BOND Ready-to-Use Primary Antibody Progesterone Receptor (16)

Catalog No: PA0312

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IVD

Instructions for Use

Please read before using this product.

Check the integrity of the packaging before use.

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℞ Only

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Intended Use

Ready-to-Use Format
For *in vitro* diagnostic use.

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

Progesterone Receptor Clone (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.

Progesterone Receptor Clone (16) is optimized for use on the Leica Biosystems automated BOND-MAX or BOND-III systems using the BOND Polymer Refine Detection kit.

Summary and Explanation

Progesterone Receptor content of breast cancer tissue is an important parameter in the prediction of prognosis and response to endocrine therapy. The introduction of antibodies to PR has allowed the immunohistochemical determination of progesterone receptor status of breast tumors to be carried out in routine histopathology laboratories. Progesterone Receptor Clone (16) [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). PR (16) primary antibody is a ready to use product that has been specifically optimized for use on the automated BOND-MAX and BOND-III systems in combination with BOND Polymer Refine Detection. The recommended staining protocol for PR (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 PR (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 and BOND-III systems reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting and reagent application.

Reagent Provided

PR (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 supernantants tested on formalin fixed, parrafin-embedded sections of breast carcinoma of known receptor status. Colonies demonstrating positive immunohistochemical staining were cloned by limiting dilution.

Dilution and Mixing

PR (16) primary antibody is optimally diluted for use on the automated BOND-MAX and BOND-III systems 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

- BOND Polymer Refine Detection (Catalog No. DS9800).
- BOND Dewax Solution (Catalog No. AR9222).
- BOND Epitope Retrieval Solution 2 (Catalog No. AR9640).
- BOND Wash Solution x10 Concentrate (Catalog No. AR9590).

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 automated BOND-MAX and BOND-III systems.

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 PR (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[1].

Specimen Preparation

All specimens must be prepared to preserve the tissue for immunohistochemical staining.

Standard methods of tissue processing should be used for all specimens.

It is recommended that tissues are prepared in formalin-based fixatives and are routinely processed and paraffin-embedded. For example, resection specimens should be blocked into a thickness of 3–4 mm and fixed for 18–24 hours in 10% neutral-buffered formalin.

The tissues should then be dehydrated in a series of alcohols and cleared through xylene, followed by impregnation with molten paraffin wax, held at no more than 60 °C. Tissue specimens should be sectioned between 3–5 µm.

To preserve antigenicity, tissue sections mounted on slides should be stained within 4–6 weeks of sectioning when held at room temperature (20–25 °C). Following sectioning, it is recommended that slides are incubated at 60 °C for one hour to assist with adherence.

In the USA, the Clinical Laboratory Improvement Act of 1988 requires in 42 CFR 493.1259(b) that "The laboratory must retain stained slides for at least ten years from the date of examination and retain specimen blocks at least two years from the date of examination."

Precautions

- This product is intended for in vitro diagnostic use.
- The concentration of ProClin™ 950 is 0.35%. It contains the active ingredient 2-methylisothiazol-3(*2H*)-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[2]. 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

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

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 provided by the user and included for each set of test conditions in each staining run. A tumor 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. The recommended positive control tissue for use with PR (16) is a weakly positive breast carcinoma and 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 provided by the user and 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 PR (16) is tonsil (endothelium).

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.

False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products.

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.

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

Positive Tissue Control

The positive tissue control stained with PR (16) 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 cross reactivity 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 non-specifically.

Patient Tissue

Examine patient specimens stained with PR (16) last. The staining pattern of PR (16) 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.

Cautionary Note: Staining may also be present in stromal cells, endothelial cells, lymphocytes and other tissue elements. Interpretation should be assessed in the context of the sample being assessed.

Assay Interpretation

For the determination of progesterone receptor expression, only a nuclear staining pattern should be evaluated. A pathologist using a brightfield microscope should perform slide evaluation. For evaluation of the immunohistochemical staining and scoring, an objective of 10x magnification is appropriate. The use of 20-40x objective magnification should be used in the conformation of the score. Cytoplasmic staining should be considered as non-specific staining and is not to be included in the assessment.

The American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical

Testing of estrogen and progesterone receptors in Breast Cancer (Arch Pathol Lab Med. 2010; 134(6): 907-922), should be used for assay interpretation.

Specifically, evaluation is defined as:

• Positive for progesterone receptor if finding of ≥ 1% of tumor cell nuclei are immunoreactive.

• Negative for progesterone receptor if finding of < 1% of tumor cell nuclei are immunoreactive in the presence of evidence that the sample can express progesterone receptor (positive intrinsic controls are seen).

• Uninterpretable for progesterone receptor if finding that no tumor nuclei are immunoreactive and that internal epithelial elements present in the sample or separately submitted from the same sample lack any nuclear staining.

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

PR (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

Immunoreactivity

The specificity of PR (16) was evaluated on 118 normal tissues cases. Characteristic staining was observed in the nuclei of cells that express high levels of the protein, a proportion of endometrial, cervical ovarian and myometrial cells, and normal breast ductal cells. Negative tissues included adrenal, bone marrow, brain (cerebellum), brain (cerebrum), esophagus, heart, liver, mesothelial cells, parathyroid, peripheral nerve, skeletal muscle, skin, small intestine, spleen, spinal cord, stomach, testis, thymus, and thyroid. Positive staining was also observed in occasional stromal cells of the bladder, lung and prostate, occasional lymphocytes in colon and rectum, acinar cells in salivary/submandibular gland, occasional renal tubular cells in the kidney, occasional hypophyseal cells of the pituitary and occasional islet cells of the pancreas.

PR (16) was evaluated on a range of tumor tissue cases. Intense staining was observed in fibroadenomas of the breast, an endometrioid adenocarcinoma of the ovary and a follicular papillary adenocarcinoma of the thyroid. Moderate staining was observed in a fibroblastic meningioma and a small cell carcinoma of the lung. Weak staining was observed in a malignant meningioma and a follicular carcinoma of the thyroid. Variable staining was observed in adenocarcinomas of the endometrium. The percentage of positive cells was low (1-10%) in the follicular papillary adenocarcinoma and follicular carcinoma of the thyroid, and high (>10%) in the fibroblastic meningioma, malignant meningioma, fibroadenomas of the breast, small cell carcinoma of the lung, endometrioid adenocarcinoma of the ovary and adenocarcinomas of the endometrium.

Reproducibility (BOND-MAX system)

Intra run reproducibility of staining with BOND Ready-to-Use Primary Antibody PR (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 PR (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).

Repeatability (BOND-III system)

Six unique breast tumor tissue cases were stained as part of intra-run repeatability testing. Of the six tissues, two had PR high expression (>10% tumor cells), 1 PR low expression (1-10% tumor cells), and 3 PR negative (<1% tumor cells). 9 slides were stained per case in two runs resulting in 54 assessments.

27 unique breast tumor tissue cases were stained as part of inter-instrument, inter-lot, and inter-day repeatability testing. Of the 27 tissues, 9 had PR high expression (>10% tumor cells), 5 PR low expression (1-10% tumor cells), and 13 PR negative (<1% tumor cells). 18 slides were stained per case in 21 runs resulting in 486 assessments.

All repeatability testing met the acceptance criteria.

Reproducibility (BOND-III system)

The Reproducibility Study was conducted at 3 sites over 5 non-consecutive days and a minimum of 20 calendar days on 135 unique FFPE breast tumor tissue cases and scored according to ASCO/CAP guidelines (≥1% cut-off) (Arch Pathol Lab Med. 2010; 134(6): 907-922) for a total of 1209 evaluations. Table 1 details the inter laboratory reproducibility of the assay. The average positive, negative and overall agreement was 96.2%, 95.7% and 95.9%, respectively, support the highly reproducible results of PR (16) staining using the BOND-III when used for the determination of PR status in a clinical setting.

Table 1. Inter Laboratory Reproducibility

	Average Positive Agreement	Average Negative Agreement	Average Overall Agreement
Inter Laboratory Reproducibility between three sites	**96.2%** **[95% CI:94.5%-97.6%]**	**95.7%** **[95% CI: 93.9%-97.3%]**	**95.9%** **[95% CI: 94.3%-97.4%]**

Each pathologist (1 per site) scored all stained slides prepared by each site. A total of 1215 slides (405 per pathologist) were scored. Table 2 details the inter observer reproducibility between three pathologists. The average positive, negative and overall agreement was 94.1%, 93.4% and 93.7%, respectively.

Table 2. Inter Observer Reproducibility

	Average Positive Agreement	Average Negative Agreement	Average Overall Agreement
Inter Observer Reproducibility between three sites	**94.1%** **[95%CI:92.0%-95.8%**	**93.4%** **[95% CI: 91.1%-95.3%]**	**93.7%** **[95% CI: 91.7%-95.5%]**

Method Comparison

PR (16) testing was performed at 3 sites on BOND-III and BOND-MAX and scored according to ASCO/CAP guidelines (≥1% cut-off) (Arch Pathol Lab Med. 2010; 134(6): 907-922). The method comparison data are presented in Table 3. The positive, negative and overall agreement was 95.5%, 95.7% and 95.6%, respectively. These results indicate PR (16) staining using the BOND-III is comparable to PR (16) staining using the BOND-MAX.

Table 3. Agreement between PR (16) on BOND-III and PR (16) on BOND-MAX

	BOND-MAX Negative	BOND-MAX Positive	Total
BOND-III Negative	**222**	**10**	**232**
BOND-III Positive	**10**	**213**	**223**
Total	**232**	**223**	**455**

Positive Percent Agreement = 213/223 = 95.5% (95% CI: 91.9%-97.5%)

Negative Percent Agreement = 222/232 = 95.7% (95% CI: 92.2%-97.6%)

Overall Percent Agreement = 435/455 = 95.6% (95% CI: 93.3%-97.1%)

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.

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