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DAKO

Specifications

DAKO Monoclonal Mouse Anti-Human Progesterone Receptor, clone PgR 636 for Immunoenzymatic Staining

IMMUNOGEN: Formalin-fixed recombinant full length A-form of human progesterone receptor[1]
CLONE: PgR 636[1]

CODE NO.: M3569
Primary Antibody
Lot No. 053
Total Protein Concentration: 12.3 mg/mL (Refractometry)
Mouse IgG Concentration: 288µg/mL (Single Radial Immunodiffusion)
Subclass: IgG_1, kappa

INTENDED USE:
For In Vitro Diagnostic Use
DAKO Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 636 (Anti-PR, PgR 636) is intended for laboratory use for the semi-quantitative detection of progesterone receptor by light microscopy in normal and pathological human paraffin-embedded tissue processed in neutral buffered formalin. This antibody is indicated for use as an aid in the management, prognosis and prediction of outcome of breast cancer. 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.

Refer to the General Instructions for Immunohistochemistry (IHC) or the Detection System Instructions of IHC procedures for: (1) Principle of Procedure, (2) Materials Required, Not Supplied, (3) Storage, (4) Specimen preparation, (5) Staining Procedure, (6) Quality Control, (7) Troubleshooting, (8) Interpretation of Staining, (9) General Limitations

SUMMARY AND EXPLANATION:
INTRODUCTION
The role of steroid hormone receptors in breast cancer is well-known.[2,3] The absence of ER and PR predicts early recurrence and poor survival of breast cancer patients.[4-7] Also, the presence of ER and PR in tumors predicts the potential for benefit from tamoxifen and other endocrine-related therapies. Measurement of ER and PR can be determined semi-quantitatively using IHC or quantitatively using DCC or EIA. Correlation between the semi-quantitative and quantitative evaluations of PR have ranged from 73 to 91% depending on the laboratory and antibody used.[8-10]

SPECIFICITY
Anti-PR, PgR 636 has been demonstrated to react with the PR-A and PR-B forms by Western blot of whole cell extracts and reacts with both free and hormone-bound PR.[1] The epitope has been mapped to the amino terminal domain shared by PR-A and PR-B.

REAGENTS PROVIDED:
M3569
Anti-PR, PgR 636 is available in a 0.2 mL or 1 mL volume as a mouse anti-human monoclonal antibody tissue culture supernatant in 50 mM Tris/HCl, pH 7.2, containing 15 mM NaN_3 and stabilizing protein.

M3569 may be used at a dilution of 1:50 when performing IHC using the DAKO LSAB®2 detection system. These are guidelines only. Optimal antibody concentrations may vary depending on specimen and preparation method, and should be determined by each individual laboratory.

MATERIALS REQUIRED, NOT SUPPLIED:
Refer to the General Instructions for IHC and/or the Detection System Instructions. In addition, use the following negative reagent control.

DAKO Mouse IgG_1, Code No. X0931

PRECAUTIONS:
1. For In Vitro Diagnostic Use.
2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, build-ups of NaN_3 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.[11]
3. Minimize microbial contamination of reagents or increase in nonspecific staining may occur.

STORAGE:
Store at 2-8 °C.

SPECIMEN PREPARATION:
Biopsy specimens may be preserved for IHC staining by formalin fixation followed by paraffin embedding.

Anti-PR, PgR 636 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. S3003) is recommended. DAKO Target Retrieval Solution (Code No. S1700) or 10x Concentrate (Code No. S1699) is recommended using a 20-40 minute heating protocol.

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STAINING PROCEDURE:
Follow the procedure for the detection system selected. When performing IHC with the LSAB2 detection system, use a 1:50 dilution in a 10-30 minute incubation with the diluted Anti-PR, PgR 636.

PERFORMANCE CHARACTERISTICS:
The cellular staining pattern for anti-PR, PgR 636 is nuclear. A positive staining result is defined as more than 10% of tumor cells with stained nuclei of any intensity.

Normal tissues: Distribution of PgR throughout normal tissue has been reported in a variety of studies. The nuclei of uterine gland cells were found to be strongly immunoreactive. Weaker immunostaining was observed in the nuclei of endometrial and prostatic stromal cells.

Immunoreactivity in a panel of normal tissues: Table 1 contains a list of positive tissues with PgR immunoreactivity. All tissues were formalin-fixed and paraffin embedded and stained with Anti-PR, PgR 636 according to the instructions in the package insert using the LSAB2 detection system (DAKO Code No. K0675). Negative tissues included adrenal (4), bone marrow (2,) brain/cerebellum (4), brain/cerebrum (3), colon (3), esophagus (3), heart (3), kidney (3), liver (3), lung (3), mesothelial cells (3), ovary (3), pancreas (3), parathyroid (3), peripheral nerve (3), salivary gland (3), skeletal muscle (3), skin (3), small intestine (3), spleen (4), stomach (3), testis (3), thymus (3), thyroid (3), and tonsil (3).

TABLE 1: Summary of PgR Normal Tissue Reactivity

TISSUE TYPE (# tested)	POSITIVE TISSUE ELEMENT STAINING AND STAINING PATTERN	
Breast (3)	Ductal epithelial cells	3+ staining intensity, 3/3 tissues
Cervix uteri (3)	Glandular epithelial cells	2+ staining intensity, 1/3 tissues
	Stromal fibroblasts	2+ staining intensity, 2/3 tissues
Pituitary (3)	Pituicytes	2+ staining intensity, 1/3 tissues
Prostate (3)	Stromal fibroblasts	2+ staining intensity, 1/3 tissues
Uterus (3)	Endometrial stroma	2+ staining intensity 3/3 tissues
	Myometrium	2+ staining intensity, 3/3 tissues
	Endometrial glands	2+ staining intensity, 2/3 tissues

A second survey of normal tissues demonstrated positivity in endometrium and weak positivity in prostate after heat-induced epitope retrieval using the LSAB+ detection system. Negative tissues included esophagus, testis, breast, liver, kidney, skeletal muscle, placenta, adrenal, tonsil, lung, colon, skin, pancreas, spleen, thyroid, stomach and cardiac muscle.[1]

Abnormal tissues: Ninety seven breast cancer tissues were tested using the DAKO anti-PR, PgR 636 with the LSAB2 detection system, which had been previously assessed for PR expression using the PR-EIA. Correlation between the 2 assays was 90.7% while specificity was 94% and sensitivity was 87.2%. In another study, 31 breast carcinomas previously tested with the DCC assay were stained using the LSAB+ detection system. Positive staining was reported for 21/23 positive tumors, while 6/8 remained negative (91% sensitivity and 75% specificity).[1]

Anti-PR, PR 636 with peroxidase/antiperoxidase detection system was used to immunostain a variety of 60 different tumor types. Breast cancer (5/11), uterine (2/2), ovarian (2/6), and endometrial (2) carcinomas stained strongly. Medullary carcinoma of the thyroid (1/2) and testicular yolk sac tumor were positive. Other tumors including melanoma, lymphoma and neuroendocrine and neural tumors were negative for PR expression.[1]

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

Intra-run reproducibility: Following the standard DAKO EnVision®+ Peroxidase Kit protocol (Code No. K4007), three slides from each tissue block randomly distributed through the staining order, were stained with Ready-to-Use DAKO® Mouse Anti-Human Progesterone Receptor, clone PgR 636 (Code No. NP008). Concurrently, one slide from each block was stained with the negative control reagent (Code No. NP015).

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

Reproducibility experiments with PgR 636 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.

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