Instructions for Use
Please read before using this product.

Check the integrity of the packaging before use.

Bond™ Ready-to-Use Primary Antibody
Progesterone Receptor (16)

Catalog No: PA0312

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Progesterone Receptor (16)

Catalog No: PA0312

Intended Use

This reagent is for in vitro diagnostic use.

Progesterone Receptor (16) monoclonal antibody is intended to be used for the qualitative identification by light microscopy of human progesterone receptor (PR) in formalin-fixed, paraffin-embedded tissue by immunohistochemical staining using the automated BOND-MAX system. Progesterone Receptor Clone (16) [PR (16)] specifically binds to the PR antigen located in the nucleus of PR positive normal and neoplastic cells.

PR (16) 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.

Summary and Explanation

PR content of breast cancer tissue is an important parameter in the prediction of prognosis and response to endocrine therapy. PR (16) is a mouse monoclonal antibody directed against the human progesterone receptor molecule. A prokaryotic recombinant protein, corresponding to the N terminal region of the A form was used as the immunogen.

Principle of Procedure

Immunohistochemical techniques can be used to demonstrate the presence of antigens in tissue and cells (see “Using BOND Reagents” in your BOND user documentation). Progesterone Receptor (16) primary antibody is a ready to use product that has been specifically optimized for use on the automated BOND-MAX system in combination with Bond Polymer Refine Detection. The recommended staining protocol for Progesterone Receptor (16) primary antibody is IHC Protocol F. Heat induced epitope retrieval is recommended using Bond Epitope Retrieval Solution 2 for 20 minutes. Bond Polymer Refine Detection utilizes a novel controlled polymerization technology to prepare polymeric HRP-linker antibody conjugates. The detection system avoids the use of streptavidin and biotin, and therefore eliminates nonspecific staining as a result of endogenous biotin.

Bond Polymer Refine Detection works as follows:

• The specimen is incubated with hydrogen peroxide to quench endogenous peroxidase activity
• Bond Ready-To-Use Primary Antibody Progesterone Receptor (16) is applied
• A post primary antibody solution enhances penetration of the subsequent polymer reagent
• A poly-HRP anti-mouse/rabbit IgG reagent localizes the primary antibody
• The substrate chromogen, 3,3'- diaminobenzidine (DAB), visualizes the complex via a brown precipitate
• Hematoxylin (blue) counterstaining allows the visualization of cell nuclei

Using Bond Polymer Refine Detection in combination with the automated BOND-MAX system reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting and reagent application.

Reagent Provided

Progesterone Receptor (16) is a mouse anti-human monoclonal antibody produced as a tissue culture supernatant, and supplied in Tris buffered saline with carrier protein, containing 0.35% ProClin™ 950 as a preservative.

Total volume = 7 mL.

Clone

16

Immunogen

Prokaryotic recombinant protein corresponding to the N-terminal region of the A form of the human progesterone receptor.

Specificity

Human progesterone receptor.

Subclass

IgG1.

Total Protein Concentration

Approx 10 mg/mL.

Antibody Concentration

Greater than or equal to 1 mg/L as determined by ELISA.

Method

PR (16) was raised against recombinant progesterone receptor protein that was expressed from cDNA derived from mRNA extracted from the cell line T47D. Balb/c mice were immunized with the resulting PR protein fragment. 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.
Dilution and Mixing
Progesterone Receptor (16) primary antibody is optimally diluted for use on the automated BOND-MAX system in combination with Bond Polymer Refine Detection. 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. Refer to “Using BOND Reagents” in your BOND user documentation.

Materials Required But Not Provided
Refer to “Using BOND Reagents” in your BOND user documentation for a complete list of materials required for specimen treatment and immunohistochemical staining using the BOND-MAX system.

Storage and Stability
Store at 2–8 °C. Do not use after the expiration date indicated on the container label.
The signs indicating contamination and/or instability of Progesterone Receptor (16) are: turbidity of the solution, odor development, and presence of precipitate.
Return to 2–8 °C immediately after use.
Storage conditions other than those specified above must be verified by the user.

Specimen Preparation
The recommended fixative is 10% neutral-buffered formalin for paraffin-embedded tissue sections.

Precautions
• This product is intended for in vitro diagnostic use.
• The concentration of ProClin™ 950 is 0.35%. It contains the active ingredient 2-methyl-4-isothiazolin-3-one, and may cause irritation to the skin, eyes, mucous membranes and upper respiratory tract. Wear disposable gloves when handling reagents.
• To obtain a copy of the Material Safety Data Sheet contact your local distributor or regional office of Leica Biosystems, or alternatively, visit the Leica Biosystems Web site, www.LeicaBiosystems.com
• 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 precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents or specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Seek medical advice.
• Consult Federal, State or local regulations for disposal of any potentially toxic components.
• Minimize microbial contamination of reagents or an increase in non-specific staining may occur.
• Retrieval, incubation times or temperatures other than those specified may give erroneous results. Any such change must be validated by the user.

Instructions for Use
Progesterone Receptor (16) primary antibody was developed for use on the automated BOND-MAX system in combination with Bond Polymer Refine Detection. The recommended staining protocol for Progesterone Receptor (16) primary antibody is IHC Protocol F. Heat induced epitope retrieval is recommended using Bond Epitope Retrieval Solution 2 for 20 minutes.

Quality Control
Refer to “Using BOND Reagents” in your BOND user documentation.

Troubleshooting
Refer to reference 3 for remedial action.
Contact your local distributor or the regional office of Leica Biosystems to report unusual staining.

Interpretation of Staining
Refer to “Using BOND Reagents” in your BOND user documentation.

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 all of the steps used to prepare and interpret the final IHC preparation.
• The manufacturer provides these antibodies/reagents at optimal dilution for use following the provided instructions for IHC on prepared tissue sections or cytologic preparation. 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 nonspecific 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.

Product Specific Limitations
Progesterone Receptor (16) has been optimized at Leica Biosystems for use with Bond Polymer Refine Detection and BOND ancillary reagents. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances. The protocol times may vary, due to variation in tissue fixation and the effectiveness of antigen enhancement, and must be determined empirically. Negative reagent controls should be used when optimizing retrieval conditions and protocol times.

Performance Characteristics
Reproducibility
Intra run reproducibility of staining with Bond Ready-To-Use Primary Antibody Progesterone Receptor (16) was determined by staining 10 sections of the same tissue using Leica Biosystems Bond Polymer Refine Detection (DS9800). 10 of 10 slides stained positively. All slides stained with similar staining specificity and intensity (varied by <1).
Inter run reproducibility of staining with Bond Ready-To-Use Primary Antibody Progesterone Receptor (16) was determined by staining 10 sections of the same tissue, on 3 different staining runs using Leica Biosystems Bond Polymer Refine Detection (DS9800). 10 of 10 slides stained positively on each run. All slides stained with similar staining specificity and intensity (varied by <1).

Immunoreactivity
During antibody development, the specificity of PR (16) was evaluated on a range of normal tissues. Characteristic staining was observed in the nuclei of cells that express high levels of the protein, a proportion of endometrial, ovarian and myometrial cells, and normal breast ductal cells. Negative tissues included adrenal, bone marrow, brain (cerebellum), brain (cerebrum), colon, esophagus, heart, kidney, liver, lung, mesothelial cells, parathyroid, peripheral nerve, salivary/submandibular gland, skeletal muscle, skin, small intestine, spleen, spinal chord, stomach, testis, thymus, and thyroid. Weak staining was observed in ovary stromal cells and occasional islet cells of the pancreas.
Additional evaluation on a panel of normal tissues was conducted using the Bond Ready-To-Use Progesterone Receptor (16) primary antibody in conjunction with Bond Refine Detection System on the Leica Biosystems BOND-MAX slide staining system. Staining results are summarized in Table 1.

Table 1: Reactivity of Progesterone Receptor (16) on 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>Small percentage of variable nuclear staining seen.</td>
<td>0–3+</td>
</tr>
<tr>
<td>Brain, Cerebellum</td>
<td>3</td>
<td>No specific immunohistochemical staining seen.</td>
<td>0</td>
</tr>
<tr>
<td>Brain, Cerebrum</td>
<td>3</td>
<td>No specific immunohistochemical staining seen. Faint non-specific background blush.</td>
<td>0</td>
</tr>
<tr>
<td>Breast</td>
<td>3</td>
<td>Variable nuclear positivity seen in a percentage of ductal components.</td>
<td>0–3+</td>
</tr>
<tr>
<td>Cervix</td>
<td>3</td>
<td>Strong nuclear positivity in cervical stromal cells and glandular epithelial cells. Faint non-specific background blush in lumen of glandular component.</td>
<td>3+</td>
</tr>
<tr>
<td>Colon</td>
<td>3</td>
<td>No specific immunohistochemical staining seen.</td>
<td>0</td>
</tr>
<tr>
<td>Esophagus</td>
<td>3</td>
<td>No specific immunohistochemical staining seen. Faint non-specific background blush in connective tissue.</td>
<td>0</td>
</tr>
<tr>
<td>Heart</td>
<td>3</td>
<td>No specific immunohistochemical staining seen. Non-specific cross reaction in cytoplasm of muscle cells.</td>
<td>0–1+</td>
</tr>
<tr>
<td>Kidney</td>
<td>3</td>
<td>Variable nuclear positivity in a small percentage of renal tubule nuclei. Faint non-specific background blush in connective tissue.</td>
<td>0–1+</td>
</tr>
<tr>
<td>Liver</td>
<td>3</td>
<td>Faint non-specific background blush in connective tissue.</td>
<td>0</td>
</tr>
<tr>
<td>Lung</td>
<td>3</td>
<td>Small percentage of variable nuclear staining seen.</td>
<td>0–1+</td>
</tr>
<tr>
<td>Tissue</td>
<td>Number of cases</td>
<td>Description of Staining</td>
<td>Staining Intensity (0–3+)</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------</td>
<td>-----------------------------------------------------------------------------------------</td>
<td>--------------------------</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>Strong nuclear positivity in ovarian stromal cell nuclei.</td>
<td>3</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3</td>
<td>Variable nuclear positivity in islet cell nuclei.</td>
<td>3</td>
</tr>
<tr>
<td>Parathyroid</td>
<td>3</td>
<td>No specific immunohistochemical staining seen. Faint non-specific immunohistochemical cross-reaction seen.</td>
<td>0–1+</td>
</tr>
<tr>
<td>Peripheral nerve</td>
<td>3</td>
<td>No specific immunohistochemical staining seen.</td>
<td>0</td>
</tr>
<tr>
<td>Pituitary</td>
<td>3</td>
<td>Small percentage of variable nuclear staining seen.</td>
<td>1–3+</td>
</tr>
<tr>
<td>Prostate</td>
<td>3</td>
<td>Small percentage of variable nuclear staining seen.</td>
<td>0–2+</td>
</tr>
<tr>
<td>Salivary/ Submandibular Gland</td>
<td>3</td>
<td>Small percentage of variable nuclear staining seen.</td>
<td>0–2+</td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>3</td>
<td>No specific immunohistochemical staining seen. Non-specific background blush seen.</td>
<td>0–2+</td>
</tr>
<tr>
<td>Skin</td>
<td>3</td>
<td>No specific immunohistochemical staining seen. Non-specific background blush seen in epithelial layer.</td>
<td>0–2+</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>3</td>
<td>Nuclear positivity in muscle layer.</td>
<td>3</td>
</tr>
<tr>
<td>Spleen</td>
<td>3</td>
<td>No specific immunohistochemical staining seen.</td>
<td>0</td>
</tr>
<tr>
<td>Stomach</td>
<td>3</td>
<td>Variable nuclear positivity in muscle layer.</td>
<td>0–2+</td>
</tr>
<tr>
<td>Testis</td>
<td>3</td>
<td>Variable nuclear positivity in capsular region and associated fibrous tissue.</td>
<td>2–3+</td>
</tr>
<tr>
<td>Thymus</td>
<td>2</td>
<td>No specific immunohistochemical staining seen.</td>
<td>0</td>
</tr>
<tr>
<td>Thyroid</td>
<td>2</td>
<td>No specific immunohistochemical staining seen.</td>
<td>0</td>
</tr>
<tr>
<td>Tonsil</td>
<td>3</td>
<td>No specific immunohistochemical staining seen.</td>
<td>0</td>
</tr>
<tr>
<td>Uterus</td>
<td>3</td>
<td>Strong nuclear positivity in endometrial/myometrial glandular tissue and stromal cell nuclei.</td>
<td>3+</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>3</td>
<td>No specific immunohistochemical staining seen. Non-specific immunohistochemical cross reaction seen in hyaline cartilage.</td>
<td>0–2+</td>
</tr>
</tbody>
</table>

Key to Staining Intensity:
0    - Negative
1+   - Weak
2+   - Moderate
3+   - Strong

A total of 87 breast cancer tissue samples were used to evaluate equivalence in staining characteristics between a number of PR primary antibodies, including PR (16) and PR (636). Correlation between PR (16) and PR (636) was 98% overall (85/87). Percent positive agreement was 98% (53/54) and percent negative agreement was 97% (32/33).

An additional study was initiated to directly compare PR (16) with PR (636) in 100 breast cancer samples. The two antibodies demonstrated an overall agreement of 96.0% (95% CL: 92.1, 99.9). Positive percent agreement was 98.4% (95% CL: 95.9, 100.0) and negative percent agreement was 91.9% (95% CL: 86.4, 97.4).

Normal Tissues
Clone 16 detects the progesterone receptor A isoform in the nuclei of cells that express high levels of the protein, a proportion of endometrial, ovarian and myometrial cells, and normal breast ductal cells.

Tumor Tissues
Clone 16 stained 8/13 breast fibroadenomas, 57/87 breast carcinomas and 3/10 ovarian tumors. No staining was observed in a variety of additional tumors evaluated (n=108).

**Progesterone Receptor (16) is recommended for determining progesterone receptor A status of breast cancer tissue.**

Characterisation of PR (16) during antibody development included a comparative evaluation of a series of 100 breast carcinomas. The tissues evaluated were routinely processed formalin-fixed, paraffin-embedded specimens stained using both PR (16) and PR (636). There was an observed concordance of staining for 96/100 cases.

Further Information
Further information on immunostaining with BOND reagents, under the headings Principle of the Procedure, Materials Required, Specimen Preparation, Quality Control, Assay Verification, Interpretation of Staining, Key to Symbols on Labels, and General Limitations can be found in “Using BOND Reagents” in your BOND user documentation.
Bibliography

2. Villanova PA. National Committee for Clinical Laboratory Standards (NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. 1991; 7(9). Order code M29-P.

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18 July 2016