**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.

**Immunogen**

Synthetic peptide corresponding to the C-terminus of the human SMAD4 protein.

**Summary and Explanation**

SMAD 4, also known as DPC4 or SMAD family member n°4, is a protein involved in cell signaling in mammals. SMAD 4 forms with SMAD 3 a complex which can bind to DNA and modify the expression of several genes related to cellular activities such as proliferation or differentiation. SMAD 4 serves as a mediator between extracellular growth factors from the TGFβ family and genes inside the cell nucleus. The abbreviation coin co-SMAD stands for common mediator. SMAD 4 is also defined as a signal transducer.

SMAD 4, is often found mutated in many cancers. The mutation can be inherited or acquired during an individual's lifetime. If inherited, the mutation affects both somatic and sexual cells. If the SMAD 4 mutation is acquired, it will only exist in certain somatic cells. Indeed, SMAD 4 is not synthesized by all cells. The protein is present in skin, pancreatic, colon, uterus and epithelial cells. It is also produced by fibroblasts. The functional SMAD 4 participates in the regulation of the TGF-β signal transduction pathway, which negatively regulates growth of epithelial cells and the extracellular matrix (ECM).

When the structure of SMAD 4 is altered, expression of the genes involved in cell growth is no longer regulated and cell proliferation can go on without any inhibition. The important number of cell divisions leads to the forming of tumors and then to multiploid colorectal cancer and pancreatic carcinoma. It is found inactivated in at least 50% of pancreatic cancers. SMAD 4 is also found mutated in the autosomal dominant disease juvenile polyposis syndrome (JPS). JPS is characterized by hamartomatous polyps in the gastrointestinal (GI) tract. These polyps are usually benign, however they are at greater risk of developing gastrointestinal cancers, in particular colon cancer.

Approximately 55% of pancreatic cancers bear deletions or mutations in SMAD4/DPC4. Patients undergoing surgical resection of their pancreatic adenocarcinoma, survival of patients whose tumors expressed SMAD4 protein was significantly longer (unadjusted median survival, 19.2 months) compared with 14.7 months without SMAD4 protein expression (P = 0.03). This SMAD4 survival benefit persisted after adjustment for prognostic factors including tumor size, margin status, lymph node status, pathological stage, blood loss, and use of adjuvant chemoradiotherapy.

**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.

**Storage**

Store at 2-8 °C. 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 0010-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.
### 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.
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>

### Symbol Key / Légende des symboles/Erläuterung der Symbole

- IVD: In Vitro Diagnostic Medical Device
- **Storage Temperature Limites de température Zulässiger temperaturbereich**
- Manufacturer Fabricant Hersteller
- **Catalog Number** Référence du catalogue Bestellnummer
- In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum
- **Read Instructions for Use** Consulter les instructions d’utilisation Gebrauchsanweisung beachten
- **Expiration Date** Utiliser jusque Verwendbar bis
- **Lot Number** Code du lot Chargenbezeichnung

### 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