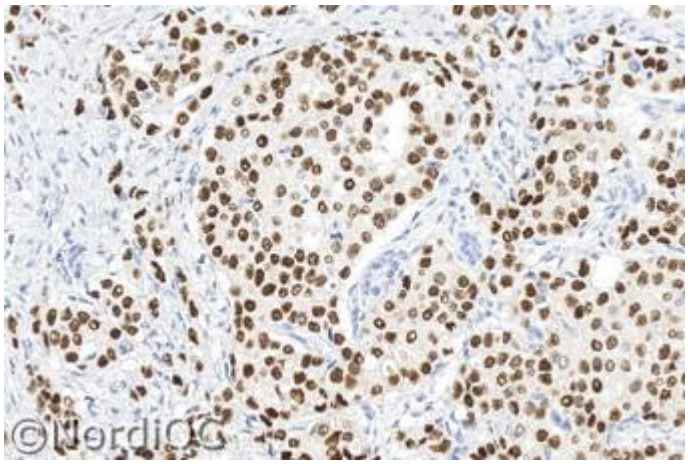


Fig. 2b
ER staining of the breast ductal carcinoma no. 5 with 90 – 100 % cells positive using an insufficient protocol, same field as in Fig. 2a. The majority of the nuclei of the neoplastic cells are stained, but also compare with Fig. 3b, same protocol.

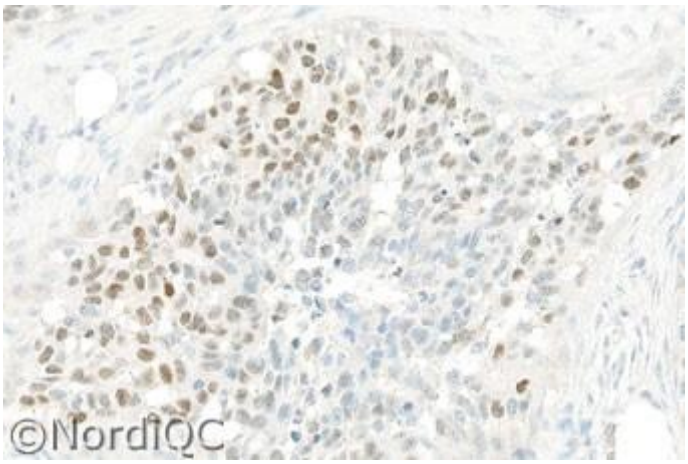


Fig. 3a
Optimal ER staining of the breast ductal carcinoma no. 3 with 40 – 60 % cells positive. A weak to moderate but distinct nuclear staining is seen in the majority of the neoplastic cells. Same protocol as in Figs. 1a and 2a.

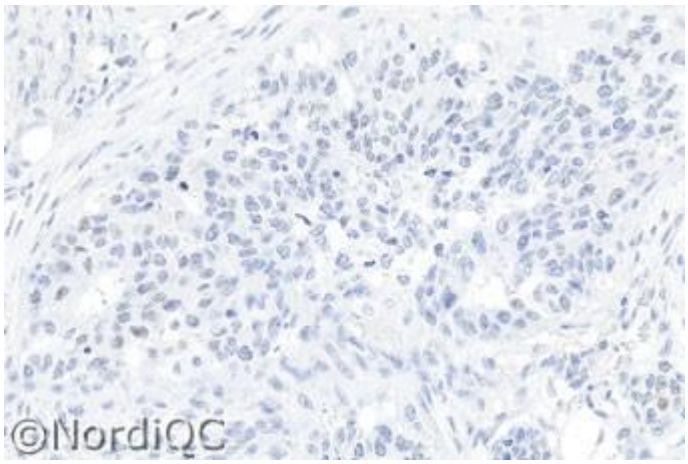


Fig. 3b
Insufficient ER staining of the breast ductal carcinoma no. 3 with 40 – 60 % cells positive using same protocol as in Figs. 1b and 2b, same field as in Fig. 3a. The neoplastic cells are false negative.

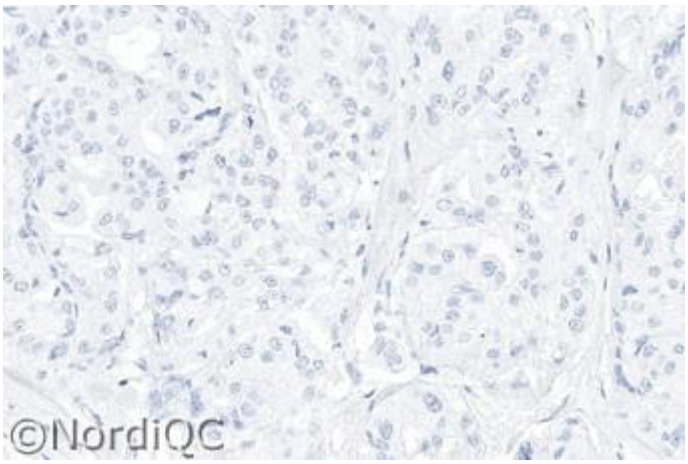


Fig. 4a
Optimal ER staining of the breast ductal carcinoma no. 2 using same protocol as in Figs. 1a – 3a. No nuclear staining is seen in the neoplastic cells and no background staining is seen. Compare the optimal result with the staining in Fig. 4b.

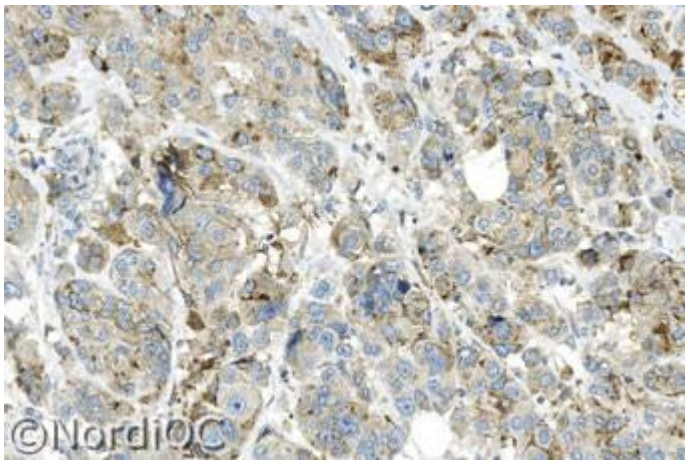


Fig. 4b
ER staining of the breast ductal carcinoma no. 2 using the mAb clone 1D5 with HIER in an alkaline buffer and a 3-step labelled polymer detection system. No nuclear staining reaction is seen in the neoplastic cells, whereas a strong intracytoplasmic staining is seen.
The result was assessed as good. An interpretation could be made, but was compromised by the excessive cytoplasmic staining.
This pattern was frequently seen when the mAb clone 1D5 was applied by a high sensitive protocol.

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