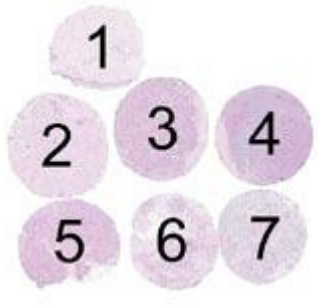
Assessment Run B10 2010

Estrogen receptor (ER)

The slide to be stained for ER comprised the following 7 tissues:

No.	Tissue	ER extension*	ER intensity*
1.	Uterine cervix	80 - 90 %	Moderate to strong
2.	Breast lobular carcinoma	60 - 80 %	Weak to moderate
3.	Breast ductal carcinoma	10 - 30 %	Weak
4.	Breast ductal carcinoma	Negative	Negative
5.	Breast ductal carcinoma	10 - 30 %	Weak
6.	Breast ductal carcinoma	60 - 80 %	Weak to moderate
7.	Breast ductal carcinoma	50 - 70 %	Moderate to 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. 2, 3, 5 & 6 as indicated above.
- A strong distinct nuclear staining of the proportion of the neoplastic cells in the breast ductal carcinoma no. 7 as indicated above.
- No nuclear staining in the neoplastic cells in the breast carcinoma no. 4 and no more than a weak cytoplasmic reaction in cells with a strong nuclear staining.

A cytoplasmic reaction in the breast ductal carcinoma no. 4 was accepted when using the mAb clone 1D5, as this did not influence the interpretation.

197 laboratories participated in this assessment. 67 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and the marks given are summarized.

Table 1. **Abs and assessment marks for ER, run B10**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone **SP1**	32 4 1 1	NeoMarkers Dako Immunologic Spring	20	10	6	2	79 %	79 %
mAb clone **6F11**	29 2 1 1	Novocastra/Leica Vector BioCare Monosan	9	7	8	9	48 %	78 %
mAb clone **1D5**	24 2 2	Dako Immunologic Zytomed	5	3	5	15	29 %	44 %
mAb clones **1D5+6F11**	4	NeoMarkers	0	1	3	0	-	-
Ready-To-Use Abs								
rmAb clone **SP1, 790-4324/25**	60	Ventana	56	3	0	1	98 %	98 %
rmAb, clone **SP1, IS/IR151**	22	Dako	8	6	5	3	64 %	100 %
rmAb clone **SP1, RM-9101-R7**	4	NeoMarkers	0	2	1	1	-	-
mAb/rmAb clones	1	Biocare	1	0	0	0	-	-

6F11+SP1, PM308								
mAb clone **6F11, PA0151**	1	Novocastra/Leica	0	0	0	1	-	-
mAb clone **1D5, IR654**	1	Dako	0	0	0	1	-	-
mAb clone **1D5, N1575**	1	Dako	0	0	0	1	-	-
mAb clones **1D5+ER-2-123, SK310/K4071**	4	Dako	0	2	1	1	-	-
Total	197		99	34	29	35	-	-
Proportion			50 %	17 %	15 %	18 %	67 %	-

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 (6/12)*, Target Retrieval Solution (TRS) pH 9 (Dako) (1/4), TRS pH 9 (3-in-1, Dako) (2/4), Cell Conditioning 1 (BenchMark, Ventana) (7/10), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1), Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/1), Diva Decloaker (Biocare) (1/2) or Citrate pH 6 (1/4) 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 30 out of 38 (79 %) 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 (4/9), TRS pH 9 (Dako) (1/3), TRS pH 9 (3-in-1, Dako) (2/4) or Bond Epitope Retrieval Solution 2 (Bond, Leica) (2/6) as the retrieval buffer. The mAb was typically diluted in the range of 1:50– 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 14 out of 18 (78 %) laboratories produced a sufficient staining.

mAb clone **1D5**: The protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (3/12) or TRS pH 9 (3-in-1, Dako) (2/6) as the retrieval buffer. The mAb was diluted in the range of 1:35– 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 8 out of 18 (44 %) laboratories produced a sufficient staining.

Ready-To-Use Abs
rmAb clone **SP1** (prod. no. 790-4324/25, Ventana): The protocols giving an optimal result were based on HIER using mild or standard Cell Conditioning 1, an incubation time of 16-40 min in the primary Ab and iView (760-091) or UltraView (760-500) as the detection system with or without amplification. Using these protocol settings 59 out of 60 (98 %) laboratories produced a sufficient staining.

rmAb clone **SP1** (prod. no. IS/IR151, Dako): The protocols giving an optimal result were all 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 20-30 min in the primary Ab and EnVision Flex (K8000), Flex+ (K8002) or EnVision REAL (K5007) as the detection system. Using these protocol settings all of 11 (100 %) laboratories produced a sufficient staining (optimal or good). One lab used an incubation time of 32 min, HIER in standard Cell Conditioning 1 (Benchmark, Ventana) and UltraView (760-500) as the detection system and produced a staining marked as optimal.

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

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody.
- Insufficient HIER (use of citrate pH 6.0 and/or too short efficient heating time).
- Less successful performance of the mAb clone 1D5 both as concentrate and in Ready-To-Use (RTU) formats.

In this assessment the prevalent feature of an insufficient staining was a generally too weak or false negative reaction, the latter especially seen in ductal carcinomas no. 3 & 5 (where a weak staining of 10-30% of the

neoplastic cells was expected). This pattern was seen in 50 out of 64 of the insufficient results (78 %). Insufficient HIER and/or a too low concentration of the primary Ab were the most common causes for the insufficient results. In 10 out of 64 of the insufficient results (16%) both a false negative and false positive staining was seen, typically due to a too low concentration of the primary Ab combined with efficient HIER in an alkaline buffer and the use of a biotin based detection system. This gave a strong cytoplasmic staining in the lobular carcinoma no. 2 while the nuclei were almost negative complicating the interpretation. Also a negative reaction was seen in the two ductal breast carcinomas no. 3 & 5 in these cases.

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. However in this run B10, the rmAb clone SP1 (both as concentrate and Ready-To-Use) gave a higher proportion of sufficient results compared to clone 6F11 and in particular to clone 1D5. It was noteworthy that the RTU systems from Dako and Ventana for the rmAb clone SP1 gave a significant higher pass rate (98-100 %) than the pass rate (79 %) when same clone was used with an in-house procedure, even with optimal protocol settings.

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**	274	158 (58%)	49 (18%)	145	99 (68%)	49 (34%)
mAb **6F11**	274	198 (72%)	103 (38%)	208	174 (84%)	103 (50%)
rmAb **SP1**	372	315 (85%)	235 (63%)	340	308 (91%)	235 (69%)

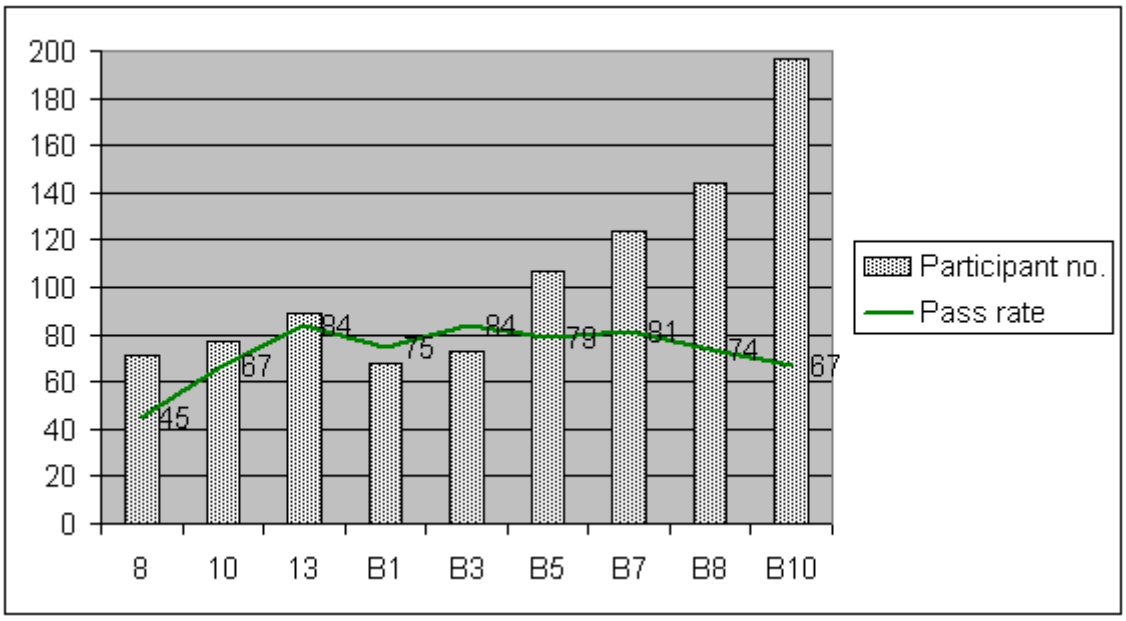
*Runs 8, 10, 13, B1, B3, B5, B7, B8 and B10.
** HIER settings and dilution range of the Ab in all assessments giving an optimal result.

As shown in the previous runs, uterine cervix was an appropriate control for ER staining: In the optimal protocols almost all the epithelial cells throughout the layers of the squamous epithelium and in the glands showed a moderate to strong and distinct nuclear reaction.

This was the 9th assessment of ER in NordiQC. A decrease in the proportion of sufficient results has been seen in the last two runs as shown in table 3. The decrease in part is caused by many new participants in the last two runs and also a more challenging material.

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
Participants, n	71	77	89	68	73	107	124	144	197
Sufficient results, %	45 %	67 %	84 %	75 %	84 %	79 %	81 %	74 %	67 %



Conclusion

The mAb clone 6F11 and in particular the rmAb SP1 were the most robust Abs for ER. In this assessment the RTU systems for ER based on the rmAb clone SP1 (Dako and Ventana) gave a higher pass rate for the demonstration of ER than the in-house protocols. Clone 1D5 constantly shows an inferior performance.
HIER is mandatory, preferable in an alkaline buffer: A non-biotin based detection system should be used.
Uterine cervix is an appropriate control for ER: Both the epithelial cells and most stromal cells must show a strong distinct nuclear reaction with minimal cytoplasmic reaction.

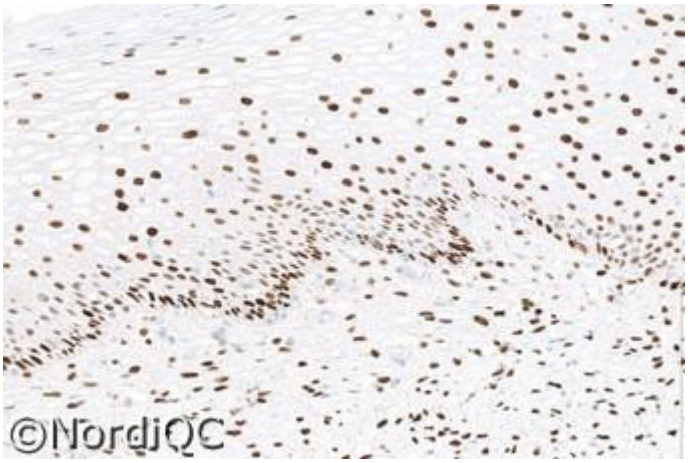


Fig. 1a
Optimal ER staining of the uterine cervix using the rmAb clone SP1 optimally calibrated and with HIER in an alkaline buffer. Virtually all the squamous epithelial cells show a distinct nuclear staining. The majority of the stromal cells are demonstrated and only endothelial and lymphoid cells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

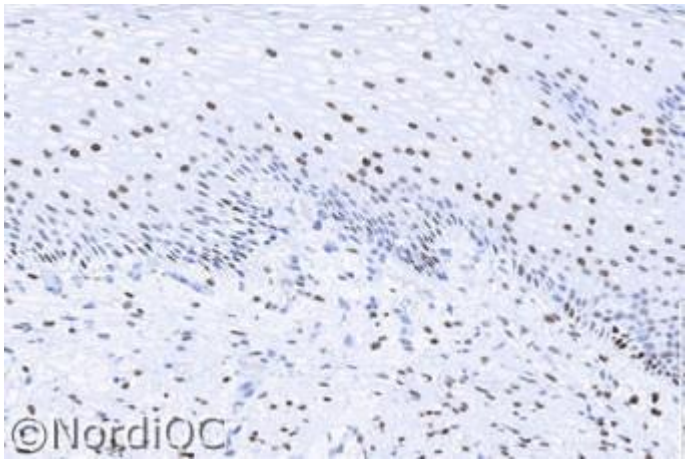


Fig. 1b
Insufficient ER staining of the uterine cervix– same field as in Fig. 1a. The proportion and intensity of the positive squamous epithelial cells and stromal cells are reduced. Also compare with Figs. 2b and 3b – same protocol. The protocol was based on the mAb clone 1D5 applied with protocol settings giving a too low sensitivity – mAb clone 1D5 too diluted and insufficient HIER.

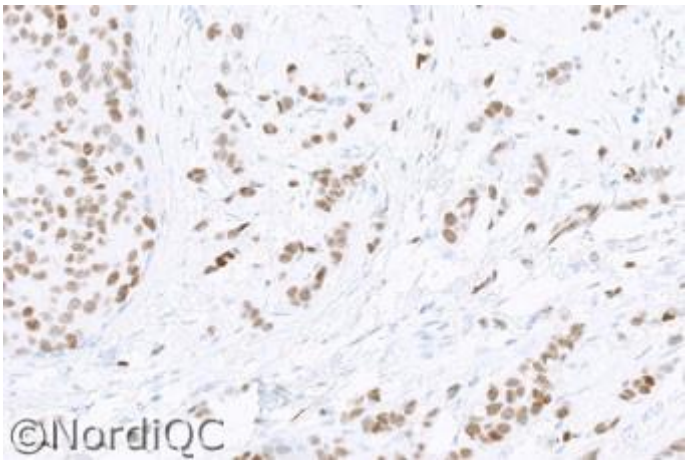


Fig. 2a
Optimal ER staining of the breast ductal carcinoma no. 6 with 60 – 80 % cells positive. The majority of the nuclei of the neoplastic cells show a weak to moderate staining. Same protocol as in Fig. 1a.

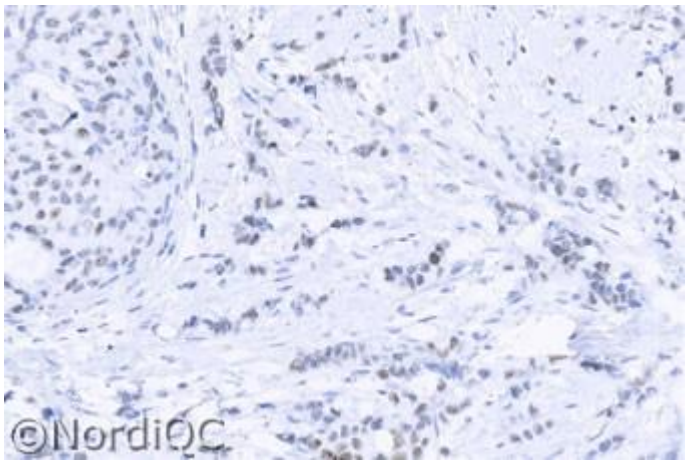


Fig. 2b
ER staining of the breast ductal carcinoma no. 6 with 60 – 80 % cells positive using an insufficient protocol – same field as in Fig. 2a. The majority of the nuclei of the neoplastic cells are stained, but weaker than seen in Fig. 2a. Also compare with Fig. 3b – same protocol.

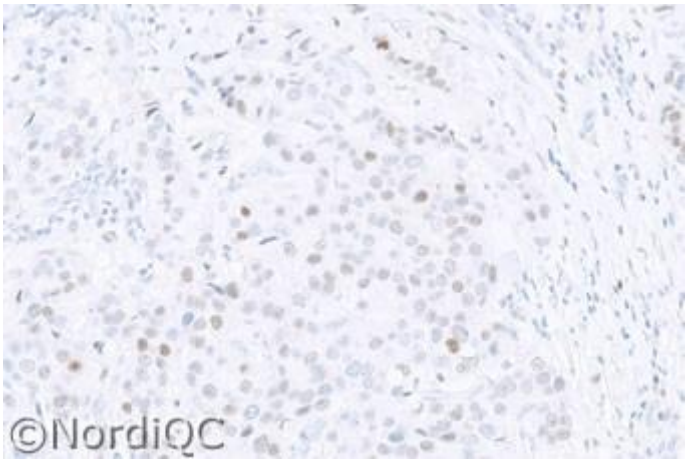


Fig. 3a
Optimal ER staining of the breast ductal carcinoma no. 3 with 10 – 30 % cells positive. A weak but distinct nuclear staining is seen in more than 10 % of the neoplastic cells. Same protocol as in Figs. 1a and 2a.

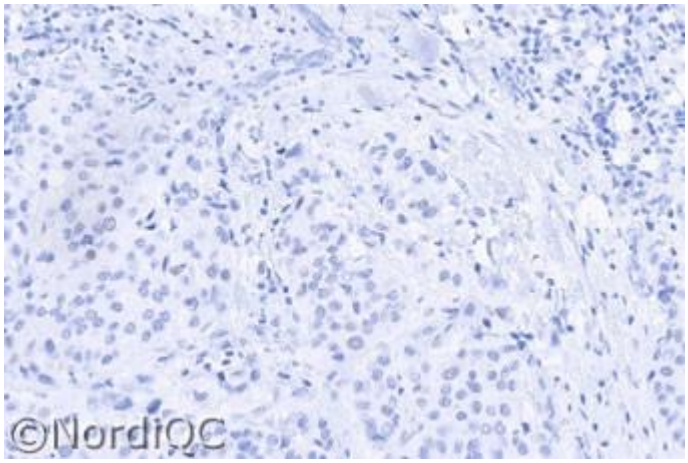


Fig. 3b
Insufficient ER staining of the breast ductal carcinoma no. 3 (with 10 – 30 % cells positive) using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. No nuclear staining reaction is seen in the neoplastic cells.

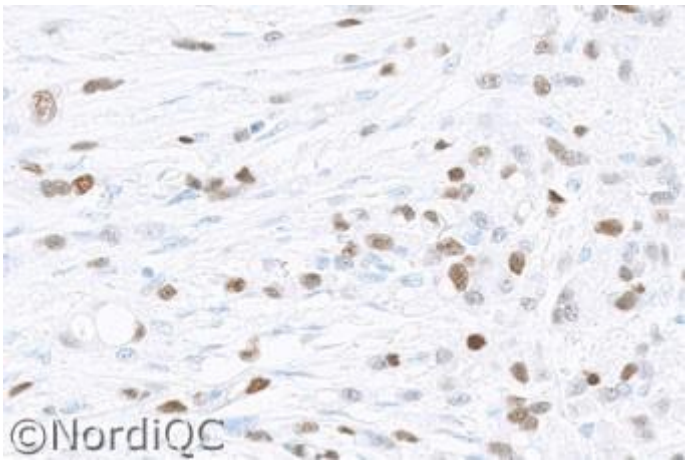


Fig. 4a
Optimal ER staining of the lobular breast carcinoma no. 2. The nuclei are strongly stained and no cytoplasmic staining reaction is seen. Same protocol as in Figs. 1a – 3a using the rmAb SP1, HIER in an alkaline buffer and a multimer (non-biotin) based detection system.
Compare the optimal result with the staining in Fig. 4b.

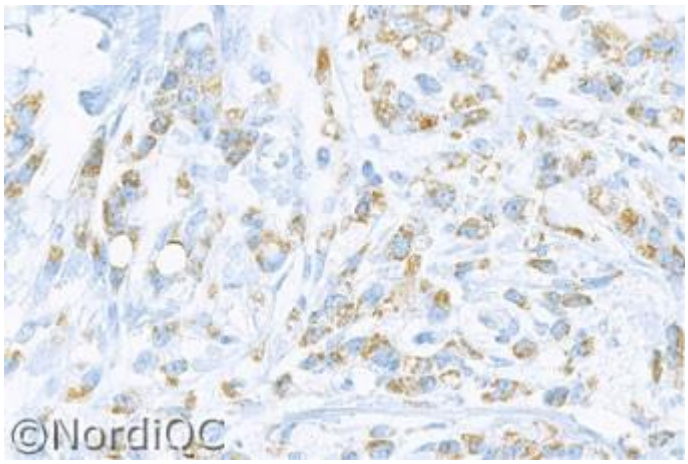


Fig. 4b
Insufficient ER staining of the lobular breast carcinoma no. 2. No nuclear staining reaction is seen in the neoplastic cells, whereas a strong cytoplasmic staining is seen due to endogenous biotin. The protocol was based on the rmAb SP1 used too diluted, HIER in an alkaline buffer and a biotin based detection system.

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.

SN/MV/LE 6-12-2010