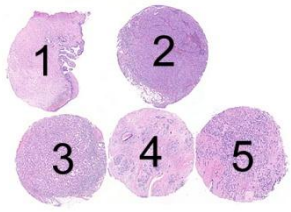
Assessment Run B8 2009

Estrogen Receptor (ER) alpha

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	Negative
3.	Breast ductal carcinoma	Negative	Negative
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 and processed according to the "Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry", AIMM, vol 16, no. 6, 2008.

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 carcinoma no. 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 3 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, as this did not influence the interpretation.

144 laboratories participated in this assessment. 74 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for ER, run B8**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone **SP1**	35 2 1 1	NeoMarkers Dako Diagnostic Biosystems Zhongshan Bio	14	13	8	4	69 %	71 %
mAb clone **6F11**	22 2 2 1	Novocastra Monosan Vector Biocare	14	7	4	2	78 %	84 %
mAb clone **1D5**	20 4	Dako Immunologic	4	9	4	7	54 %	76 %
mAb clones **1D5+6F11**	2	NeoMarkers	0	1	1	0	-	-
Ready-To-Use Abs								
rmAb clone **SP1, 790-4324/25**	40	Ventana	33	5	1	1	95 %	97 %
rmAb clone **SP1, IR151**	7	Dako	1	3	2	1	57 %	67 %
mAb clone **1D5, IS654**	1	Dako	1	0	0	0	-	-
mAb clones **1D5 + ER-2-123, K4071/SK310**	2	Dako	0	1	0	1	-	-
mAb clone **6F11** + rmAb clone **SP1, IP308**	1	BioCare	0	1	0	0	-	-
mAb clone **6F11, PA0151**	1	Novocastra	0	0	0	1	-	-

Total	144		67	40	20	17	107	-
Proportion			46 %	28 %	14 %	12 %	74 %	-

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 (2/12)*, Target Retrieval Solution (TRS) pH 9 (EnVision FLEX TRS high pH, Dako, (4/10), Cell Conditioning 1 (BenchMark, Ventana) (3/4), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/2), EDTA/EGTA pH 8 (1/1)* or Citrate pH 6 (3/7) 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 25 out of 35 (71 %) 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 (4/8), TRS pH 9 (EnVision FLEX TRS high pH, Dako, (4/6), Bond Epitope Retrieval Solution 2 (Bond, Leica) (3/6), Cell Conditioning 1 (BenchMark, Ventana) (1/4) or Citrate pH 6 (2/3) as retrieval buffer. The mAb was typically diluted in the range of 1:30– 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 21 out of 25 (84 %) laboratories produced a sufficient staining (optimal or good).

mAb **1D5**: the protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (1/12) or TRS pH 9 (EnVision FLEX TRS high pH, Dako, (3/8) 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 13 out of 17 (76 %) 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 on the BenchMark XT or the Ultra 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. 1 laboratory used the Ab with HIER in Citrate pH 6, UltraView + amplification using the Nexes. Using these protocol settings 38 out of 39 (97 %) laboratories produced a sufficient staining.

rmAb clone **SP1**, prod. no IR151, Dako: The protocol giving an optimal result was 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) as the detection system. Using these protocol settings 4 out of 6 (67 %) laboratories produced a sufficient staining.

mAb clone **1D5**, prod. no IS654, Dako: The protocol giving an optimal result was 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) as the detection system.

The most frequent causes of insufficient stainings were:
- Too low concentration of the primary antibody.
- Insufficient HIER (use of citrate pH 6.0 and/or too short efficient heating time)
- Excessive retrieval impairing the morphology

In this assessment the prevalent feature of an insufficient staining was a general too weak reaction or complete false negative reaction especially in the ductal carcinoma no. 4 with 60-80% positivity. This pattern was seen in 89 % (33 out of 37) of the insufficient results. As found 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 the epithelial cells throughout the layers of the squamous epithelium and in the glands showed a moderate to strong and distinct nuclear reaction compared to the protocols giving insufficient results in which both the proportion of positive cells and the intensity was significantly reduced. In this run, 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. An optimal staining could both be obtained, when the Abs were used as a concentrate and applied in an in-house protocol or applied as a Ready-To-Use format.
In table 2 the overall performance and accumulated pass rates of the three most widely used markers for ER in the NordiQC assessments are listed.
As observed in the previous assessment of ER an insufficient staining could also be due to excessive HIER typically as a consequence of too long heating time and/or too high temperature hampering the morphology and thus complicating the interpretation. This pattern was seen in 11 % of the insufficient results.

Table 2. **Results for the three most used Abs in eight 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**	244	150 (62 %)	44 (18 %)	127	91 (72 %)	44 (35 %)
mAb **6F11**	240	182 (76 %)	94 (39 %)	190	164 (86 %)	101 (53 %)
rmAb **SP1**	247	210 (85 %)	151 (61 %)	231	208 (90 %)	151 (65 %)

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

This was the 8' assessment of ER in the NordiQC breast module and a relative constant proportion of sufficient results have been obtained in the previous 6 runs as shown in table 3, despite many new participants:

Table 3. **Sufficient over-all results for ER in the eight NordiQC runs**

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

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 shall 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 shall show a strong distinct nuclear reaction with minimal cytoplasmic reaction.

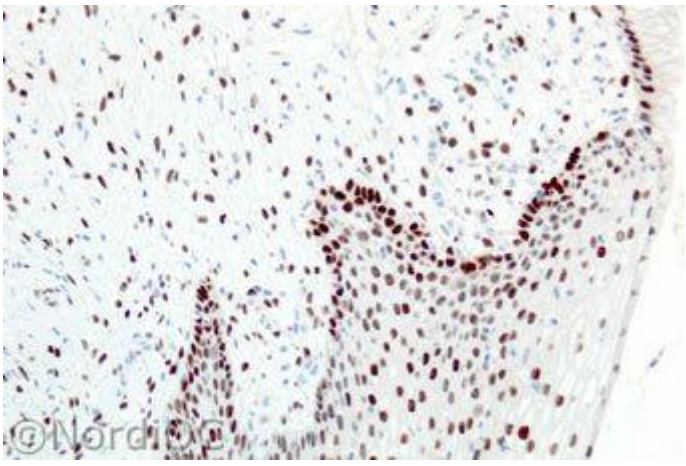


Fig. 1a
Optimal ER staining of the uterine cervix using the rmAb clone SP1. Virtually all the squamous and columnar epithelial cells show a distinct nuclear staining. The majority of the stromal cells are demonstrated and only endothelial and lymphoid cells are negative.

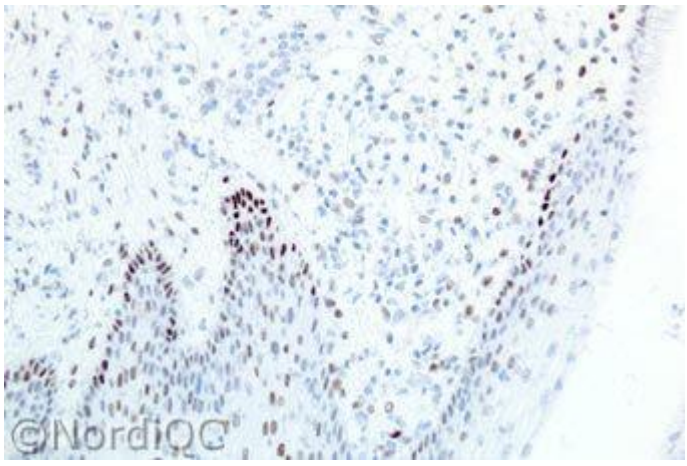


Fig. 1b
Insufficient ER staining of the uterine cervix – same field as in Fig. 1a. Only scattered (mainly basal) epithelial and stromal cells show a weak to moderate nuclear staining. Also compare with Figs. 2b and 3b – same protocol. The protocol was based on the mAb clone 1D5 and HIER in citrate pH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

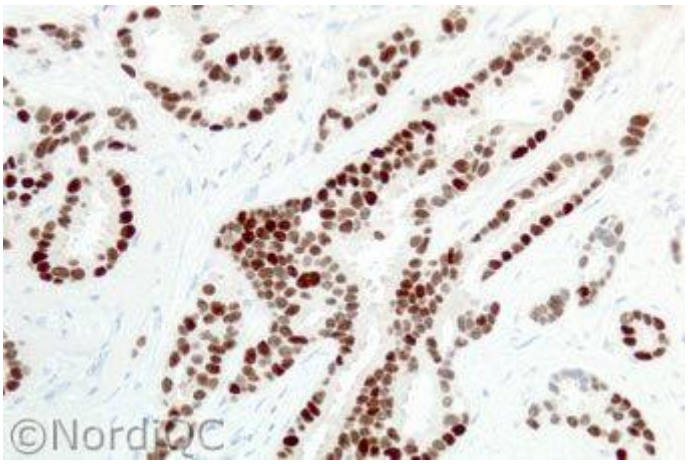


Fig. 2a
Optimal ER staining of the breast ductal carcinoma with 90 – 100 % cells positive. Virtually all the nuclei of the neoplastic cells are strongly stained with a weak cytoplasmic reaction – note the cytoplasmic reaction is only seen in the neoplastic cells, while the background is negative. Same protocol as in Fig. 1a.

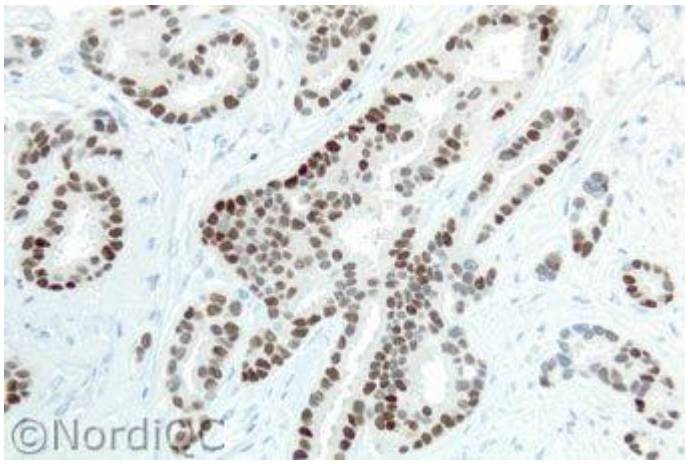


Fig. 2b
ER staining of the ductal breast carcinoma with 90 – 100 % cells positive using an insufficient protocol – same field as in Fig. 2a. Virtually all the nuclei of the neoplastic cells are stained. However, compare with Fig. 3b – same protocol.

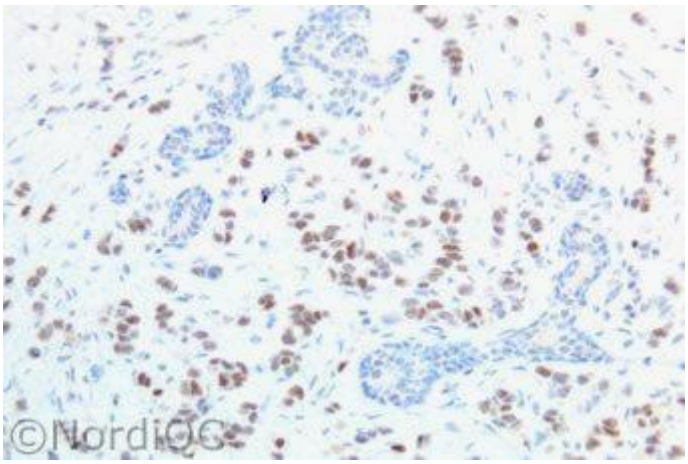


Fig. 3a
Optimal ER staining of the breast ductal carcinoma with 60 – 80 % cells positive. The majority of the nuclei show a weak to moderate staining. Same protocol as in Figs. 1a and 2a.

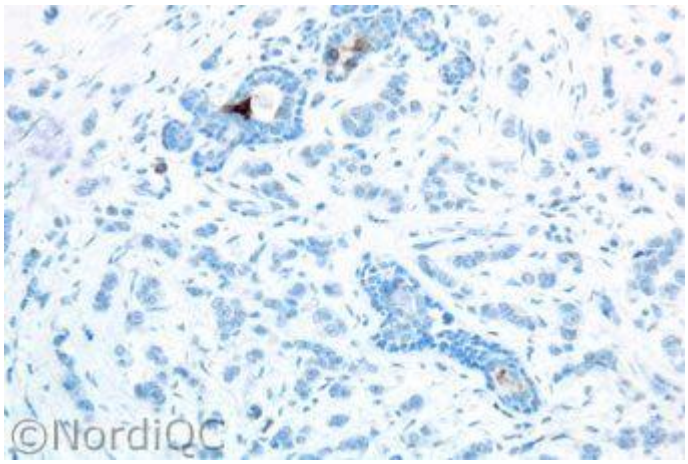


Fig. 3b
Insufficient ER staining of the breast ductal carcinoma with 60 – 80 % cells positive using same protocol as in Fig. 1b and 2b. No nuclear staining reaction is seen in the neoplastic cells. A cytoplasmic reaction is seen in scattered epithelial cells of the normal glands.

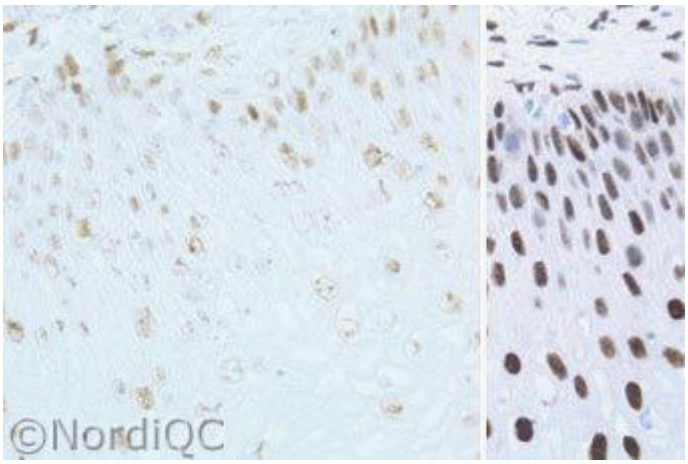


Fig. 4a
Left: Insufficient staining of the uterine cervix using the rmAb clone SP1 with excessive HIER. The nuclei of the epithelial cells show a severe impairment of the morphology complicating the interpretation. The nuclei show a granular positivity and many nuclei are almost "empty" and only the nuclear membrane can be identified.
Right: Optimal staining of the uterine cervix using same clone after an appropriate HIER setting. The nuclei are preserved and the positive nuclear reaction can without difficulty be interpreted.

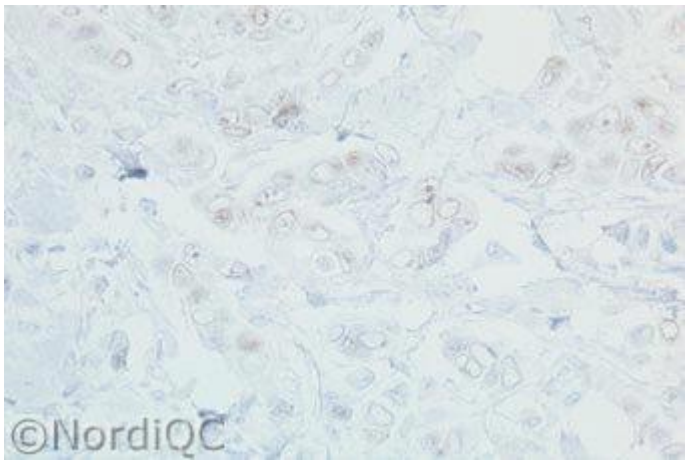


Fig. 4b
Insufficient staining of the breast ductal carcinoma with 60 – 80 % cells positive using same insufficient protocol as in Fig. 4a left. The nuclei of the neoplastic cells show a severe impairment of the morphology complicating the interpretation.

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