

**Monoclonal Mouse
Anti-Human
Prostate-Specific Membrane Antigen**
Clone 3E6

Code M3620

Intended use

For in vitro diagnostic use.

Monoclonal Mouse Anti-Human Prostate-Specific Membrane Antigen, Clone 3E6, is intended for use in immunohistochemistry (IHC). The antibody labels PSMA-expressing cells in normal and neoplastic tissues (1, 2). Results aid in the classification of prostate adenocarcinoma (1, 2). Differential classification is aided by the results from a panel of antibodies. 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. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

Synonyms for antigen

PSMA, PSM', FOLH, GCP2 (2-9).

Summary and explanation

Prostate-specific membrane antigen (PSMA) is a 750 amino acid type II membrane glycoprotein with folate hydrolase and neuropeptidase activity. The *PSMA* gene is located on the short arm of chromosome 11 and several alternatively spliced variants have been identified, including the cytosolic variant PSM' (5, 8, 9). PSMA is expressed in normal and malignant prostatic epithelium and in a subset of non-prostatic tissues (4-6). PSMA expression has also been reported on the neovasculature of a variety of non-prostatic solid tumors (1, 5-7).

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure, Materials Required, Not Supplied, Storage, Specimen Preparation, Staining Procedure, Quality Control, Troubleshooting, Interpretation of Staining, General Limitations.

Reagent provided

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

Clone: 3E6 (1). Isotype: IgG1, kappa.

Mouse IgG concentration: see label on vial.

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

LNCaP membrane preparation and immunoaffinity-purified PSMA (3).

Specificity

Monoclonal mouse anti-PSMA has been demonstrated to react in Western blotting with PSMA from LNCaP cell lysate, seminal fluid and with recombinant baculovirus expressed PSMA. Clone 3E6 also binds a 100 kDa protein in LNCaP lysates, corresponding to PSM'. Monoclonal anti-PSMA clone 3E6 recognizes an epitope present in the 57-134 amino acid region of the extracellular portion of the PSMA molecule, as determined by Western blot analysis of baculovirus expressed PSMA fragments (3).

Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide (NaN₃), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide 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.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused solution 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

Paraffin sections: The antibody can be used for labeling paraffin-embedded tissue sections fixed in formalin. Tissue specimens should be cut into sections of approximately 4 µm.

Pre-treatment: Pre-treatment of formalin-fixed, paraffin-embedded tissue sections with heat-induced epitope retrieval (HIER) is required. Optimal results are obtained by pretreating tissues with HIER using diluted EnVision FLEX Target Retrieval Solution, High pH (50x) (Code K8004). Deparaffinization, rehydration and epitope retrieval can be performed in Dako PT Link. For details, please refer to PT Link User Guide. The following parameters should be used for PT Link: Pre-heat temperature: 65 °C; epitope retrieval temperature and time: 97 °C for 20 (±1) minutes; cool down to 65 °C. Remove slide rack from PT tank and immediately dip slides in jar/tank (e.g., PT Link Rinse Station (Code PT109)) containing diluted room temperature EnVision FLEX Wash Buffer (20x) (Code K8007). Leave slides in Wash Buffer for 1-5 minutes.

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides (Code K8020) is recommended. After staining, the sections must be dehydrated, cleared and mounted using a permanent mounting method.

Staining procedure

These are guidelines only. Optimal conditions may vary depending on specimen type and preparation method, and should be validated individually by each laboratory. The performance of this antibody should be established by the user when utilized with other manual staining systems or automated platforms.

Dilution: The recommended dilution of Monoclonal Mouse Anti-Human PSMA, Clone 3E6, Code M3620, is 1:50. Dilute the antibody in Dako Antibody Diluent (Code S0809). Incubate pretreated tissue sections for 20 minutes at room temperature.

Negative control: The recommended negative control reagent is Dako Negative Control, Mouse IgG1 (Code X0931), diluted to the same Ig concentration as the primary antibody. Unless the stability of the diluted antibody and negative control has been established in the actual staining procedure, dilute these reagents immediately prior to use.

Visualization: The recommended visualization system is EnVision FLEX, High pH (Code K8000/K8010) using a 20 minute incubation at room temperature. Follow the procedure enclosed with the selected visualization system(s).

Counterstaining: The recommended counterstain is EnVision FLEX Hematoxylin (Code K8008/K8018).

Quality control: Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include prostate and the cells/structures should display reaction patterns as described for this tissue in the "Performance characteristics" section.

Staining interpretation

The cellular staining pattern is cytoplasmic and/or membranous.

Performance characteristics

Normal tissues: In prostate, glandular epithelial cells show a moderate to strong cytoplasmic and/or membranous staining reaction.

Tissue Type (# tested)	Labeled Tissue Elements	Tissue Type (# tested)	Labeled Tissue Elements
Adrenal (3)	0/3	Pancreas (3)	0/3
Bone marrow (3)	0/3	Parathyroid (3)	2/3 ductal cells (1%), cytoplasmic
Breast (3)	3/3 Glandular epithelial cells (40%), cytoplasmic	Pituitary (3)	0/3
Cerebellum (3)	1/3 Neural stromal cells (50%), cytoplasmic	Prostate (3)	3/3 Glandular epithelial cells (60%), cytoplasmic and/or membranous
Cerebrum (3)	0/3	Salivary gland (3)	3/3 Glandular epithelial cells (4%), cytoplasmic
Cervix (3)	0/3	Skeletal muscle	0/3
Colon (3)	0/3	Skin (3)	0/3
Esophagus (3)	0/3	Small intestine (3)	0/3
Heart (3)	0/3	Spleen (3)	1/3 follicular cells (10%), cytoplasmic; macrophage (40%), cytoplasmic
Kidney (3)	3/3 Proximal tubule cells (75%), cytoplasmic	Stomach (3)	0/3
Liver (3)	3/3 hepatocytes (15%), cytoplasmic	Testis (3)	0/3
Lung (3)	0/3	Thymus (3)	0/3
Mesothelial cells (3)	0/3	Thyroid (3)	0/3
Nerve, peripheral (3)	0/3	Tonsil (3)	3/3 Follicular cell (10%), cytoplasmic
Ovary (3)	0/3	Uterus (3)	1/3 vascular endothelium (60%), cytoplasmic










Abnormal tissues:

In prostate adenocarcinoma, glandular epithelial cells showed a moderate to strong cytoplasmic and/or membranous staining reaction (92/102) (2).

References

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Explanation of symbols

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



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