**Intended Use**

For In Vitro Diagnostic Use.

This antibody is intended for use in immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

* The IMP-3 antibody, clone EP286, has been manufactured using Epitomics RabMab® technology covered under Patent No.'s 5,675,063 and 7,402,409.

**Immunogen**

A synthetic peptide corresponding to the human IMP-3 protein.

**Summary and Explanation**

Insulin-like growth factor 2 mRNA-binding protein 3 is a protein that in humans is encoded by the IGF2BP3 gene. The protein encoded by this gene is primarily found in the nucleus, where it can bind to the 5' UTR of the insulin-like growth factor II leader 3 mRNA and may repress translation of insulin-like growth factor II during late development. The encoded protein contains several K Homology domains, which are important in RNA binding and are known to be involved in RNA synthesis and metabolism. IMP3 is normally expressed in early embryonic tissues. The IHC of IMP3 may help in the classification of Non-small Cell Lung Carcinomas and Pancreatic Adenocarcinomas as well as subtypes of carcinomas from other organs such as Renal Cell Carcinoma, Adenocarcinoma of the Uterine Cervix, Endometrial Carcinoma, Adenocarcinoma of the Esophagus, Malignant Melanoma, Merkel Cell Carcinoma, Urothelial Carcinoma, Neuroendocrine Carcinoma of the Lung, and triple negative breast cancer.

Various studies have found that IMP3 is a marker for malignancy and is correlated with increased tumor aggressiveness and reduced overall survival. The diagnosis of Pancreatic Ductal Adenocarcinoma (PDAC) in core needle biopsies can be challenging, and immunohistochemical studies of IMP3 expression have been found to be a potential new marker for the diagnosis of PDAC in core needle biopsies. IMP3 expression for the prognostic evaluation of non-small cell lung carcinomas has been found to exhibit mainly cytoplasmic staining pattern in the NSCLC tissues with a positive rate of IMP3 protein expression of 74.7% in the NSCLC tissues, a significantly higher than the rate of 19.9% in the adjacent non-tumor tissues. In the early- and late-stage NSCLC the disease-free and overall survival rates of the patients with IMP3 expression were significantly lower than those of the patients without IMP3 expression. IMP3 plays a significant role in the progression of NSCLC, and it may potentially be used as an independent biomarker for prognostic evaluation. IMP3 may be a useful diagnostic marker in the assessment of endometrial cancers and their precursor lesions, particularly when the amount of available tissue material is limited and a concern of type II cancer arises. High frequency of IMP3 expression is present in decidualized endometrial stroma of gestational endometrium and chorionic villi in early pregnancy.

**Precautions**

1. For professional users only. Ensure results are interpreted by a medical professional.
2. This product contains sodium azide (NaN3), a toxic chemical which may react with plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent sodium azide build-up.
3. Ensure proper handling procedures are used with reagent. Always wear proper laboratory equipment such as laboratory coat and gloves when handling reagents.
4. Unused solution should be disposed of according to local and federal regulations.
5. Do not ingest reagent. If reagent ingested, contact a poison control center immediately.
6. For complete recommendations for handling biological specimens please refer to the CDC document, “Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories” (8).

**Storage**

Store at 2-8 ºC. Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

**Specimen Preparation**

**Paraffin sections:** The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation to ensure best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033) or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

**Frozen sections and cell preparations:** The antibody can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

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**Presentation**

**Catalog Num.** | **Antibody Type** | **Dilution** | **Volume/Qty**
--- | --- | --- | ---
BSB 2964 | Tinto Prediluted | Ready-to-Use | 3.0 mL
BSB 2965 | Tinto Prediluted | Ready-to-Use | 7.0 mL
BSB 2966 | Tinto Prediluted | Ready-to-Use | 15.0 mL
BSB 2967 | Concentrated | 1:25 - 1: 100 | 0.1 mL
BSB 2968 | Concentrated | 1:25 - 1: 100 | 0.5 mL
BSB 2969 | Concentrated | 1:25 - 1: 100 | 1.0 mL
BSB 2970 | Control Slides | Not Applicable | 5 slides

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**Antibody Type** | **Isotype** | **Reactivity** | **Localization** | **Species Reactivity**
--- | --- | --- | --- | ---
Rabbit Monoclonal | IgG | Paraffin, Frozen | Cytoplasmic, Nuclear | Human, Predicted: Mouse, Rat

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**Species Reactivity**

Human, Predicted: Mouse, Rat

**Localization**

Cytoplasmic, Nuclear

**Reactivity**

Paraffin, Frozen

**Clone**

EP286

**Inset:** IHC of IMP-3 on a FFPE Liver Tissue
Staining Procedure

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positive charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.
3. Deparaffinize, dehydrate and rehydrate tissues.
4. Subject tissues to heat epitope retrieval using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
5. Any of three heating methods may be used:
   a. TintOretriever Pressure Cooker or Equivalent
      Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA, and place in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.
   b. TintOretriever PT Module or Water Bath Method
      Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.
   c. Conventional Steamer Method
      Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a Steamer, cover and steam for 30-60 minutes.
6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15–20 minutes.
7. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with IHC wash buffer or DI water.
9. Continue IHC staining protocol.

Recommended IHC Protocol

<table>
<thead>
<tr>
<th>Step</th>
<th>ImmunoDetector AP/HRP</th>
<th>PolyDetector AP/HRP</th>
<th>PolyDetector Plus HRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxidase/AP Blocker</td>
<td>5 min.</td>
<td>5 min.</td>
<td>5 min.</td>
</tr>
<tr>
<td>Primary Antibody</td>
<td>30-60 min.</td>
<td>30-60 min.</td>
<td>30-60 min.</td>
</tr>
<tr>
<td>1st Step Detection</td>
<td>10 min.</td>
<td>30-45 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>2nd Step Detection</td>
<td>10 min.</td>
<td>Not Applicable</td>
<td>15 min.</td>
</tr>
<tr>
<td>Substrate-Chromogen</td>
<td>5-10 min.</td>
<td>5-10 min.</td>
<td>5-10 min.</td>
</tr>
<tr>
<td>Counterstain</td>
<td>Varies</td>
<td>Varies</td>
<td>Varies</td>
</tr>
</tbody>
</table>

Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a medical professional.

References