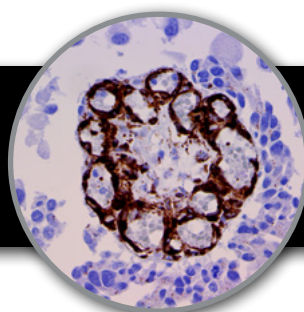


Osteonectin/SPARC

Clone: BSB-93

Mouse Monoclonal



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Inset: IHC of Osteonectin/SPARC on a FFPE Bladder TCC 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

A synthetic peptide corresponding to the N-terminus of the human SPARC protein.

Summary and Explanation

Osteonectin also known as secreted protein acidic and rich in cysteine (SPARC) or basement-membrane protein 40 (BM-40) is a protein that in humans is encoded by the SPARC gene. Osteonectin is a glycoprotein in the bone that binds calcium. It is secreted by osteoblasts during bone formation, initiating mineralization and promoting mineral crystal formation. Fibroblasts, including periodontal fibroblasts, synthesize Osteonectin. This protein is synthesized by macrophages at sites of wound repair and platelet degranulation, so it may play an important role in wound healing.

Osteonectin also increases the production and activity of matrix metalloproteinases, a function important to invading cancer cells within bone. Additional functions of Osteonectin beneficial to tumor cells include angiogenesis, proliferation and migration. Overexpression of Osteonectin is reported in many human cancers such as breast, prostate and colon. A correlation between Osteonectin overexpression and ampullary cancers and chronic pancreatitis has been reported. A study designed to examine the expression and functional role of Osteonectin in primary and metastatic Pancreatic Ductal Adenocarcinoma (PDAC) showed a 31-fold increase in Osteonectin mRNA levels in PDAC and a 16-fold increase in chronic pancreatitis as compared with the normal pancreas ($P < 0.01$). By immunohistochemistry, faint immunoreactivity was detected in the normal pancreas. In contrast, strong staining of the cancer cells was observed in addition to extensive Osteonectin immunoreactivity in surrounding fibroblasts and in the extracellular matrix. In metastatic tissues, strong immunoreactivity was observed in fibroblasts and in extracellular matrix surrounding metastatic cancer cells, whereas the signal was absent in most tumor cells. In vitro studies showed that osteonectin was able to inhibit cancer cell growth while promoting invasiveness of pancreatic tumor cells. Another study set out to examine both the transcript levels of Osteonectin and the presence of the molecule in breast cancer tissue and to demonstrate if a link existed between the levels of Osteonectin and clinical outcome. Protein levels of Osteonectin were assessed using immunohistochemistry and levels were correlated with nodal status, grade, prognosis and long-term survival (10 years). Transcript levels of Osteonectin were found to be significantly higher in tumor tissue when compared to normal background breast tissue. Node-positive tumors also exhibited higher levels of Osteonectin than node-negative tumors. Over a 6 year follow-up, high levels of Osteonectin was seen to be significantly associated with the overall survival of the patients and it was concluded that Osteonectin plays a crucial role in tumor development in breast cancer and as such has a significant bearing on patient prognosis and long-term survival.

Antibody Type	Mouse Monoclonal	Clone	BSB-93
Isotype	IgG1	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic	Control	Adrenal, Testis, Placenta, TCC, Testicular Cancer, Cervical Cancer
Species Reactivity	Human		

Presentation

Osteonectin/SPARC 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.

Presentations

Catalog Num.	Antibody Type	Dilution	Volume/Qty
BSB 3258	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 3259	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 3260	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 3261	Concentrated	1: 50 - 1:200	0.1 mL
BSB 3262	Concentrated	1: 50 - 1:200	0.5 mL
BSB 3263	Concentrated	1: 50 - 1:200	1.0 mL
BSB 3264	Control Slides	Not Applicable	5 slides

Precautions

1. For professional users only. Ensure results are interpreted by a medical professional.
2. This product contains sodium azide (NaN_3), 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" (4).

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 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 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.

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

1. Villareal XC, et al. Structure of human osteonectin based upon analysis of cDNA and genomic sequences. *Biochemistry*. 1989; 28 (15): 6483-91.
2. Guweidhi A, et al. Osteonectin influences growth and invasion of pancreatic cancer cells. *Annals of Surgery*. 2005; 242 (2): 224-34.
3. Watkins G, et al. Increased levels of SPARC (osteonectin) in human breast cancer tissues and its association with clinical outcomes. *Prostaglandins Leukot Essent Fatty Acids*. 2005 Apr; 72 (4):267-72.
4. 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.

Recommended IHC 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	Varies	Varies	Varies

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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