Novocastra™ Lyophilized Mouse Monoclonal Antibody Progesterone Receptor Clone 16

Catalog No: NCL-PGR-312

Instructions for Use
Please read before using this product.

Check the integrity of the packaging before use.

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IVD

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Intended Use

For in vitro Diagnostic Use.

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

PGR 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.

Summary and Explanation

Progesterone Receptor (PGR) 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 PGR has allowed the determination of receptor status of breast tumors to be carried out in routine histopathology laboratories. PGR Clone 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 of human progesterone receptor, was used as the immunogen. Antibody characterization studies demonstrated that PGR Clone 16 reacts with both A and B forms of human progesterone receptor in Western blotting procedures, but only the A form is detected in immunohistochemical procedures.

Principle of Procedure

The product is used 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, endogenous peroxidase activity is blocked and 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- or ABC-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). 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

NCL-PGR-312 is a lyophilized tissue culture supernatant containing 15 mM sodium azide as a preservative. The user is required to reconstitute the contents of the vial with sterile distilled water prior to use.

Immunogen

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

Clone

16

Ig Class

IgG1

Total Protein Concentration

Refer to vial label for batch specific total protein concentration.

Antibody Development

Greater than or equal to 324.0 mg/L as determined by ELISA. Refer to vial label for batch specific Ig concentration.

Method

PGR Clone 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 PGR protein fragment. Screening was conducted by ELISA, with ELISA positive supernantants 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:

NCL-PGR-312 is a lyophilized tissue culture supernatant containing 15 mM sodium azide as a preservative. The user is required to reconstitute the contents of the vial with the correct volume of sterile distilled water as indicated on the vial label.

Suggested dilution: 1:100–1:200 for 60 minutes at 25 ˚C. High temperature antigen retrieval using 0.01 M citrate retrieval solution (pH 6.0) is recommended. This is provided as a guide and users should determine their own optimal working dilutions. 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

Refer to the Detection System instructions for use for a list of Materials Required But Not Provided.

Storage

Store unopened antibody at 2–8 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. The reconstituted antibody is stable for at least two months when stored at 2–8 °C. For long term storage, it is recommended that aliquots of the reconstituted antibody are stored frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use. 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.
Heat induced epitope retrieval using 0.01 M citrate retrieval solution (pH 6.0) is recommended.

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.
- The molarity of sodium azide in this reagent is 15 mM. A Material Safety Data Sheet (MSDS) is available upon request for sodium azide.
- 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 precautions[1].
- 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

Incubate tissue sections with primary antibody reagent for 60 minutes at 25 °C. Refer to the “Methodology” section of the Detection System instructions for use for explanation of recommended staining procedure.

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 wax-embedded as soon as possible in the same manner as the patient sample(s).

Troubleshooting

Refer to reference 3 for remedial action.

Contact your local distributor or the regional office of Leica Biosystems to report unusual staining

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[2]. The recommended positive control tissue for use with PGR Clone 16 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 PGR Clone 16 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 Tech Support USA 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 PGR Clone 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 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.

Nonspecific 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].

Patient Tissue

Examine patient specimens stained with PGR Clone 16 last. The staining pattern of PGR Clone 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.

Scoring System

Slides should be scored using the Quick Score System in which the tumor is scored according to the proportion of cell nuclei stained and the intensity of the staining (Leake R et al. Immunohistochemical detection of steroid receptors in breast cancer: a working protocol. J Clin Pathol. 2000; 53(8):634–635).

Slide	Score	Definition
Intensity	3	Strong staining
	2	Moderate staining
	1	Weak staining
	0	Negative
Proportion	5	67–100%
	4	34–66%
	3	11–33%
	2	1–10%
	1	<1%
Quick Score = Intensity + Proportion A score of 3 or over is classed as receptor positive.		

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[4].
- 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 at optimal dilution for use 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 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.

Performance Characteristic
Immunoreactivity
Normal Tissues

During antibody development, the specificity of PGR Clone 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 cord, stomach, testis, thymus, and thyroid. Weak staining was observed in ovary stromal cells and occasional islet cells of the pancreas

Tumor Tissues

A total of 189 breast tissue specimens in two separate studies were used to compare staining results between PGR Clone 16 and DAKO PgR 636. Overall concordance was 98% (95% CI .95, 1.0). Percent positive agreement was 100% (95% CI .97, 1.0) and percent negative agreement was 96% (95% CI .89, .99).

Bibliography

1. National Committee for Clinical Laboratory Standards (NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, P.A. 1991; 7(9). Order code M29-P.
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4. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen: a possible source of error in immunohistochemistry. American Journal of Clinical Pathology. 1980; 73:626.
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Amendments to Previous Issue

Not applicable.

Explanation of Symbols

	**Manufacturer**		**Temperature limitations**		**Total Protein Concentration**
	***In vitro* diagnostic device**		**Batch number**		
	**Consult instructions for use**		**Use by**		

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