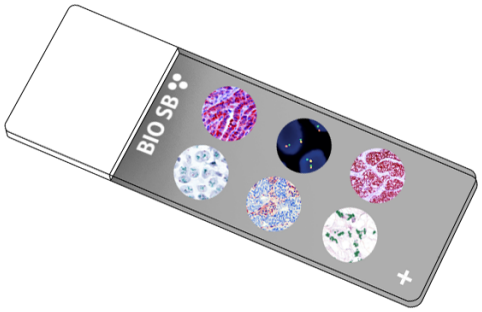
Bio SB
BIOSCIENCE FOR THE WORLD

Osteonectin/SPARC Control Slides

CE IVD



Intended Use
For In Vitro Diagnostic Use.

Summary and Explanation
Osteonectin also known as secreted protein acidic and rich in cysteine 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 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 matrices 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 examined both the transcript levels of Osteonectin and the presence of the molecule in breast cancer tissue and to determine whether 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.

Presentation
Five slides of Osteonectin/SPARC positive tissues, each mounted on Hydrophilic Plus Slides, provided in a plastic mailer.

Catalog No.	*Quantity*
BSB-9317-CS	5 slides
BSB 3264	5 slides

Storage Store at 20-25°C

Precautions
1. For professional users only. Results should be interpreted by a qualified medical professional.
2. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
4. Dispose of unused solution with copious amounts of water.
5. 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).

Stability
This product is stable up to the expiration date on the product label.
Do not use after expiration date listed on the package label.

IHC Protocol
1. 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).
2. 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.
3. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.

4. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
5. Wash slides with ImmunoDNA washer or DI water.
6. 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

Abbreviated IF Protocol

Step	Incubation Time
Rinse slides in IF wash buffer	5 minutes
Drain and wipe excess IF wash buffer off slide	
Conduct remaining steps in the dark	
Apply Antibody	30-60 minutes
Rinse with 3 changes of IF wash buffer	3x15 minutes each
Coverslip with IF mounting medium	

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. 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. https://www.cdc.gov/mmwr/pdf/other/su6101.pdf

Symbol Key / Légende des symboles/Erläuterung der Symbole

EC REP — QAdvis EAR AB, Ideon Science Park, Scheelevägen 17, SE-223 70 Lund, Sweden	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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