IMP3 (RM)

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3180-120816

BIOCARE MEDICAL

Catalog Number:	**ACI 3180 A, B**	**API 3180 AA**
Description:	0.1, 0.5 ml, concentrated	6.0 ml, prediluted
Dilution:	1:100	Ready-to-use
Diluent:	Renoir Red	N/A

Intended Use:
For In Vitro Diagnostic Use
IMP3 (RM) [EP286] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of IMP3 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. 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:
IMP3 (insulin-like growth factor II mRNA-binding protein 3) is a 580 amino acid oncofetal RNA binding protein containing four K homology domains which is encoded by a 4350 bp mRNA transcript produced by the IGF2BP3 gene on chromosome 7p11.5 (1,2). Its relevance as a novel biomarker in many kinds of cancer has been recently published and data also suggest that IMP3 may play an important role in malignant transformation (3). Cytoplasmic expression of IMP3 has been associated with a more aggressive phenotype in many cancers including triple negative (basal-like) breast cancers, colon cancers, lung cancer and prostate cancer (4-7). IMP3 has also been used in discriminating between benign vs malignant cancers and IMP3 has been shown to be a marker of high-grade dysplasia in esophageal adenocarcinoma (3, 8-10).

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, an enzyme labeled polymer is added to bind to the primary antibody. The detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Rabbit monoclonal
Species Reactivity: Human; others not tested
Clone: EP286
Isotype: IgG
Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.
Epitope/Antigen: A synthetic peptide corresponding to the human IMP3 protein
Cellular Localization: Cytoplasmic/nuclear
Positive Tissue Control: Lung squamous carcinoma and normal pancreas
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative

Storage and Stability:
Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations:
Peroxide Block:
Block for 5 minutes with Biocare's Peroxidazed 1.

Pretreatment: Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 60 minutes at RT.

Probe: N/A

Polymer: Incubate for 30 minutes at RT with a secondary-conjugated polymer.

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Biocare's Warp Red.

Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Note:
This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.
Performance Characteristics:
Cross-reactivity on normal tissue and sensitivity and specificity on diseased tissue is summarized in Tables 1 and 2, respectively.
Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. 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.
Quality Control:
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011
Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper

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Precautions Cont'd:
plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (11)

2. 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. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (12)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:
1. Mueller-Pillasch F*, et al.* Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein. Oncogene. 1997;14(22):2729–33.
2. Nielsen J, *et al.* A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. Mol Cell Biol. 1999;19(2):1262–70.
3. Lu D, *et al.* An oncofetal protein IMP3: a new molecular marker for the detection of esophageal adenocarcinoma and high-grade dysplasia. Am J Surg Pathol. 2009 Apr;33(4):521-5.
4. Walter O, *et al.* IMP3 is a novel biomarker for triple negative invasive mammary carcinoma associated with a more aggressive phenotype. Hum Pathol. 2009 Nov;40(11):1528-33.
5. Li D, *et al.* IMP3 is a novel prognostic marker that correlates with colon cancer progression and pathogenesis. Ann Surg Oncol. 2009 Dec;16(12):3499-506.
6. Beljan Perak R, *et al.* IMP3 can predict aggressive behaviour of lung adenocarcinoma. Diagn Pathol. 2012 Nov 28;7:165.
7. Chromecki TF, *et al.* Prognostic value of insulin-like growth factor II mRNA binding protein 3 in patients treated with radical prostatectomy. BJU Int. 2012 Jul;110(1):63-8.
8. Lee AF, *et al.* IMP3 and GLUT-1 immunohistochemistry for distinguishing benign from malignant mesothelial proliferations. Am J Surg Pathol. 2013 Mar;37(3):421-6.
9. Danialan R*, et al.* The utility of PAX8 and IMP3 immunohistochemical stains in the differential diagnosis of benign, premalignant, and malignant endocervical glandular lesions. Gynecol Oncol. 2013 Aug;130(2):383-8.
10. Mentrikoski MJ, *et al.* Diagnostic utility of IMP3 in segregating metastatic melanoma from benign nevi in lymph nodes. Mod Pathol. 2009 Dec;22(12):1582-7.
11. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
12. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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Table 1: Cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebellum	0	1
Cerebral Cortex	0	1
Pituitary	0	1
Adrenal Gland	0	1
Thymus	1	1
Tonsil	1	1
Thyroid	0	1
Esophagus	0	1
Stomach	0	1
Small Intestine	0	1
Colon	0	1
Appendix	0	1
Pancreas	0	1
Spleen	0	1
Ovary	0	1
Cervix	0	1
Endomyometrium	0	1
Fallopian Tube	0	1
Placenta	1	1
Kidney	0	1
Urethra	0	1
Breast	0	1
Prostate	0	1
Testis	1	1
Myocardium	0	1
Smooth Muscle	0	1
Skeletal Muscle	0	1
Lymph Node	0	1
Aorta	0	1
Lung	0	1
Skin	0	1
Liver	0	1

Table 2: Sensitivity and specificity was determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Lung Adenocarcinoma	9	11
Lung Squamous carcinoma	9	10
Breast cancer	1	4
Colon cancer	0	4
Prostate cancer	1	4