Monoclonal Mouse
Anti-Human Estrogen Receptor α
Clone 1D5
Code No. M 7047
Lot 032. Edition 06.03.02

Intended use
For in vitro diagnostic use. DAKO Monoclonal Mouse Anti-Human Estrogen Receptor, Clone 1D5 (Anti-ER, 1D5), is intended for laboratory use to semi-quantitatively identify by light microscopy an epitope located on the N-terminal domain of the estrogen receptor in normal and pathological human cryostat and paraffin-embedded tissue processed in neutral buffered formalin. Positive results aid in the classification of normal and abnormal cells/tissues and serve as an adjunct to conventional histopathology. The clinical interpretation of any positive staining or its absence should be complemented by morphological and histological studies with proper controls. Evaluations should be made within the context of the patient’s clinical history and other diagnostic tests by a qualified individual.

A variety of immunohistochemical staining methods are suitable for use with this antibody.

Introduction
Steroid receptors exhibit a high affinity and specificity for their ligands. The human estrogen receptor (ER) is a dimeric protein of 65 kDa located primarily on the membrane of cell nuclei and belongs to a class of trans-acting proteins which stimulate transcription by binding to specific DNA elements, also known as hormone response elements. Through binding estrogen, the ER is induced to stimulate gene transcription, hence is also known as an inducible enhancer factor (1).

Measurement of the ER has been shown to be prognostically relevant for predicting overall survival and predicting relapse-free survival (2-4). The information gained by this assay can aid in assessing the likelihood of response to therapy as well as in the prognosis and management of breast cancer patients (2-5).

Refer to the General Instructions for immunohistochemistry (IHC) or the Detection System Instructions of IHC procedures for:

Reagent provided
Monoclonal mouse antibody provided in liquid form as cell culture supernatant dialysed against 0.05 mol/L Tris/HCl, pH 7.2, and containing 15 mmol/L NaN₃. Package size is 1 mL.

Clone: 1D5 (6). Isotype: IgG1, kappa.

Mouse IgG concentration: 290 mg/L, Total protein concentration: 22.5 g/L

Immunogen
Soluble recombinant human estrogen receptor protein (6).

Specificity
Anti-ER, 1D5 was produced in BALB/c mice using recombinant estrogen receptor (RER), a protein with molecular mass of 67 kDa (6). Anti-ER, 1D5 was shown to react with an epitope located in the N-terminal domain of ER. Anti-ER, 1D5 specifically binds to an antigen located primarily on the surface of cell nuclei of normal and some neoplastic mammary epithelial cells. Anti-ER, 1D5 does not recognize the ERβ (7).

Precautions
1. For In Vitro Diagnostic Use.

2. This product contains sodium azide (NaN₃), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, build-ups of NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing (8).

3. Minimize microbial contamination of reagents or increase in nonspecific staining may occur

Storage
Store at 2-8 °C or aliquot into convenient volumes and freeze at -20 °C. Avoid repeated freezing and thawing. Frozen antibodies may be stored in small aliquots until periodic assay verifications detect unacceptable changes in reactivity.

Fresh dilutions of the antibody should be made prior to use and are stable for up to eight hours at room temperature (20-25 °C). Unused portions of antibody preparations should be discarded after eight hours.

Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the user must verify the conditions (9).

There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens.

Specimen preparation
Formalin sections: Biopsy specimens may be preserved for IHC staining by formalin fixation followed by paraffin embedding.
DAKO Monoclonal Mouse Anti-Human Estrogen Receptor, code No. M 7047 can be used on tissues fixed in neutral buffered formalin, methacarn or Carnoy’s fixative prior to paraffin embedding. The deparaffinized tissue sections must be treated with heat prior to the IHC staining procedure (10). Target retrieval involves immersion of tissue sections in a pre-heated buffer solution and maintaining heat, either in a water bath (95-99 °C), a steamer (95-99 °C) or an autoclave (121 °C). For greater adherence of tissue sections to glass slides, the use of silanized slides (DAKO code No. S 3003) is recommended. DAKO Target Retrieval Solution (code No. S 1700) or 10 x Concentrate (code No. S 1699) is recommended using a 20 minutes heating protocol.

**Staining procedure**

**Dilution:** DAKO Monoclonal Mouse Anti-Human Estrogen Receptor, code No. M 7047, may be used at a dilution of 1:35 when performing IHC using the DAKO EnVision, DAKO EnVision Doublestain or DAKO LSAB2 detection systems. These are guidelines only. Optimal antibody concentrations may vary depending on specimen preparation method, and should be determined by each individual laboratory. As negative control, DAKO Mouse IgG1, code No. X 0931, diluted to the same mouse IgG concentration as the primary antibody, is recommended.

**Visualization:** Follow the procedure for the detection system selected. The primary antibody dilution specified is suitable for a 10-minutes incubation when using an EnVision, EnVision Double stain or LSAB2 detection system.

**Staining interpretation:** The cellular staining pattern for anti-ER, 1D5 is nuclear. Cytoplasmic staining is considered to be background, non-specific staining, if seen.

There has been a variety of staining scales reported in the literature. Staining intensity has been reported using a staining intensity scale, and using the scale in conjunction with the percentage of cells staining (H score). The intensity score when stratified and compared to outcome, provided a greater statistical significance for DFS (p = 0.003 versus p = 0.01) compared to positive versus negative evaluation (2). Therefore, DAKO recommends using a semi-quantitative scoring system, e.g., 0-3 or negative, weak, moderate, and strong.

**Product specific limitations**

1. Wash buffers containing high levels of detergent can decrease the staining intensity with anti-ER, 1D5.
2. Occasional lymphoid tumours and non-lymphoid neoplasms such as melanomas are labelled.

**Performance characteristics**

**Reproducibility:** Eight serial sections from each of three different formalin-fixed, paraffin embedded blocks of breast carcinoma (prescreened for low antigen density) were collected for testing. Testing was performed as follows:

**Intra-run reproducibility:** Following the standard DAKO LSAB®2 Peroxidase Kit protocol (code No. K 0677), three slides from each tissue block were stained with Ready-to-Use DAKO® Mouse Anti-Human Estrogen Receptor, clone 1D5 (code No. N 1575). Concurrently, one slide from each block was stained with the supplied negative control reagent.

**Inter-run reproducibility:** Staining one slide from each tissue block, the above procedure was repeated on two additional days. Concurrently, one slide from each tissue block was stained with the supplied negative control reagent.

Reproducibility experiments with anti-ER, 1D5 yielded consistent results with intra- and inter-run testing. Consistent test conditions were maintained throughout the study and reagents were stored at 2-8°C between test runs.

**Normal tissues:** Distribution of ER throughout normal tissue has been reported in a variety of studies, summarized in a review article (11). The functionality of the ER is also explored in this review article. Immunoreactivity in a panel of normal tissues: Table 1 contains a summary of ER immunoreactivity with the recommended panel of normal tissues. All tissues were formalin-fixed and paraffin embedded and stained with Anti-ER, 1D5 according to the instructions in the package insert.

**TABLE 1**

<table>
<thead>
<tr>
<th>TISSUE TYPE</th>
<th>POSITIVE TISSUE ELEMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Tissue #)</td>
<td>STAINING AND STAINING PATTERN</td>
</tr>
<tr>
<td>Adrenal (4)</td>
<td>None</td>
</tr>
<tr>
<td>Bone Marrow (2)</td>
<td>None</td>
</tr>
<tr>
<td>Brain/Cerebellum (4)</td>
<td>None</td>
</tr>
<tr>
<td>Brain/Cerebrum (3)</td>
<td>None</td>
</tr>
<tr>
<td>Breast (3)</td>
<td>Mammary gland (1-2+ staining intensity, 2/3 tissues)</td>
</tr>
<tr>
<td>Cervix uteri (3)</td>
<td>Squamous epithelium (1/3 tissues)</td>
</tr>
<tr>
<td>Colon (3)</td>
<td>None</td>
</tr>
<tr>
<td>Esophagus (3)</td>
<td>None</td>
</tr>
<tr>
<td>Heart (3)</td>
<td>None</td>
</tr>
<tr>
<td>Kidney (3)</td>
<td>None</td>
</tr>
<tr>
<td>Liver (3)</td>
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</tr>
<tr>
<td>Lung (3)</td>
<td>None</td>
</tr>
<tr>
<td>Mesothelial Cells (3)</td>
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</tr>
<tr>
<td>Ovary (3)</td>
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</tr>
<tr>
<td>Pancreas (3)</td>
<td>None</td>
</tr>
<tr>
<td>Parathyroid (3)</td>
<td>None</td>
</tr>
<tr>
<td>Peripheral Nerve (3)</td>
<td>None</td>
</tr>
<tr>
<td>Pituitary (3)</td>
<td>None</td>
</tr>
<tr>
<td>Prostate (3)</td>
<td>Fibromuscular stroma, 1+ staining intensity, cytoplasmic pattern, 1/3 tissues</td>
</tr>
<tr>
<td>Salivary Gland (3)</td>
<td>None</td>
</tr>
<tr>
<td>Skeletal Muscle (3)</td>
<td>None</td>
</tr>
</tbody>
</table>

(3)
Skin (3) None
Small Intestine (3) None
Spleen (4) None
Stomach (3) None
Testis (3) None
Thymus (3) None
Thyroid (3) None
Tonsil (3) None
Uterus (3) Endometrium (1/3 tissues, 1+ staining intensity)

Reported staining in all tissues was nuclear, unless otherwise noted.

Abnormal Tissues: In pathological tissues, anti-ER, 1D5 was examined for specificity and sensitivity for breast cancer as well as for the evaluation of patients for endocrine therapy. Numerous studies of cases of breast cancer showed anti-ER, 1D5 to be safe and effective in this endeavor (2, 3, 10, 12-17). On frozen tissues, anti-ER, 1D5 immunoreacted with 63/93 (67.7%) cases of breast cancer and only 1/30 (3.3%) nonbreast cancers reacted positively (6). Direct comparison of anti-ER, 1D5 on formalin-fixed, paraffin-embedded (FFPE) specimens to the Abbott Estrogen receptor immunocytochemical assay using H222 on frozen sections of the same tumors has been completed by 7 different laboratories on more than 1000 specimens (2, 3, 10, 19-21). The Anti-ER, 1D5 specificity ranged from 51 to 88% while sensitivity ranged from 89% to 100%. When outcome to tamoxifen therapy or other measure of hormone reactivity is considered, one study reported that Anti-ER, 1D5 correlated with outcome to tamoxifen (7) while a second study reported the Anti-ER, 1D5 had a positive predictive value of 64% and negative predictive value of 92% (3).

Comparison of breast carcinoma ER concentration using anti-ER, 1D5 compared to dextran-coated charcoal (DCC) was performed by six different investigators. The relative scale for comparison is reported in Table 2. A total of 1,376 cases were assessed. Concordance was seen in 86.4% (1,188) of the cases, while 1D5+/DCC – was found in 8.6% (94) of the cases and 1D5-/DCC+ was found in 6.8% (94) of the cases (3, 6, 13, 17, 23, 24).

TABLE 2. Correlation Between The IHC Score and DCC Mean Values

<table>
<thead>
<tr>
<th>IHC Score</th>
<th>DCC Mean (fmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>+</td>
<td>22</td>
</tr>
<tr>
<td>++</td>
<td>33</td>
</tr>
<tr>
<td>+++</td>
<td>90</td>
</tr>
<tr>
<td>++++</td>
<td>365</td>
</tr>
</tbody>
</table>

* Cut-off positivity at ≤ 10 fmol/mg

Anti-ER, 1D5 was used to detect ER presence in a variety of tumors. No staining was found in carcinomas other than breast (0/14), malignant melanomas (0/2) or lymphoid tumors (0/6), while limited positivity was noted for sarcomas (1/8) (6).

References


