

The slide to be stained for estrogen receptor alpha (ER) comprised:

1. Breast fibrocystic disease, 2. Uterine cervix, 3-5. Ductal breast carcinoma (ER-positivity verified in four reference laboratories: 3: 10–30 %, 4: 40–60 %, 5: 80–100 %).

Criteria for assessing an ER staining as optimal included:

A distinct nuclear staining reaction in the three ductal breast carcinomas comprising a proportion of cells corresponding the above mentioned ranges. A weak cytoplasmic staining of cells with strong nuclear staining was accepted, as was staining of necrotic tissue. The glandular tissue in the fibrocystic breast disease should display a heterogeneous nuclear reaction and the stromal cells of the uterine cervix a widespread positivity.

77 laboratories submitted stainings. At the assessment 19 achieved optimal staining (25 %), 33 good (43 %), 20 borderline (26 %) and 5 (6%) poor staining.

The following Ab's were used:

mAb clone 6F11 (Novocastra, n=27; Ventana, n=13).  
 mAb clone 1D5 (DakoCytomation, n=30; Immunotech, n=4).  
 mAb clone SP1 (NeoMarkers, n=3).

Optimal stainings could be obtained with all 3 clones (6F11: 11/40, 1D5: 7/34, and SP1: 1/3). In the protocols giving optimal results, all used HIER (MWO, n=14; pressure cooker, n=3; water bath, n= 2). Using MWO the efficient heating time (at 100°C) was 15-25 min, the total heating time 20–30 min.

Using a pressure cooker the efficient heating time was 12 min (108°C) or 3 min (120°C). The heating buffer in the optimal protocols was Tris-EDTA/EGTA pH 9 (n=17) or Citrate pH 6 (n=2). Using clone 6F11, optimal stainings was obtained with a concentration in the range of 1:10–1:200. With clone 1D5, the concentration was in the range of 1:25–1:75 (both ranges depending on the total sensitivity of the protocol). Clone SP1 was used in 1:200.

Generally, clone 1D5 gave a more pronounced cytoplasmic reaction than clones 6F11 and SP1.

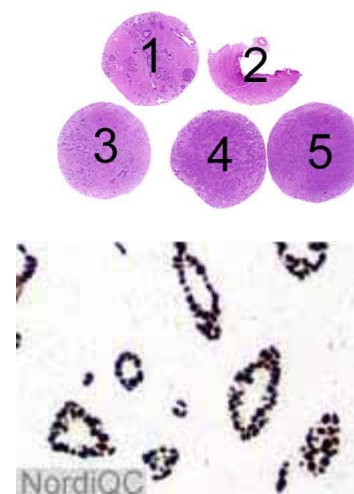
The most prevalent feature of the insufficient stainings (25/77) was a false negative reaction of the ductal breast carcinoma with 10–30 % positivity. Almost all laboratories were able to detect ER in the specimen with 80-100 % positivity.

The most frequent causes of insufficient stainings (often in combination) were:

- Insufficient HIER, especially too short efficient heating time (<15 min) often in combination with citrate pH 6
- Too low concentration of the primary Ab.

This ER assessment is the second in NordiQC. The proportion of insufficient (borderline or poor) stainings was reduced from 55 % in run 8 to 32 % in the present run 10. However, it should be emphasized that the tumour cases are not identical.

The two main recommendations given to the laboratories producing an insufficient staining in run 8 was to optimize HIER and increase the Ab concentration. 13 (out of 25) laboratories changed their protocols according to these recommendations, of which 10 improved their score in the present run. Among the 12 laboratories not following the recommendations, only three improved their marks.



Optimal ER staining (mAb clone SP1 - same reaction could be obtained with the clones 6F11 and 1D5) of the ductal breast carcinoma with the high expression of ER. > 80 % of the nuclei show a strong staining.

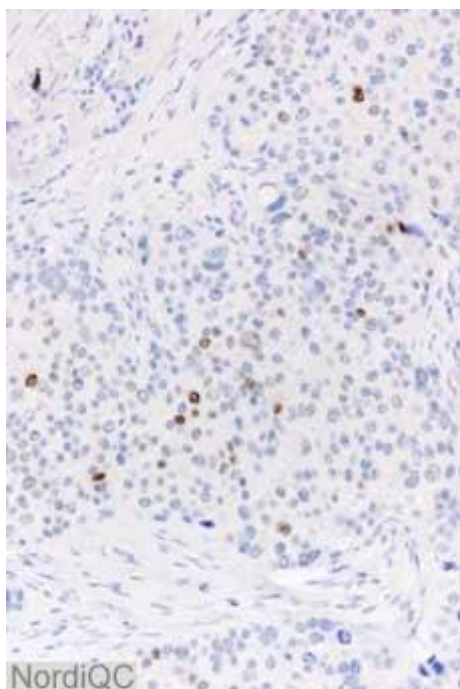


Fig. 1a  
Optimal ER staining (mAb clone 1D5) of the ductal breast carcinoma with the low expression of ER.  
> 10 % of the nuclei show a weak to moderate staining.

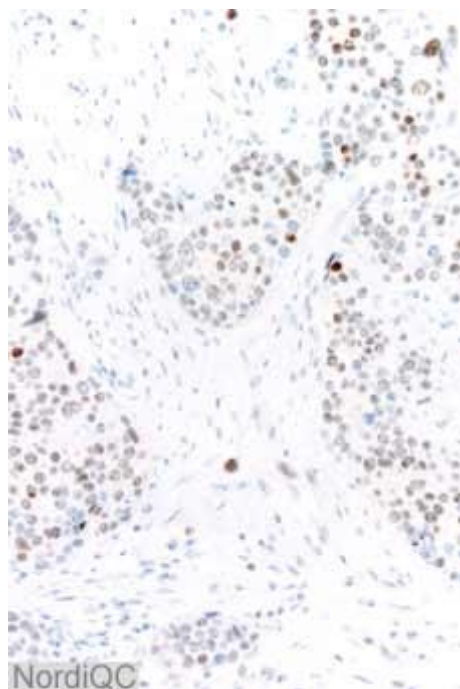


Fig. 1b  
Optimal ER staining (mAb clone 6F11) of the ductal breast carcinoma with the low expression of ER.  
> 10 % of the nuclei show a moderate staining.

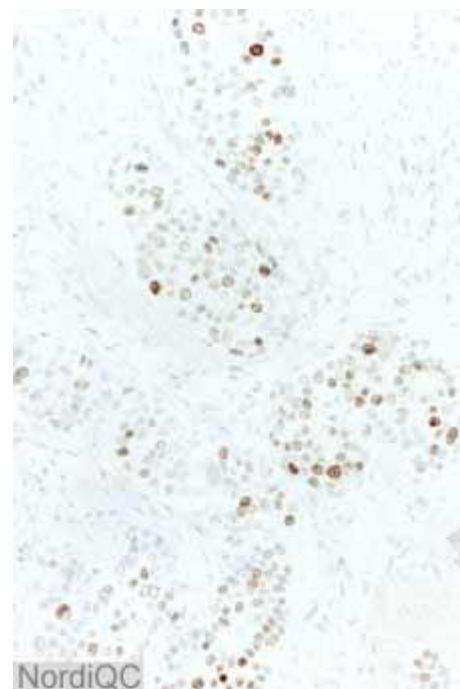


Fig. 1c  
Optimal ER staining (mAb clone SP1) of the ductal breast carcinoma with the low expression of ER.  
> 10 % of the nuclei show a moderate staining.

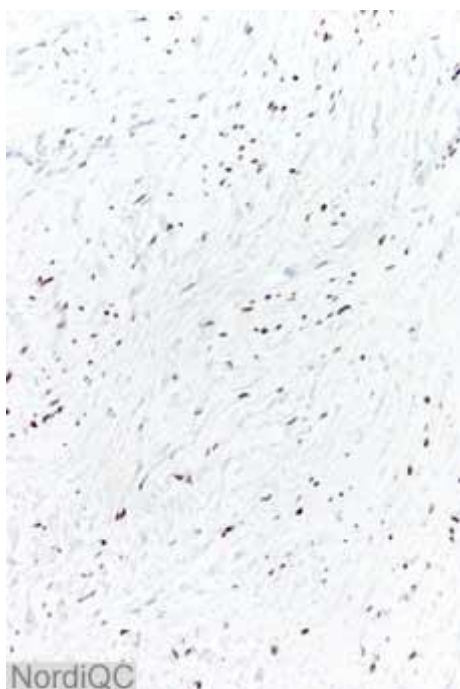


Fig. 2a  
Optimal ER staining (mAb clone 1D5) of the uterine stromal cells.  
Almost all nuclei are strongly stained.

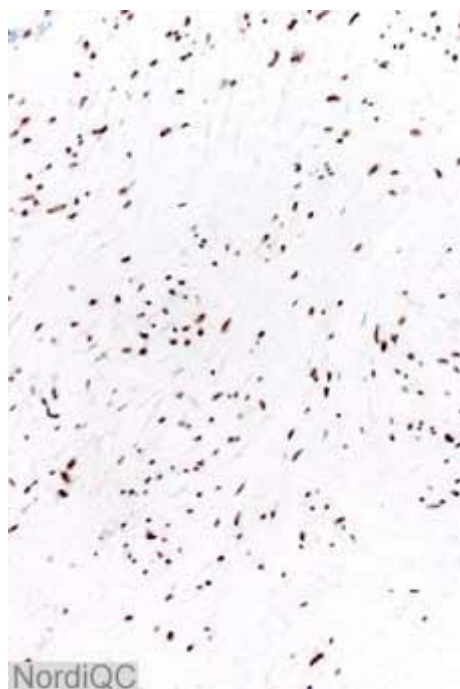


Fig. 2b  
Optimal ER staining (mAb clone 6F11) of the uterine stromal cells. Almost all nuclei are strongly stained.



Fig. 2c  
Optimal ER staining (mAb clone SP1) of the uterine stromal cells. Almost all nuclei are strongly stained.

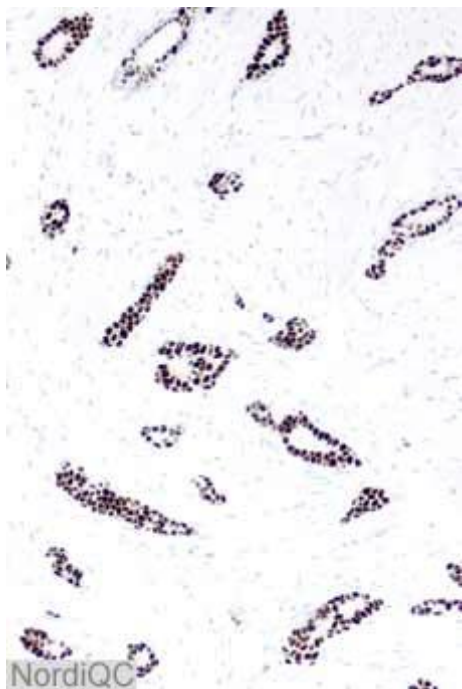


Fig. 3a  
 Good ER staining (mAb clone 1D5) of the ductal breast carcinoma with the high expression of ER.  
 > 80 % of the nuclei show a moderate or strong staining.

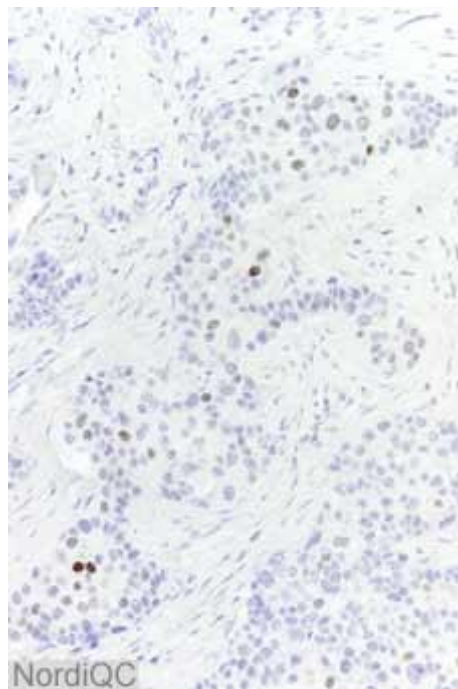


Fig. 3b  
 Good ER staining (mAb clone 1D5) of the ductal breast carcinoma with the low expression of ER.  
 > 10 % of the nuclei show a weak positive staining.



Fig. 3c  
 Good ER staining (mAb clone 1D5) of the uterine stromal cells. Almost all nuclei are moderately stained.

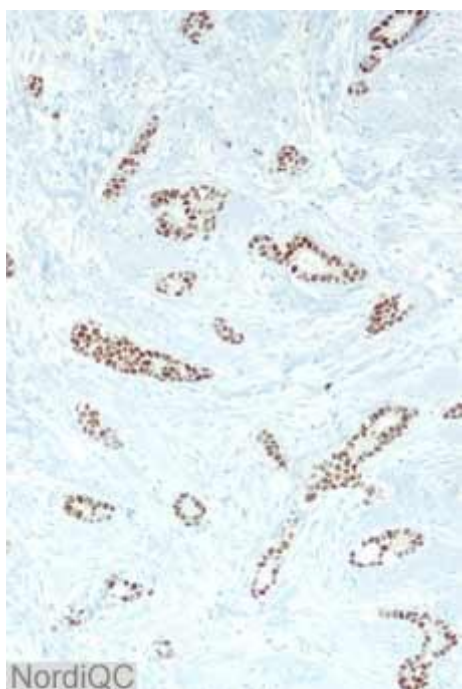


Fig. 4a  
 ER staining of the ductal breast carcinoma with the high expression of ER using an insufficient protocol. > 10 % of the nuclei show a positive staining – however compare with fig. 4b.

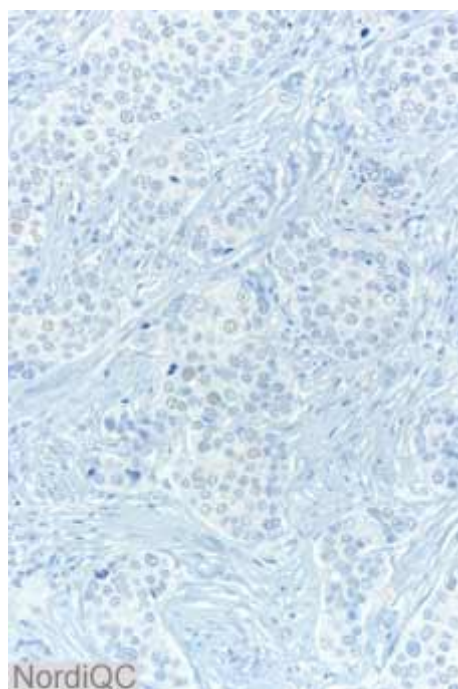


Fig. 4b  
 Insufficient ER staining of the ductal breast carcinoma with the low expression of ER. The nuclei are virtually unstained.

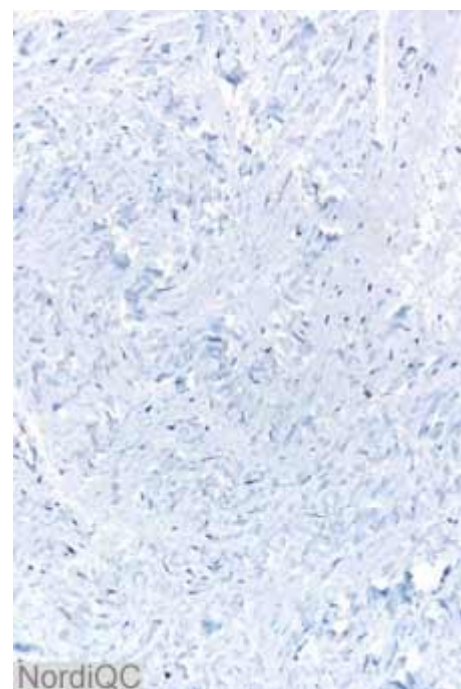


Fig. 4c  
 Insufficient ER staining of the uterine stromal cells. Almost all nuclei are negative.

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