Monoclonal Mouse Anti-Human Progesterone Receptor Clone PgR 636

Code M3569

Intended use
For In Vitro Diagnostic Use.

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.

Summary and explanation
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]

Refer to Dako's *General Instructions for Immunohistochemical Staining* 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.

Reagent provided
Monoclonal Mouse antibody provided in liquid form as tissue culture supernatant in 0.05 mol/L Tris-HCl, pH 7.2 and 0.015 mol/L sodium azide. This product contains stabilizing protein.

Clone: PgR 636[1] Isotype: IgG_1, kappa
Mouse IgG concentration mg/L: See label on vial.

When performing IHC with the LSAB™2 detection system, use a 1:50 dilution in a 10- to 30-minute incubation with the diluted Anti-PR, PgR 636. These are guidelines only. Optimal antibody concentrations may vary depending on specimen and preparation method, and should be determined by each individual laboratory.

The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

Immunogen
Formalin-fixed recombinant full-length A-form of human progesterone receptor[1]

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.

Materials required, but not supplied
Refer to Dako's *General Instructions for Immunohistochemical Staining* and/or the detection system instructions. In addition, use the following negative reagent control:
Mouse IgG_1 (code X0931)

Precautions
1. For professional users.
2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, NaN_3 may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused reagents should be disposed of according to local, State, and Federal regulations.

Storage
Store at 2–8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

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 (code S3003) is recommended. Target Retrieval Solution (code S1700) or 10x Concentrate (code S1699) is recommended using a 20- to 40-minute heating protocol.

Anti-Progesterone receptor can also be used to label cryostat sections or cell smears.

Staining procedure
Follow the procedure for the detection system selected.

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

Performance characteristics
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 636 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 LSAB™2 detection system (code 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 636 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, testes, 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 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 previously determined positive tumors, while 6/8 remained negative (91% sensitivity and 75% specificity).[1]

Anti-PR, PgR 636 with a 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
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 EnVision™+ Peroxidase Kit protocol (code K4007), three slides from each tissue block randomly distributed through the staining order were stained with ready-to-use Mouse Anti-Human Progesterone Receptor, clone PgR 636. Concurrently, one slide from each block was stained with a matched negative control reagent.

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 a matched negative control reagent.

Reproducibility experiments with anti-PR, PgR 636 yielded consistent results with intra-run and inter-run testing. Consistent test conditions were maintained throughout the study and reagents were stored at 2–8 °C between test runs.

References

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Explanation of symbols

REF	Catalogue number		Temperature limitation	IVD	In vitro diagnostic medical device
	Manufacturer	LOT	Batch code	Σ	Contains sufficient for <n> tests
	Use by		Consult instructions for use	EC REP	Authorized representative in the European Community

Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051
United States

Tel. +44 161 492 7050
www.agilent.com

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