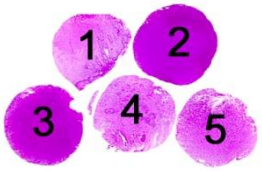
Assessment Run B7 2009

Estrogen Receptor alpha (ER)

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

No.	Tissue	ER-positivity*	ER-intensity*
1.	Uterine cervix	80-90 %	Moderate to strong
2.	Breast ductal carcinoma	Negative (<1 %)	Negative
3.	Breast ductal carcinoma	60-80 %	Weak to moderate
4.	Breast ductal carcinoma	60-80 %	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.

Criteria for assessing an ER staining as optimal included:

- A moderate to strong, distinct nuclear staining of both the columnar and 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 of the appropriate proportion of the neoplastic cells in the breast ductal carcinomas no. 3 - 4.
- A strong distinct nuclear staining of the appropriate proportion of the neoplastic cells in the breast ductal carcinoma no. 5.
- 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.

124 laboratories participated in this assessment. 81 % achieved a sufficient mark. The antibodies (Abs) and marks are summarized in Table 1.

Table 1. **Abs and scores for ER, run B7**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone **SP1**	29 1 1 1	NeoMarkers Dako Diagnostic Biosystems Master Diagnostica	16	8	6	2	75 %	80 %
mAb clone **6F11**	20 1	Novocastra Sanova	8	10	2	1	86 %	100 %
mAb clone **1D5**	23 2 1	Dako Zytomed Immunovision	0	16	8	2	62 %	-
mAb clones **1D5+6F11**	1	NeoMarkers	0	1	0	0	-	-
Ready-To-Use Abs								
rmAb clone **SP1, 790-4324/25**	30	Ventana	27	3	0	0	100 %	100 %
rmAb clone **SP1, IR151**	8	Dako	3	3	2	0	75 %	86 %
rmAb clone **SP1, ZA0102**	1	Zymed	0	0	1	0	-	-
mAb clones **1D5 + ER-2-123, K1904/SK310**	2	Dako	2	0	0	0	-	-
mAb clone **6F11 + rmAb clone SP1, IP308/PM308**	2	BioCare	0	2	0	0	-	-
mAb clone **6F11, 760-2596**	1	Ventana	1	0	0	0	-	-
Total	124		57	43	19	5	100	

Proportion			46 %	35 %	15 %	4 %	81 %	92 %

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

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

Concentrated Abs
rmAb **SP1**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (6/9)*, Target Retrieval Solution (TRS) pH 9 (EnVision FLEX TRS high pH, Dako, (5/7), Cell Conditioning 1 (BenchMark, Ventana) (2/5), EDTA/EGTA pH 8 (2/2)* or Citrate pH 6 (1/6) as retrieval buffer. The rmAb was typically diluted in the range of 1:25– 1:250 depending on the total sensitivity of the protocol employed. Using these protocol settings 24 out of 30 (80 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb **6F11**: the protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (6/9), TRS pH 9 (EnVision FLEX TRS high pH, Dako, (1/3), or Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/5) as retrieval buffer. The mAb was typically diluted in the range of 1:20– 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 14 out of 14 (100 %) laboratories produced a sufficient staining (optimal or good).

Ready-To-Use Abs
rmAb clone **SP1**, prod. no 790-4324/25, Ventana: The protocols giving an optimal result were all based on HIER using Cell Conditioning 1, mild or standard, an incubation time of 16-32 min in the primary Ab and iView or ultraView as the detection system. Using these protocol settings all of 30 (100 %) laboratories produced a sufficient staining.

rmAb clone **SP1**, prod. no IR151, Dako: The protocols giving an optimal result were all based on HIER using TRS pH 9 (EnVision FLEX TRS high pH) for 20 min in the PT-Link, an incubation time of 20 min in the primary Ab and EnVision Flex (K8000/K8002) as the detection system. Using these protocol settings 6 out of 7 (86 %) laboratories produced a sufficient staining.

mAb clones **1D5 + ER-2-123**, prod. no K1904/SK310, Dako (pharmDx™ kit): The protocols giving an optimal result were all based on HIER using TRS pH 6.1 in a pressure cooker, an incubation time of 20 or 30 min in the primary Ab and EnVision (K5207/SK310) as the detection system. Using these protocol settings both of two laboratories produced an optimal staining.

mAb clone **6F11**, prod. no 760-2596, Ventana: The protocol giving an optimal result was based on HIER using Cell Conditioning 1, mild, an incubation time of 32 min in the primary Ab and ultraView as the detection system.

The most frequent causes of an insufficient staining in this run were:
- Too low concentration of the primary antibody
- Insufficient HIER (use of citrate pH 6.0 and/or too short heating time)
- Excessive HIER.

In this assessment the prevalent feature of an insufficient staining was a general too weak reaction or completely false negative reaction in the ductal carcinomas no. 3 & 4 (which should show 60-80% positivity). As shown in the previous runs the uterine cervix could be used as an appropriate control and critical stain quality indicator for the ER staining. In the optimal protocols almost all epithelial cells showed a moderate to strong and distinct nuclear reaction (compared to protocols giving insufficient results in which both the proportion of positive cells and the intensity was significantly reduced). In concordance with previous NordiQC assessments of ER it was observed that the mAb clone 6F11 and the rmAb clone SP1 (both as concentrate and Ready-To-Use) gave a higher proportion of sufficient results compared to the mAb clone 1D5. In table 2 the overall performance is listed of the 4 most widely used Abs for ER in the NordiQC assessments. In this assessment no protocol based on the mAb clone 1D5 resulted in an optimal staining. When the clone 1D5 was used in an otherwise correctly calibrated system a moderate to strong aberrant cytoplasmic staining was seen in the ER negative breast ductal carcinoma no. 2 complicating the interpretation. The majority of the laboratories are now using efficient HIER based on an alkaline buffer, which in all assessments has shown to be valuable to provide an optimal sensitivity for the ER demonstration. However, the HIER method has to be adjusted to give both a high sensitivity and an acceptable morphology. In the current run some laboratories obtained an insufficient result due to excessive HIER (typically a too long heating time and/or too high temperature).

Table 2. **Results for the four most used Abs in seven ER tests in NordiQC**

	All ER assessments* All protocol settings			All ER assessments* Optimal protocol settings**		
	Protocols	**Sufficient**	**Optimal**	**Protocols**	**Sufficient**	**Optimal**
mAb clone **1D5**	219	136 (62%)	39 (18%)	109	77 (71%)	39 (36%)
mAb clone **1D5 + ER-2-123**	10	9 (90%)	4 (40%)	10	9 (90%)	4 (40%)
mAb **6F11**	212	161 (76%)	80 (38%)	165	143 (87%)	80 (49%)
rmAb **SP1**	161	141 (87%)	103 (64%)	151	141 (93%)	103 (73%)

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

This was the 7' assessment of ER in NordiQC breast module and a relative constant proportion of sufficient results have been obtained in the last 5 runs as shown in table 3:

Table 3. **Sufficient over-all results with ER in seven NordiQC runs**

	Run 8 2003	Run 10 2004	Run 13 2005	Run B1 2006	Run B3 2007	Run B5 2008	Run B7 2009
Participants, n	71	77	89	68	73	107	124
Sufficient results, %	45%	67%	84%	75%	84%	79%	81%

Conclusion

The mAb clone 6F11 and the rmAb SP1 seem to be the most robust Abs for ER. HIER is mandatory, preferable in an alkaline buffer and must be performed to provide an optimal balance between sensitivity and preserved morphology. The concentration of the Ab must be carefully calibrated on an appropriate control such as the uterine cervix in which both the epithelial cells and most stromal cells must show a moderate-strong distinct nuclear reaction with minimal cytoplasmic reaction.

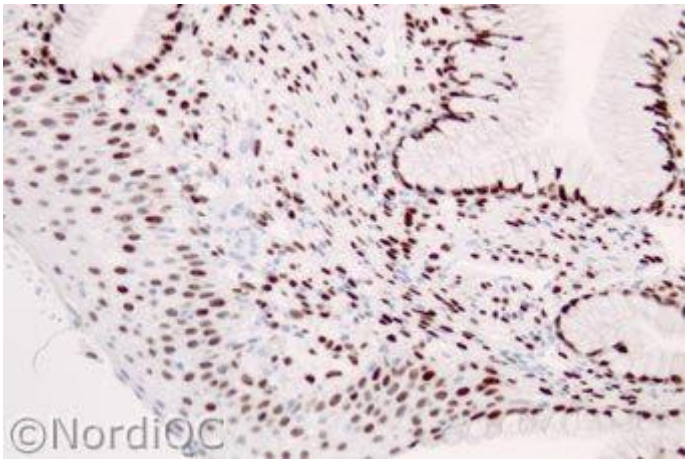


Fig. 1a
Optimal ER staining of the uterine cervix using the rmAb clone SP1. Virtually all the squamous and columnar epithelial cells show a distinct nuclear staining. The majority of the stromal cells are demonstrated and only endothelial and lymphoid cells are negative.

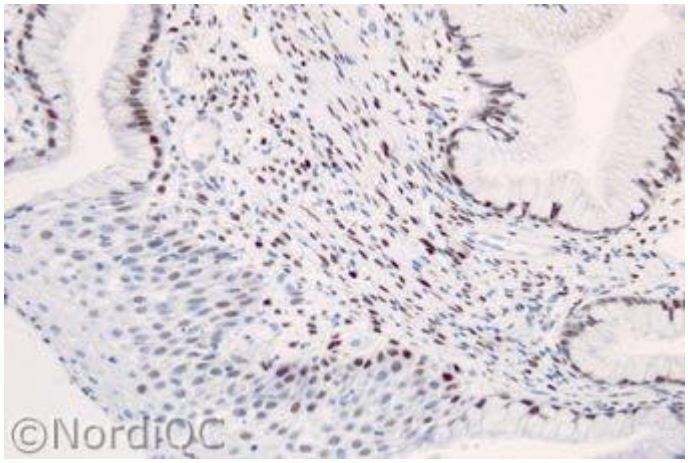


Fig. 1b
Insufficient ER staining of the uterine cervix – same field as in Fig. 1a. Only scattered epithelial and stromal cells show a weak to moderate nuclear staining. The protocol was based on the mAb clone 1D5 and HIER in citrate pH 6.0. Also compare with Figs. 2b and 3b – same protocol.

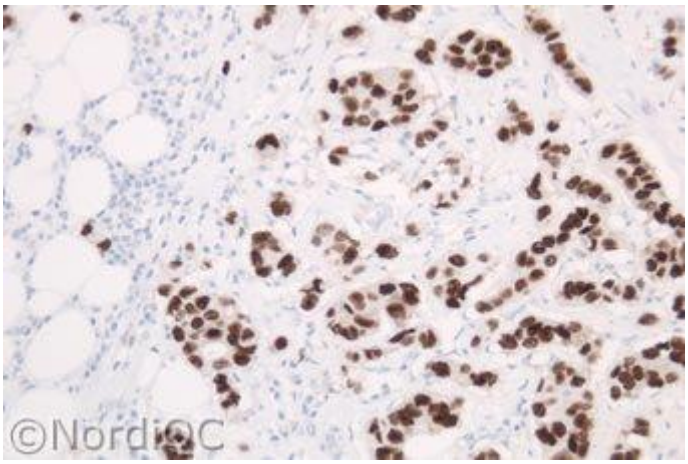


Fig. 2a
Optimal ER staining of the breast ductal carcinoma no. 5. 90 – 100 % of the neoplastic cells show a moderate to strong nuclear staining. A weak cytoplasmic reaction is seen in cells with positive nuclei, while the background is negative. Same protocol as in Fig. 1a.

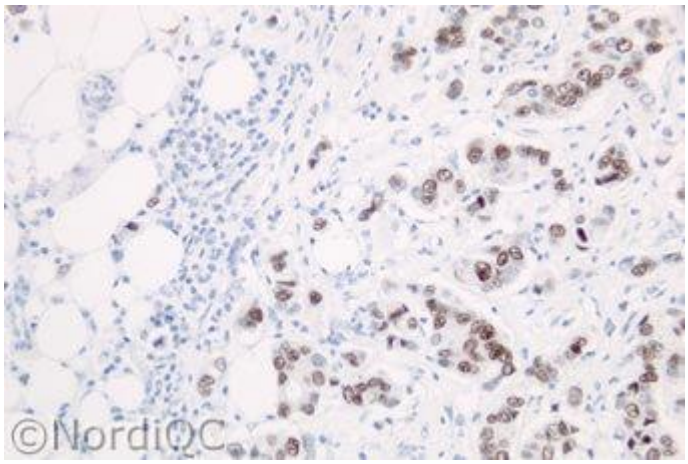


Fig. 2b
ER staining of the ductal breast carcinoma no. 5 – same field as in Fig. 2a. The majority of the nuclei of the neoplastic cells are stained, but weaker than seen in Fig. 2a. Also compare with Fig. 3b – same protocol.

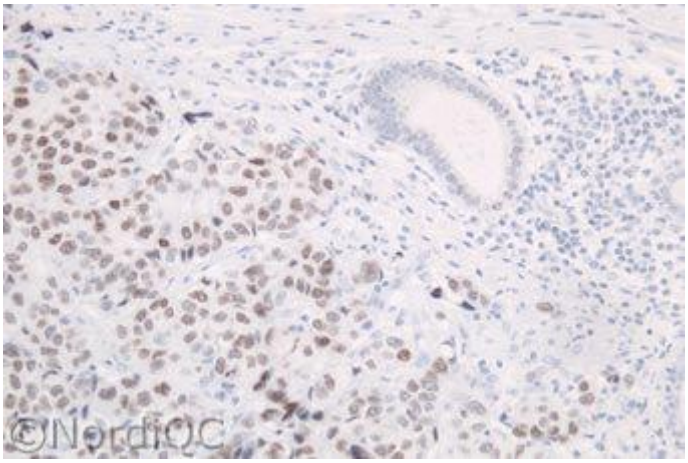


Fig. 3a
Optimal ER staining of the breast ductal carcinoma no. 3. 60 – 80 % of the neoplastic cells show a weak to moderate nuclear staining. Same protocol as in Figs. 1a and 2a.

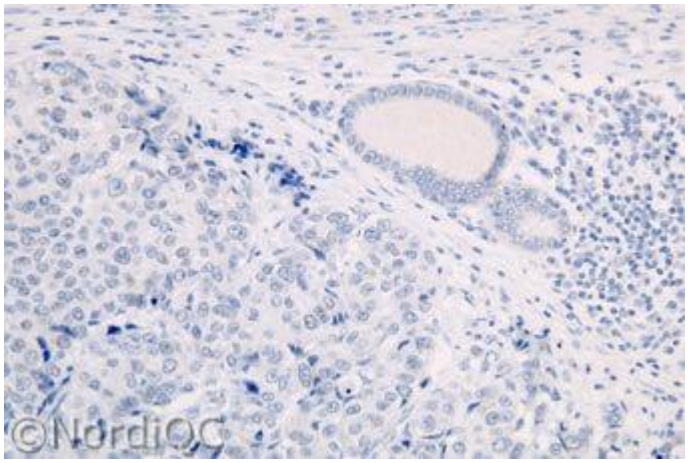


Fig. 3b
Insufficient ER staining of the breast ductal carcinoma no. 3 using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a. Only a very faint nuclear staining is seen in scattered cells.

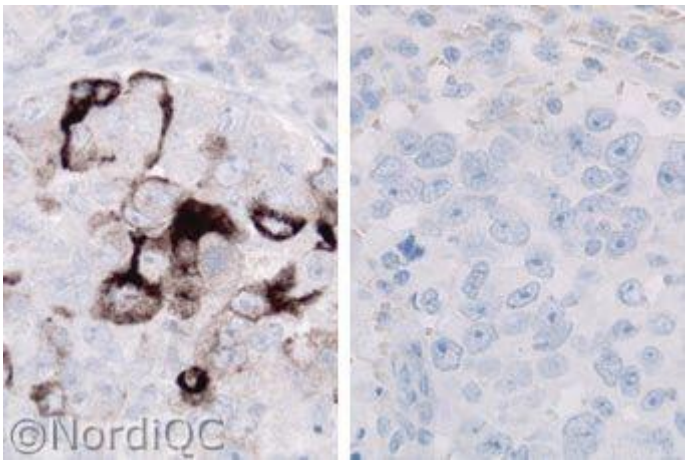


Fig. 4a
Different staining patterns of the ER negative breast ductal carcinoma no. 2.
Left: The neoplastic cells show a strong cytoplasmic staining. This pattern was frequently seen, when the mAb clone 1D5 was used.
Right: No staining is seen in the neoplastic cells. This pattern was seen when the mAb clone 6F11 and the rmAb SP1 was used.

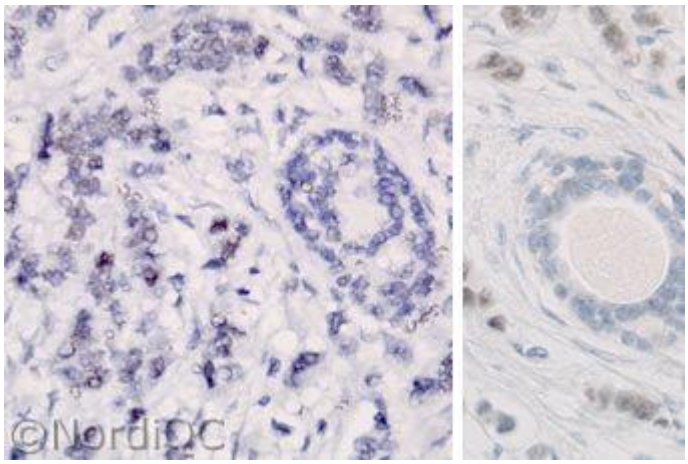


Fig. 4b
Insufficient staining (borderline) of the breast ductal carcinoma no. 4 using the rmAb clone SP1 with excessive HIER. The nuclei of both the neoplastic cells and the entrapped normal epithelial cells show a severe impairment of the morphology complicating the interpretation.
Insert right: Optimal staining of the same tumour using same clone but with appropriate HIER settings. The majority of the neoplastic cells show a weak but distinct nuclear staining.

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