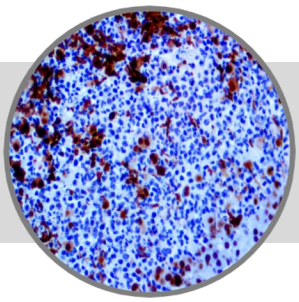
SMAD4/DPC4

Clone: BSB-63

Mouse Monoclonal



CE IVD

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Inset: IHC of SMAD4/DPC4 on a FFPE Pancreatic Cancer Tissue

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 N-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.

SMAD4, 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. 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 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. In 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) as 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.

Antibody Type	Mouse Monoclonal	**Clone**	BSB-63
Isotype	IgG1	**Reactivity**	Paraffin, Frozen
Localization	Cytoplasmic, Nuclear	**Control**	Pancreas, Thyroid, Placenta, Cervix, TCC & Lymphoblastic Lymphoma
	Species Reactivity	Human	

Presentation

SMAD4/DPC4 is a mouse monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

Catalog No.	*Antibody Type*	*Dilution*	*Volume/Qty*
BSB 3204	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 3205	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 3206	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 3207	Concentrated	1:50 - 1:200	0.1 mL
BSB 3208	Concentrated	1:50 - 1:200	0.5 mL
BSB 3209	Concentrated	1:50 - 1:200	1.0 mL

Control Slides Available

Catalog No.	*Quantity*
BSB 3210	5 slides

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN_3) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to Safety Data Sheet for this product.
9. 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" (see References in this document).

Storage Store at 2-8°C (Control Slides: Store at 20-25°C)

Stability

This product is stable up to the expiration date on the product label. 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 for 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.

Staining Procedure

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively 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 induced epitope retrieval (HIER) 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 on trivet 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 ImmunoDNA washer or DI water.
9. Continue IHC staining protocol. Wash slides between each step with ImmunoDNA washer solution.

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP
Peroxidase/AP Blocker	5 min.	5 min.	5 min
Primary Antibody	30-60 min.	30-60 min.	30-60 min.
1st Step Detection	10 min.	30-45 min.	15 min.
2nd Step Detection	10 min.	Not Applicable	15 min.
Substrate-Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain / Coverslip	Varies	Varies	Varies

Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.

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 qualified medical professional.

References

1. Lin X, et al. "Activation of transforming growth factor-beta signaling by SUMO-1 modification of tumor suppressor Smad4/DPC4". The Journal of Biological Chemistry 2003; 278(21): 18714–18719.
2. Cotran RS, Kumar V, Fausto N, Robbins SL, Abbas AK (2005). Robbins and Cotran pathologic basis of disease (7th ed.). St. Louis, Mo: Elsevier Saunders.
3. Andrew V. Biankin, et al. DPC4/Smad4 Expression and Outcome in Pancreatic Ductal Adenocarcinoma. American Society of Clinical Oncology 2002. vol. 20, 23: 4531-4542.
4. M. Tascilar et al., The SMAD4 Protein and Prognosis of Pancreatic Ductal Adenocarcinoma. Clin. Cancer Res. 2001; 7: 4115–4121.
5. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012.

Symbol Key / Légende des symboles/Erläuterung der Symbole

EC REP EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	Storage Temperature Limites de température Zulässiger Temperaturbereich	Manufacturer Fabricant Hersteller	REF Catalog Number Référence du catalogue Bestellnummer
IVD 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 Lot Number Code du lot Chargenbezeichnung

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