Instructions for Use
Please read before using this product.

Check the integrity of the packaging before use.

Estrogen Receptor Clone 6F11 (ER 6F11)
Origin™ Mouse Monoclonal Antibody
Catalog No: ORG-8871
Intended Use

For in vitro Diagnostic Use.

Leica Biosystems Estrogen Receptor Clone 6F11 (ER 6F11) mouse monoclonal antibody is intended for laboratory use to qualitatively identify by light microscopy, estrogen receptor (ER) antigen in sections of formalin fixed, paraffin embedded tissue. ER 6F11 specifically binds to the ER antigen located in the nucleus of ER positive normal and neoplastic cells.

Origin™ ER 6F11 is indicated as an aid in the management, prognosis and prediction of therapy outcome of breast cancer. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Origin™ antibodies are optimized for use with the Ventana® Medical Systems, NexES® and BenchMark™ Immunohistochemistry Staining Systems in combination with Ventana® Detection Kits.

Summary and Explanation

Estrogen receptor (ER) content of breast cancer tissue is an important parameter in the prediction of prognosis and response to endocrine therapy. The introduction of monoclonal antibodies to ER has allowed the determination of receptor status of breast tumors to be carried out in routine histopathology laboratories. ER 6F11 is a mouse monoclonal antibody directed against the human estrogen receptor molecule. A prokaryotic recombinant protein, corresponding to the full -length alpha form of human ER molecule was used as the immunogen. ER 6F11 has been shown to react with a 66 kD protein from MCF-7 cell lysates via Western blot1.
**Principle of Procedure**

Origin™ ER 6F11 is recommended for use in an immunohistochemical (IHC) procedure, which allows the qualitative identification by light microscopy of antigens in sections of formalin-fixed, paraffin-embedded tissue, via sequential steps with interposed washing steps. Prior to staining sections are subjected to epitope retrieval. The section is subsequently incubated with the primary antibody. A biotin-conjugated secondary antibody formulation that recognizes mouse immunoglobulins is used to detect the primary antibody. A streptavidin-peroxidase conjugate is then applied and binds to the biotin present on the secondary antibody. Sections are further incubated with the substrate/chromogen, 3,3’-diaminobenzidine (DAB) and DAB Substrate Buffer.

Reaction with the peroxidase produces a visible brown precipitate at the antigen site. Sections are counterstained with hematoxylin and coverslipped. Results are interpreted using a light microscope.

**Reagent Provided**

Origin™ ER 6F11 is a ready to use mouse monoclonal antibody presented in phosphate buffer pH 7.3 with 3 mg/ml carrier protein and 0.05% ProClin® 300.

**Immunogen**

Prokaryotic recombinant protein corresponding to the full-length alpha form of the human estrogen receptor molecule.

**Clone**

6F11

**Ig Class**

IgG1

**Total Protein Concentration**

Refer to vial label for batch specific Ig and total protein concentrations.

**Antibody Development**

ER 6F11 was raised against recombinant ER protein that was expressed from cDNA derived from mRNA extracted from the cell line MCF-7. Balb/c mice were immunized with the resulting (His)$_6$-tagged ER recombinant antigen. Screening was conducted by ELISA, with ELISA positive supernatants tested on formalin-fixed, paraffin-embedded sections of breast carcinoma of known receptor status. Colonies demonstrating positive immunohistochemical staining were cloned by limiting dilution.
Reconstitution, Mixing, Dilution, Titration:
Origin™ ER 6F11 is optimized for use with the Ventana® Medical Systems, NexES® and BenchMark™ Immunohistochemistry Staining Systems in combination with Ventana® Detection Kits. Further dilution may result in loss of antigen staining. The user must validate any such change. Differences in tissue processing and technical procedures in the user’s laboratory may produce significant variability in results necessitating regular performance of in-house controls (see Quality Control section).

Materials Required But Not Provided
1. Standard solvents used in Immunohistochemistry
2. PBS Buffer
3. Epitope retrieval solution
4. Ventana® NexES® or BenchMark™ Automated Staining System
   - Bar code labels
   - Ventana® Prep Kit Dispenser
   - Cell conditioning fluids
   - Detection Kit
   - Wash solution
   - Negative control reagent
   - Liquid coverslip
5. Mounting solution and coverslips

Storage
Store at 2-8 °C. Return to 2-8 °C immediately after use. Do not use after expiration date indicated on the vial label. Storage conditions other than those specified above must be verified by the user.

Specimen Preparation and Treatment Prior to Staining
The recommended fixative is 10% neutral-buffered formalin for paraffin-embedded tissue sections.

Warnings and Precautions
• This reagent has been prepared from the supernatant of cell culture. As it is a biological product, reasonable care should be taken when handling it.
• ProClin® 300 may cause irritation to skin, eyes, mucous membranes and upper respiratory tract. The concentration of ProClin® 300 in this product is 0.05% and does not meet the OSHA criteria for hazardous substance.

• Consult federal, state or local regulations for disposal of any potentially toxic components.

• Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions².

• Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens.

• If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Seek medical advice.

• Minimize microbial contamination of reagents or an increase in non-specific staining may occur.

• Incubation times or temperatures, other than those specified, may give erroneous results. Any such changes must be validated by the user.

Instructions for Use

Prior to undertaking this methodology, users must be trained in Immunohistochemical techniques on Ventana automated staining systems. Origin™ ER 6F11 is optimized for use with the Ventana® Medical Systems, NexES® and BenchMark™ Immunohistochemistry Staining Systems in combination with Ventana® Detection Kits and Prep Kit Dispensers. Before use, the Origin™ primary antibody loaded into a Ventana Prep Kit Dispenser – see instructions supplied with Ventana Prep Kit Dispenser. Apply the expiry date as indicated on the Origin™ vial label. Table 1 summarizes the requirements for immunostaining.

Table 1 - Requirements for formalin-fixed, paraffin-embedded tissues

<table>
<thead>
<tr>
<th></th>
<th>BenchMark™</th>
<th>NexES®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat Induced Epitope Retrieval</td>
<td>CC1 Solution</td>
<td>To be determined by user</td>
</tr>
<tr>
<td>Enzyme Digestion</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Enzyme Incubation</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Primary Antibody Incubation</td>
<td>16-32 minutes</td>
<td>16-32 minutes</td>
</tr>
</tbody>
</table>
Quality Control

Differences in tissue processing and technical procedures in the user’s laboratory may produce significant variability in results, necessitating regular performance of in-house controls in addition to the following procedures.

Controls should be fresh autopsy/biopsy/surgical specimens formalin-fixed, processed and paraffin-embedded as soon as possible in the same manner as the patient sample(s).

Positive Tissue Control

Used to indicate correctly prepared tissues and proper staining techniques. One positive tissue control should be included for each set of test conditions in each staining run. A tissue with weak positive staining is more suitable than a tissue with strong positive staining for optimal quality control and to detect minor levels of reagent degradation\(^3\). The recommended positive control tissue for use with ER 6F11 is endometrium.

If the positive tissue control fails to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control

Should be examined after the positive tissue control to verify the specificity of the labeling of the target antigen by the primary antibody. The recommended negative control tissue for use with ER 6F11 is tonsil. Alternatively, the variety of different cell types present in most tissue sections frequently offers negative control sites, but this should be verified by the user. Non-specific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically\(^4\). False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products.

They may also be caused by endogenous enzymes such as pseudoperoxidase (erythrocytes), endogenous peroxidase (cytochrome C), or endogenous biotin (eg. liver, breast, brain, kidney) depending on the type of immunostain used. To differentiate endogenous enzyme activity or non-specific binding of enzymes from specific immunoreactivity, additional patient tissues may be stained exclusively with substrate chromogen or enzyme complexes (avidin-biotin, streptavidin, labeled polymer) and substrate-chromogen, respectively.

If specific staining occurs in the negative tissue control, results with the patient specimens should be considered invalid.
Negative Reagent Control
Use a non-specific negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate non-specific staining and allow better interpretation of specific staining at the antigen site. Normal mouse sera diluted to the same concentration as the primary antibody may be used as a negative control reagent.

Troubleshooting
Contact Leica Biosystems Technical Service (800) 248-0123 to report unusual staining results.

Assay Verification
Prior to initial use of an antibody or staining system in a diagnostic procedure, the user should verify the antibody’s specificity by testing it on a series of in-house tissues with known immunohistochemical performance characteristics representing known positive and negative tissues. Refer to the quality control procedures previously outlined in this section of the product insert and to the quality control recommendations of the CAP Certification Program for Immunohistochemistry and/or the NCCLS IHC guideline. These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Tissues listed in the Performance Characteristics section are suitable for assay verification.

Interpretation of Staining

Positive Tissue Control
The positive tissue control stained with ER 6F11 should be examined first to ascertain that all reagents are functioning properly. If the positive tissue controls fail to demonstrate positive staining, any results with the test specimens should be considered invalid.

Negative Tissue Control
The negative tissue control should be examined after the positive tissue control to verify the specificity of the labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody crossreactivity to cells/cellular components. If specific staining (false-positive staining) occurs in the negative external tissue control, results with the patient specimen should be considered invalid.

Non-specific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically.
Patient Tissue
Examine patient specimens stained with ER 6F11 last. The staining pattern of ER 6F11 is nuclear. Positive staining intensity should be assessed within the context of any non-specific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed. If necessary, use a panel of antibodies to identify false-negative reactions.

General Limitations:

• Immunohistochemistry is a multistep diagnostic process that consists of specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.

• Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false-negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.

• Excessive or incomplete counterstaining may compromise proper interpretation of results.

• The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist who is familiar with the proper use of IHC antibodies, reagents and methods to interpret the all of the steps used to prepare and interpret the final IHC preparation.

• The manufacturer provides these antibodies/reagents for use at optimal dilution following the provided instructions for IHC on prepared tissue sections. Any deviation from recommended test procedures may invalidate declared expected results; appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.

• This product is not intended for use in flow cytometry. Performance characteristics have not been determined for flow cytometry.

• Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit non-specific staining with horseradish peroxidase.
• Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues.

• Normal/nonimmune sera from the same animal source as secondary antisera used in blocking steps may cause false-negative or false-positive results due to autoantibodies or natural antibodies.

• False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (e.g. liver, breast, brain, kidney) depending on the type of immunostain used.

Performance Characteristics

Immunoreactivity

Normal Tissues

• ER 6F11 detects the estrogen receptor alpha (ER) antigen in the nuclei of cells that express high levels of ER, a proportion of endometrial, ovarian and myometrial cells, and normal breast ductal cells. Table 2 contains a summary of ER immunoreactivity with the recommended panel of normal tissues.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Number of cases</th>
<th>Description of Staining</th>
<th>Staining Intensity (0-3+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Brain, Cerebellum</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Brain, Cerebrum</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Breast</td>
<td>5</td>
<td>Duct nuclei in 4/5 tissues</td>
<td>1+</td>
</tr>
<tr>
<td>Cervix</td>
<td>3</td>
<td>Squamous epithelium and muscle</td>
<td>2+</td>
</tr>
<tr>
<td>Colon</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Esophagus</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Heart</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Kidney</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Lung</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Mesothelial cells</td>
<td>1</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Ovary</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral nerve</td>
<td>2</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Pituitary</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Prostate</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Salivary/Submandibular gland</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>4</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Skin</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Small intestine</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Spleen</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Stomach</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Testis</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Thymus</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Thyroid</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Tonsil</td>
<td>4</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
<tr>
<td>Uterus</td>
<td>7</td>
<td>Endometrial glands and stromal cells in 6/7 tissues</td>
<td>2+</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>3</td>
<td>No staining of tissue elements</td>
<td>0</td>
</tr>
</tbody>
</table>

Key to Staining Intensity: 0 – Negative  1+ – Weak  2+ – Moderate  3+ – Strong
Published Immunoreactivity
Characterization of ER 6F11 during antibody development included a comparative evaluation of a series of 55 sequential breast carcinomas. The tissues evaluated were routinely processed formalin-fixed, paraffin-embedded specimens stained using both ER 6F11 and ER 1D5. There was an observed concordance of staining between ER 6F11 and ER 1D5 for 50/55 cases.

Estrogen receptor status was evaluated in 592 cases using routinely prepared paraffin-embedded tissue samples from primary breast carcinomas with ER 6F11 and ER 1D5. Overall, ER 1D5 and ER 6F11 showed a 97.5% concordance rate.

Bibliography


Trademarks

Origin™ and Novocastra™ are trademarks of Leica Biosystems. Ventana®, NexES® and BenchMark™ are trademarks of Ventana® Medical Systems, Inc. ProClin® 300 is a registered trademark of Rohm and Haas Company. Origin™ Antibodies are developed solely by Novocastra™ Laboratories Ltd, and do not imply approval or endorsement by Ventana® Medical Systems, Inc. Novocastra™ and Ventana® are not affiliated or related in any way.
Explanation of Symbols

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Temperature limitations</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro diagnostic device</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consult instructions for use</td>
<td>Use by</td>
<td></td>
</tr>
</tbody>
</table>

Amendments to Previous Issue

Contact telephone number in “Troubleshooting” section updated.

**Date of Issue** 2 Oct 2009 (ORG-8871/USA&Can/UK), (Form 768 rev- 5/08/03).