

The slide to be stained for estrogen receptor alpha (ER) comprised:
 1: lung adenocarcinoma, 2: ovarian serous carcinoma, 3-5: breast ductal carcinoma.

Criteria for assessing an ER staining as optimal included:

A strong and distinct nuclear staining reaction for ER in almost all of the neoplastic cells in the ductal breast carcinoma with the high expression of ER, a weak to moderate staining reaction in most neoplastic cells in the ductal carcinoma with the low expression of ER, and a heterogeneous staining of nuclei in the serous ovarian carcinoma. A weak cytoplasmic reaction of cells with strong nuclear staining was accepted, as was staining of necrotic tissue in the lung adenocarcinoma.



71 laboratories submitted stainings. At the assessment 11 achieved optimal staining (15 %), 21 good (30 %), 15 borderline (35 %) and 14 (20%) poor staining.

All 71 laboratories used either mAb clone 6F11 (36) or 1D5 (35). Clone 6F11 was obtained from Novocastra (27) and Ventana (9), 1D5 from DakoCytomation (27), Immunotech (7) and Immunovision (1).

Optimal stainings could be obtained with both clones. In the optimal protocols all used HIER, primarily based on MWO with Tris-EDTA/EGTA pH 9 as the heating buffer with a total heating time (i.e., from starting the MWO until it is turned off) of 20 – 30 min. If citrate pH 6 was used as the heating buffer, the total heating time in optimal stainings was 20 – 40 min.

Using clone 6F11 optimal stainings was obtained with a concentration of 1:10 – 1:75. Using 1D5, the concentration was in the range of 1:25 – 1:75, depending on the incubation time (25 – 60 min.).

In almost all stainings, the ductal breast carcinoma with the high ER expression was labelled. In cases where this tumour showed weaker staining, the other breast carcinoma and the serous carcinoma typically showed false negative reactions.

The probable causes of insufficient stainings were multiple, and often several suboptimal steps in the individual protocols could be identified.

In 22 out of the 29 protocols giving an insufficient result, the total heating time was ≤ 15 min and/or the antibody concentration was 1:200 – 1:1000.

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

- Insufficient HIER (too short heating time, particularly in combination with Citrate pH6)
- Too high or too low concentration of primary antibody.

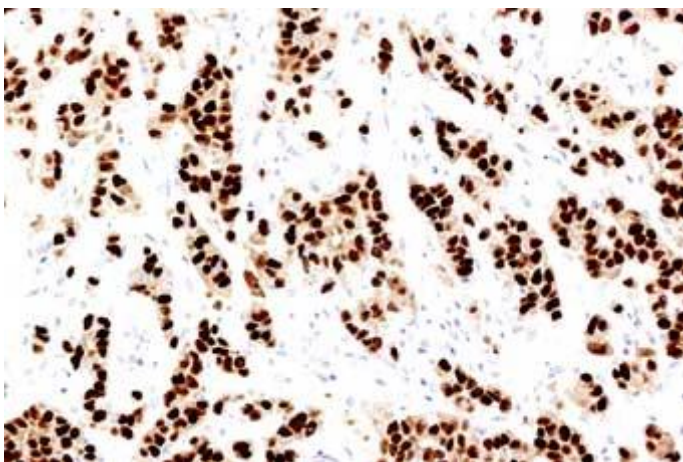


Fig. 1a
 Optimal ER staining (mAb clone 1D5) of the ductal breast carcinoma with the high expression of ER. All nuclei are strongly stained with only a weak intracytoplasmic reaction.

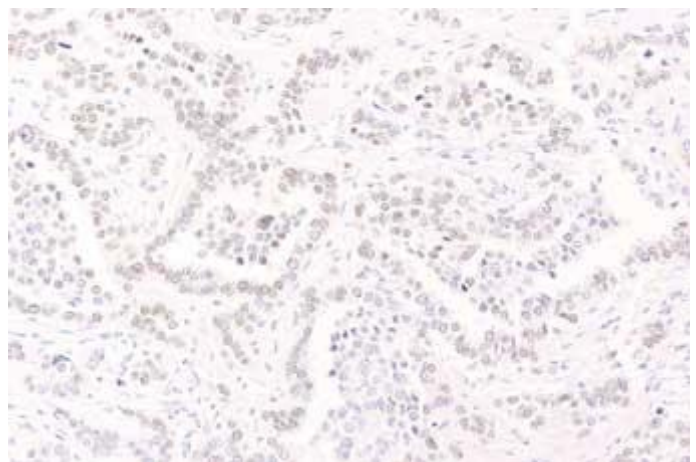


Fig. 1b
 Insufficient ER staining (mAb clone 1D5) of the ductal breast carcinoma with the high expression of ER. The nuclei of the tumour cells are almost negative. Compare with Fig.1a.

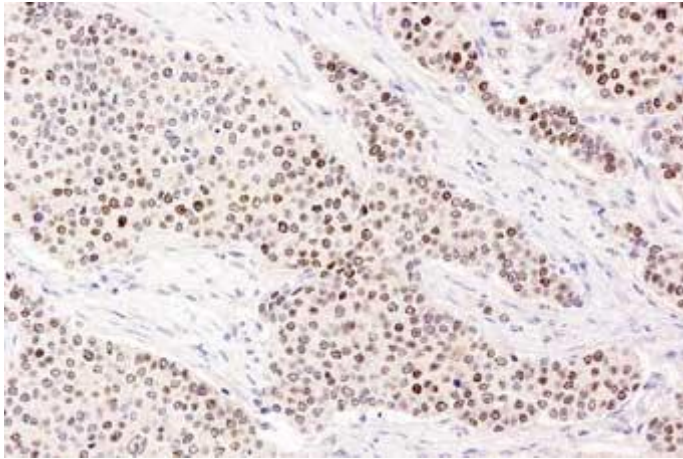


Fig. 2a
Optimal ER staining (mAb clone 6F11) of the ductal breast carcinoma with the low expression of ER. Almost all nuclei are moderately stained with only a weak intracytoplasmic reaction.

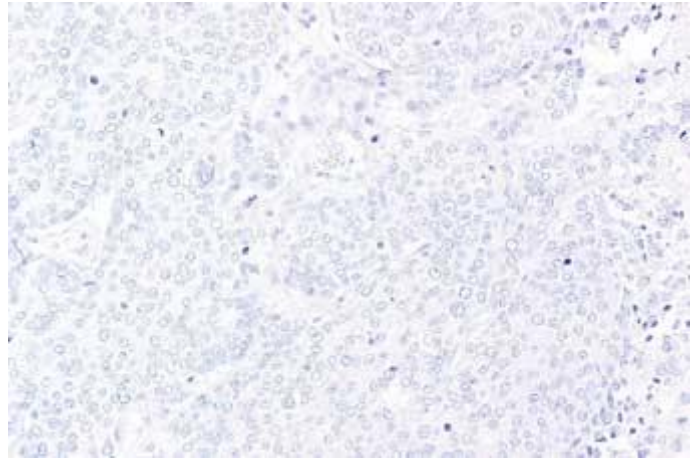


Fig. 2b
Insufficient ER staining (mAb clone 6F11) of the ductal breast carcinoma with the low expression of ER. The tumour cells are all negative. Compare with Fig. 2a..

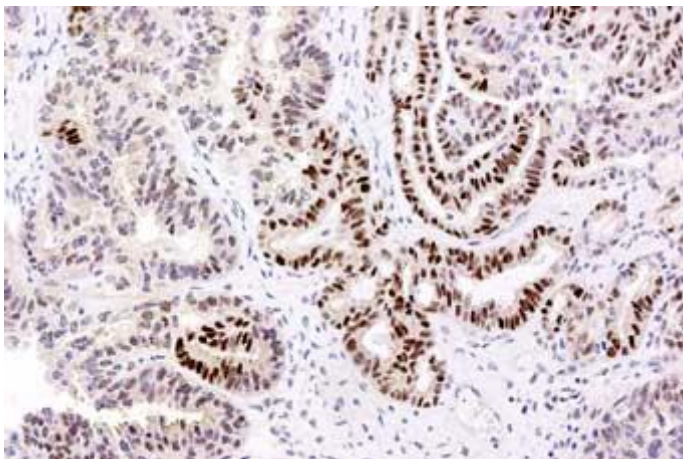


Fig. 3a
Optimal ER staining (mAb clone 6F11) of the serous ovarian carcinoma. The tumour cells show a heterogeneous, focally strong staining.

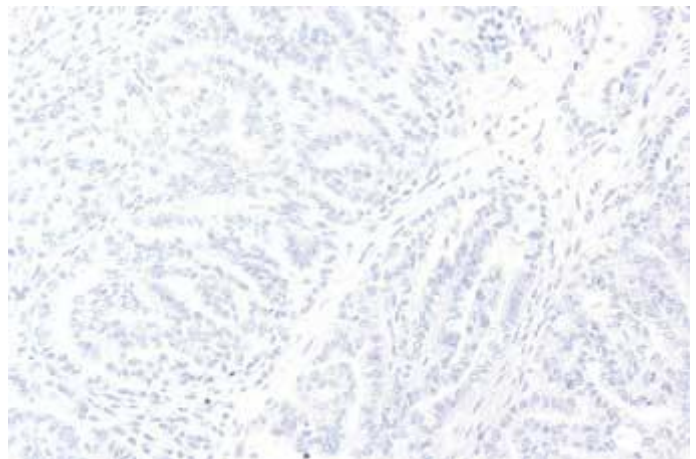


Fig. 3b
Insufficient ER staining (mAb clone 6F11) of the serous ovarian carcinoma. The tumour cells are all negative. Compare with Fig. 3a.

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