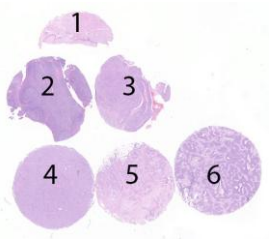
## Assessment Run B19 2015

# 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. | Tonsil | < 2-5% | Weak to strong |
| 3. | Breast carcinoma | 0% | Negative |
| 4. | Breast carcinoma | 40-60% | Weak to moderate |
| 5. | Breast carcinoma | 60-80% | Weak to strong |
| 6. | Breast carcinoma | 90-100% | Moderate to strong |




*ER-status and staining pattern as characterized by NordiQC reference laboratories using 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 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.
- An at least weak to moderate nuclear staining reaction of dispersed germinal centre and squamous epithelial cells of the tonsil.
- At least weak to moderate distinct nuclear staining reaction in the appropriate proportion of the neoplastic cells in the breast carcinomas no. 4, 5 and 6.
- No nuclear staining reaction of neoplastic cells in the breast carcinoma no. 3.
- 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. 4, 5 and 6 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 % but < 10 % of the neoplastic cells showed a nuclear staining reaction in one or more of the breast carcinomas no. 4, 5 & 6.

The staining reactions were classified as **poor** if a false negative (<1% positive cells) or false positive (>1% positive cells) staining reaction was seen in one of the breast carcinomas.

**Participation**

| | |
|---|---|
| Number of laboratories registered for ER, run B19 | 359 |
| Number of laboratories returning slides | 345 (96%) |

**Results**
345 laboratories participated in this assessment. 234 (68%) of these achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining results were:
- Insufficient HIER - too short efficient HIER time and/or use of a non-alkaline buffer
- Too low concentration of the primary Ab.
- Less successful primary Ab.

**Conclusion**
The mAb clone **6F11** and rmAb clones **EP1** and **SP1** could all be used to provide an optimal result for 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 and squamous epithelial cells must show a moderate to strong and distinct nuclear staining reaction. Lymphocytes and all endothelial cells must be negative.

Table 1. **Antibodies and assessment marks for ER, run B19**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **1D5** | 7<br>2<br>1 | Dako<br>Immunologic<br>Zytomed | 0 | 0 | 2 | 8 | - | - |
| mAb clone **6F11** | 36 | Leica/Novocastra | 2 | 13 | 5 | 16 | 42% | 50% |
| rmAb clone **EP1** | 17 | Dako | 2 | 9 | 2 | 4 | 65% | 100% |
| rmAb clone **SP1** | 30<br>4<br>3<br>2 | Thermo/Neomarkers<br>Immunologic<br>Cell Marque<br>Spring Bioscience | 16 | 10 | 7 | 6 | 67% | 74% |
| Unknown | 1 | Unknown | 1 | 0 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **1D5 IR/IS657** | 14 | Dako | 0 | 0 | 3 | 11 | - | - |
| mAb clones **1D5 + ER-2-123 K4071** | 2 | Dako | 0 | 1 | 0 | 1 | - | - |
| mAb clone **6F11 PA0151** | 3 | Leica/Novocastra | 0 | 0 | 1 | 2 | - | - |
| mAb clone **6F11 PDM048** | 1 | DBS | 0 | 0 | 0 | 1 | - | - |
| rmAb **EP1 IR/IS084** | 54 | Dako | 9 | 17 | 21 | 7 | 48% | 63% |
| rmAb clone **EP1 AN710-5M** | 1 | Biogenex | 0 | 0 | 0 | 1 | - | - |
| rmAb clone **SP1 790-4324/5** | 162 | Ventana | 75 | 75 | 9 | 3 | 93% | 95% |
| rmAb clone **SP1 IR151*** | 1 | Dako | 0 | 0 | 0 | 1 | - | - |
| rmAb clone **SP1 ILM30142-R25** | 1 | Immunologic | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **SP1 KIT-0012** | 1 | Maixin | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **SP1 MAD-000306QD** | 1 | Master Diagnostica | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **SP1 RM-9101-R7** | 1 | Thermo/Neomarkers | 0 | 1 | 0 | 0 | - | - |
| Total | 345 | | 107 | 127 | 50 | 61 | - | |
| Proportion | | | 31% | 37% | 14% | 18% | 68% | |

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

**Detailed analysis of ER, Run B19**
The following protocol parameters were central to obtain optimal staining:

**Concentrated antibodies**
mAb clone **6F11**: Protocols with optimal results were based on HIER using Cell Conditioning 1 (CC1; Ventana) (1/6)* or Tris-EDTA/EGTA pH 9 (1/5) as retrieval buffer. The mAb was typically diluted in the range of 1:40-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 4 of 8 (50%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP1**: Protocols with optimal results were based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (1/7) or Bond Epitope Retrieval Solution 2 (BERS2; Leica) (1/1) as retrieval buffer. The rmAb was diluted in the range of 1:30-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 4 of 4 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP1**: Protocols with optimal results were all based on HIER using TRS pH 9 (3-in-1) (Dako) (2/5), TRS 9 (Dako) (2/2), CC1 (Ventana) (4/8) BERS2 (Leica) (4/8), Tris-EDTA/EGTA pH 9 (2/3) or Citrate pH 6 (2/6) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 depending

on the total sensitivity of the protocol employed. Using these protocol settings 20 of 27 (74%) 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** | - | - | 1/5 (20%) | - | 0/6 (0%) | 0/7 (0%) |
| rmAb clone **EP1** | 1/3 | - | - | - | 1/1 | - |
| rmAb clone **SP1** | 1/5 (20%) | 0/2 | 4/6 (67%) | - | 4/6 (67%) | 0/1 |

* 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**
rmAb clone **EP1**, product no. **IR/IS084**, 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 20 min. at 97-99°C), 20 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 17 of 27 (63%) laboratories produced a sufficient staining result (optimal or good).

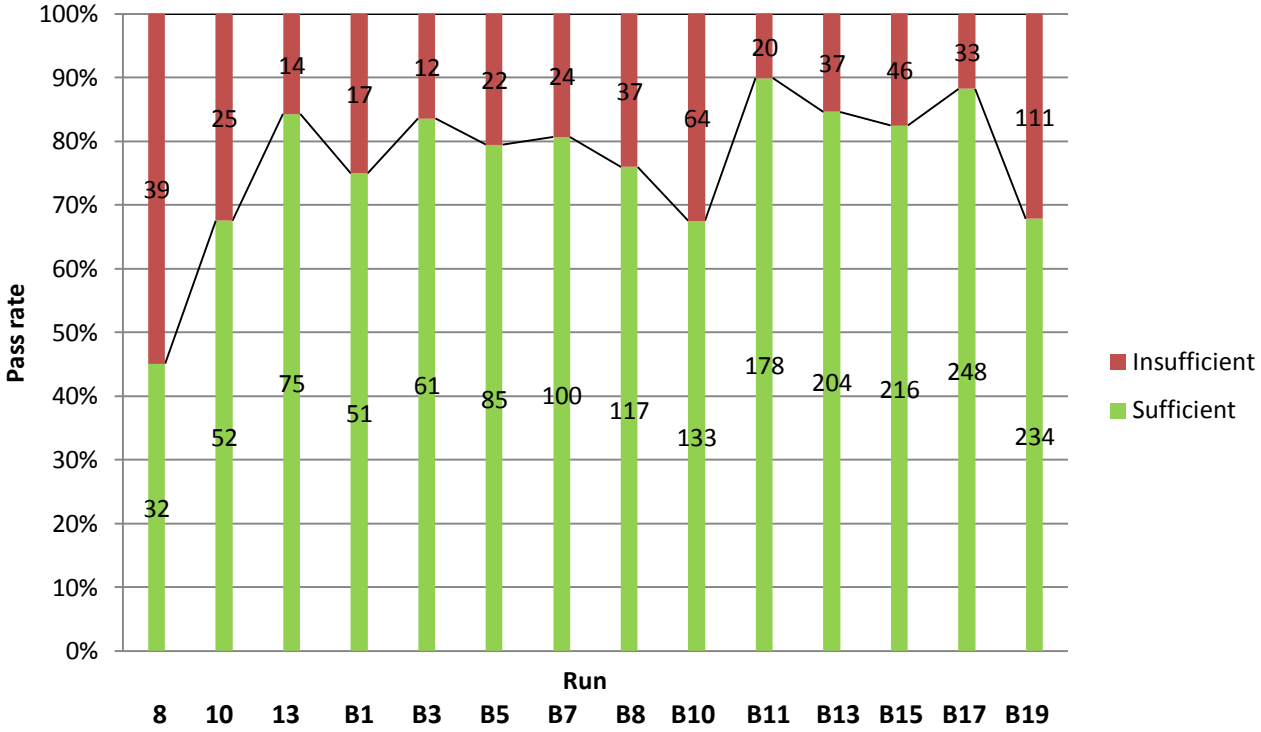
rmAb clone **SP1**, product no. **790-4324/5**, Ventana, BenchMark XT, GX, ULTRA:
Protocols with optimal result typically based on HIER using Cell Conditioning 1 (efficient heating time 30-64 min.), 12-44 min. incubation of the primary Ab and UltraView (760-500) +/- amplification kit or OptiView (760-700) as detection system. Using these protocol settings 134 of 141 (95%) laboratories produced a sufficient staining result.

rmAb clone **SP1**, product no. **KIT-0012**, Maixin, Manual staining
One protocol with an optimal result was based on HIER (Pressure Cooker) using Citrate pH 6 and 60 min. incubation of the primary Ab and KIT-5230 as detection system.

**Comments**
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 96% of the insufficient results (107 of 111).
Virtually all laboratories were able to demonstrate ER in the high and moderate level ER expressing breast carcinomas. Tissue core no. 5 and 6 were thus expected to show a staining reaction in 60-80% and 90-100% of the neoplastic cells. Demonstration of ER in the breast carcinomas no. 4 in which a weak nuclear staining reaction of 40% of the neoplastic cells was expected, was much more challenging and required a carefully calibrated protocol.
The rmAb clone SP1 and in particular the Ventana Ready-To-Use (RTU) format of the rmAb clone SP1 was most successful and provided a high proportion of sufficient and optimal results.
Using the rmAb SP1 in a laboratory developed (LD) assay, optimal results could be obtained on all 3 main IHC platform from Dako, Leica and Ventana, see table 2.
Also the mAb clone 6F11 and rmAb clone EP1 provided optimal results by a LD assay. Irrespective of the clone applied, efficient HIER, preferable in an alkaline buffer, was a central parameter for optimal results. Using HIER in a non-alkaline buffer, such as citrate pH 6, a pass rate of 22% (5 of 23 protocols) and 9% optimal were seen, whereas HIER in an alkaline buffer provided a pass rate of 51% (48 of 93) and 19% optimal.
In this assessment no sufficient result was obtained by the use of the mAb clone 1D5. All 24 protocols based on the mAb clone 1D5 were assessed as insufficient irrespective of the protocol settings otherwise being identical to the settings giving a suffcient staining performance for e.g. the rmAb clones SP1 and EP1. Typically a false negative staining result was observed. The Ventana RTU format of the rmAb clone SP1 gave a pass rate of 93% and optimal result could be obtained both by the official recommended protocol settings using 16 min. incubation of the primary Ab, HIER in CC1 for 64 min. and UltraView as detection kit. An optimal result could also be obtained by laboratory defined modifications of the protocol, typically adjusting the incubation time of the primary Ab or using a reduced HIER time. When using the recommended settings for HIER in CC1 for 64 min. a higher proportion of optimal results was seen compared to the use of reduced HIER time (typically using 32 min). Protocols based on HIER in CC1 for 64 min. provided a proportion of optimal results of 67% (30 of 45) and 39% (45 of 117) if HIER was less than 64 min. Similar observations were made for the Dako RTU format of the rmAb clone EP1. If HIER was performed as recommended for 20 min. in TRS High pH a pass rate of 67% (20 of 30) and 23% optimal were seen. If a reduced HIER time was applied (typically 10 min. at 97°C) a pass rate of 14% (2 of 14) was seen and 7% optimal. The pass rate for the Dako RTU format was inferior to the level seen for the Ventana RTU format for the demonstration of ER.

**Performance history**
This was the 14th NordiQC assessment of ER. The proportion of sufficient results was reduced as compared to the latest runs (Figure 1).

Fig. 1. **Participant numbers and pass rates for ER during 14 runs**




The decrease of the number of sufficient results can be caused by many factors. The combination of many laboratories participating for the first time and slightly more challenging material circulated might be the main parameters. A slight difference regarding the pass rates was thus observed for the laboratories participating in the ER assessment for the first time compared to the laboratories also participating in the latest assessment run B17, 2014. For the laboratories participating for the first time the pass rate was 51% (44 of 86 laboratories), whereas the pass rate was 73% (190 of 259 laboratories) for the laboratories participating in both runs.

**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 reaction in the breast carcinoma no. 4 was seen.
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 alone as they express high levels of ER.
In this assessment tonsil was included for the first time to evaluate the potential as tissue control for ER. It was observed that dispersed germinal centre and squamous epithelial cells were distinctively demonstrated in virtually all protocols providing an optimal result in the other tissues. Tonsil will be included in next assessments to further evaluate this observation and potential of tonsil as additional tissue control for the calibration of the ER protocol.

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.

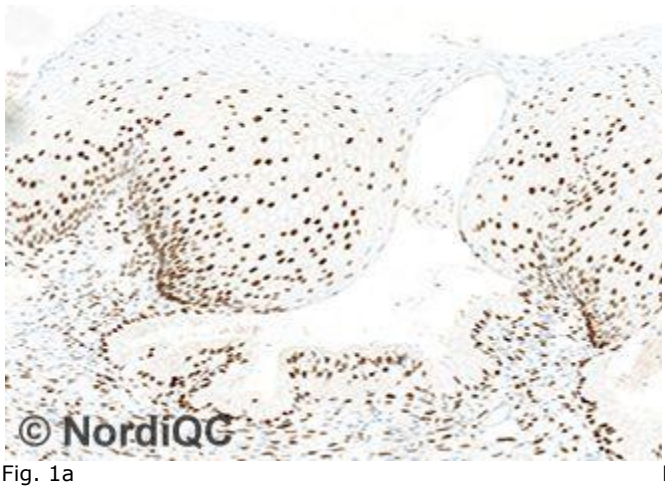



Fig. 1a
Optimal ER staining result of the uterine cervix using the rmAb clone SP1 optimally calibrated and with HIER in an alkaline buffer. Virtually all the squamous and columnar epithelial cells show a moderate to strong, distinct nuclear staining reaction. The majority of the stromal cells are demonstrated and only endothelial and lymphoid cells are negative. Also compare with Figs. 2a – 5a, same protocol.

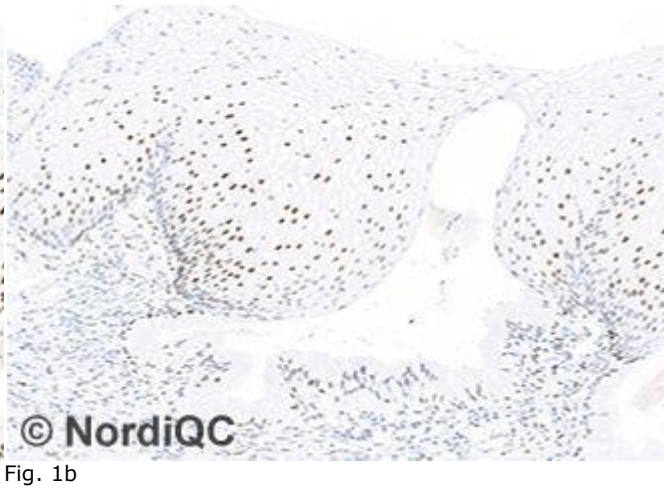



Fig. 1b
Insufficient ER staining result of the uterine cervix, same field as in Fig. 1a. The proportion and intensity of the staining reaction in the squamous and especially in columnar epithelial cells is reduced. Also compare with Figs. 2b - 4b, same protocol. The protocol was based on the rmAb clone SP1 applied with protocol settings giving a too low sensitivity – most likely due to a too dilute titre of the primary Ab.

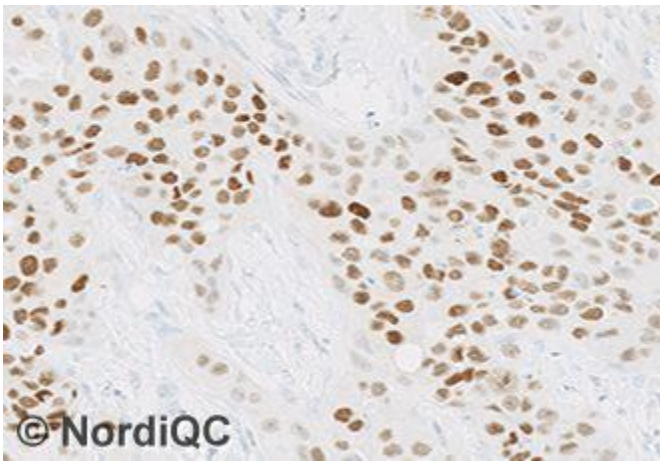



Fig. 2a
Optimal ER staining result of the breast ductal carcinoma no. 5 with 60 – 80% cells positive using same protocol as in Fig. 1a. The vast majority of the neoplastic cells show a strong, distinct nuclear staining reaction with only a weak cytoplasmic staining reaction. No background staining is seen.

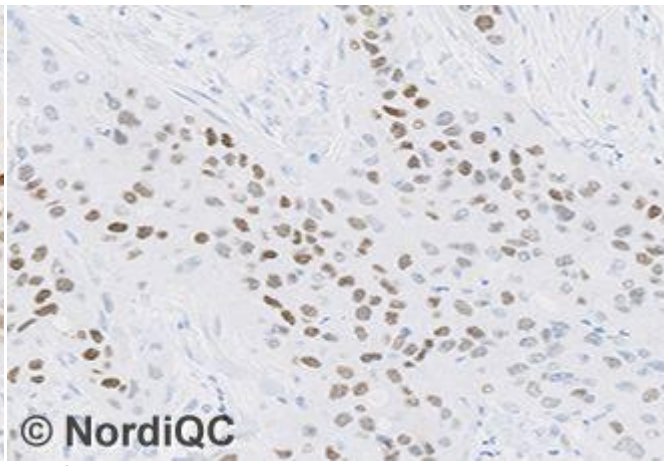



Fig. 2b
ER staining result of the breast ductal carcinoma no. 5 with 60 – 80% cells positive using the same protocol as in Fig. 1b – same field as in Fig. 2a. The majority of neoplastic cells are demonstrated. However also compare with Figs. 3b and 4b – same protocol.

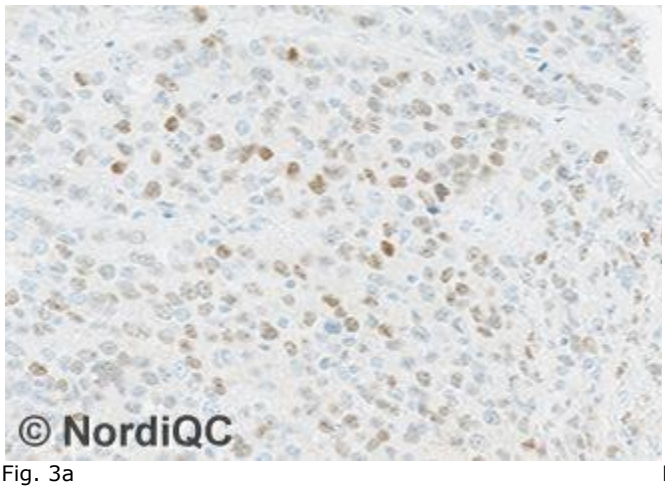



Fig. 3a
Optimal ER staining result of the breast ductal carcinoma no. 4 with 40 – 60% cells positive. A weak but distinct nuclear staining reaction in the appropriate proportion of the neoplastic cells is seen. Same protocol as in Figs. 1a and 2a.

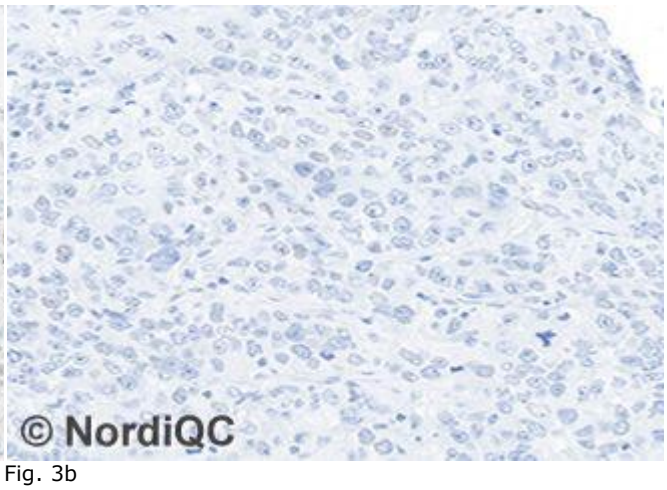



Fig. 3b
Insufficient ER staining result of the breast ductal carcinoma no. 4 with 40 – 60% cells positive using same protocol as in Figs. 1b and 2b – same field as in Fig. 4a. A false negative staining reaction is seen.

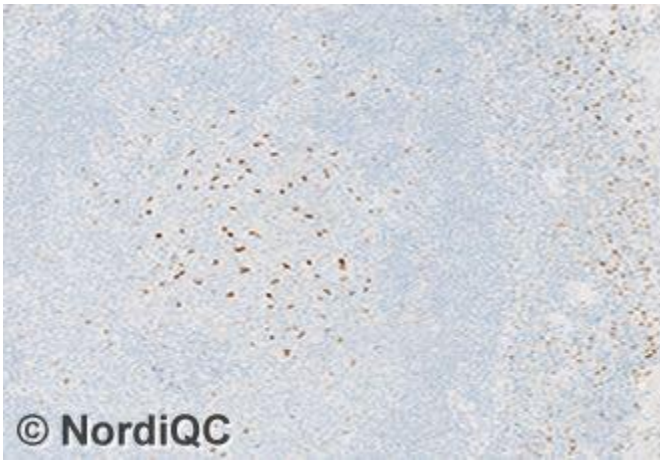



Fig 4a
Optimal ER staining result of the tonsil using same protocol as in Figs. 1a – 3a. A weak to moderate nuclear staining reaction of dispersed germinal centre and squamous epithelial cells is seen. The nuclear staining reaction can be seen at low magnification, x100. Lymphocytes are negative.

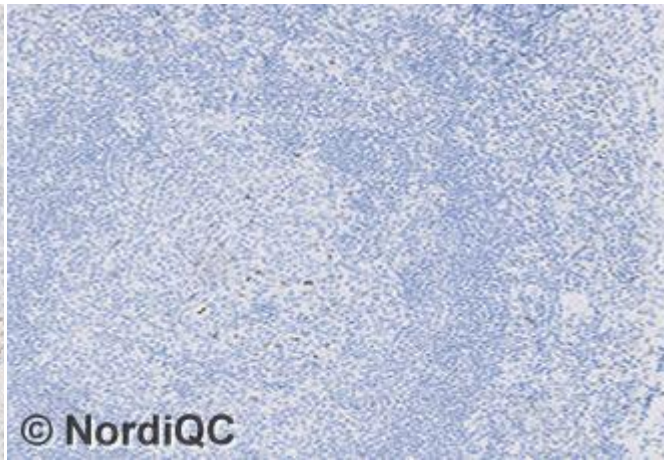



Fig 4b
Insufficient ER staining result of the tonsil using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a. Compared to the result obtained in Fig. 4a, only a faint nuclear staining reaction in a significantly reduced proportion of cells is seen.

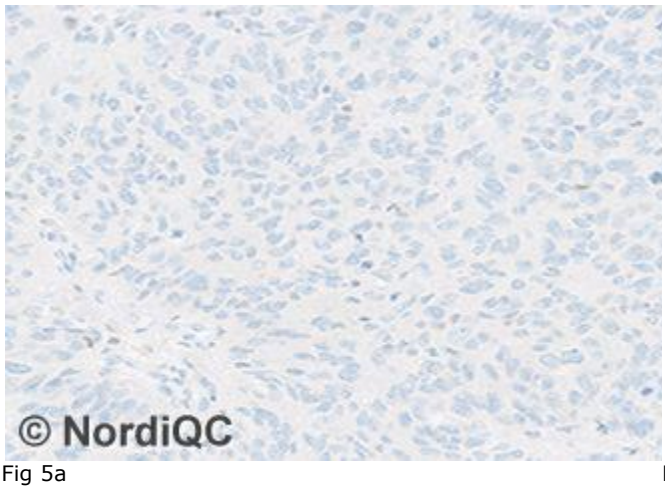



Fig 5a
Optimal ER staining result of the breast carcinoma no. 3 with no ER expression. Only dispersed stromal cells show a weak nuclear staining reaction, while all neoplastic cells are unstained. Same protocol as in Figs. 1a – 4a.

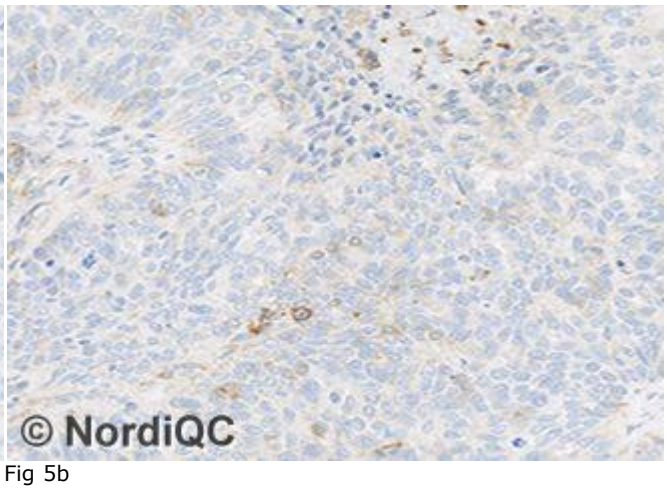



Fig 5b
ER staining result of the breast carcinoma no. 3 with no ER expression using the mAb clone 1D5. The neoplastic cells show a weak to moderate aberrant cytoplasmic staining reaction compromising the interpretation. This pattern was typically seen when the clone was applied by a protocol providing a high and required sensitivity e.g based on HIER in alkaline buffer and 3-step polymer based detection system.

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