Mouse SPARC Antibody
Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF942

DESCRIPTION
Species Reactivity: Mouse
Specificity: Detects mouse SPARC in direct ELISAs and Western blots.
Source: Polyclonal Goat IgG
Purification: Antigen Affinity-purified
Immunogen: Mouse myeloma cell line NS0-derived recombinant mouse SPARC/Osteonectin Ala18-Ile302
Accession #: P07214
Formulation: Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.
*Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

APPLICATIONS
Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

Recommended Concentration Sample

Western Blot 0.2 μg/mL See Below
Immunocytochemistry 5-15 μg/mL See Below
Immunohistochemistry 1-15 μg/mL See Below

Intracellular Staining by Flow Cytometry 0.25 µg/10^6 cells Ba/lb/C-3T3 mouse embryonic fibroblast cell line fixed with paraformaldehyde and permeabilized with saponin

CyTOF-ready Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.

DATA
Western Blot
Detection of Mouse SPARC by Western Blot. Western blot shows lysates of C2C12 mouse myoblast cell line and mouse placenta tissue. PVDF membrane was probed with 0.2 μg/mL of Goat Anti-Mouse SPARC Antigen Affinity-purified Polyclonal Antibody (Catalog # AF942) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for SPARC at approximately 35-37 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry
SPARC/Osteonectin in Mouse Embryo. SPARC/Osteonectin was detected in immersion fixed frozen sections of mouse embryo (E15) using Mouse SPARC/Osteonectin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF942) at 1.7 μg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to developing cartilage. View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

Immunocytochemistry
SPARC in C2C12 Mouse Cell Line. SPARC was detected in immersion fixed C2C12 mouse myoblast cell line using Goat Anti-Mouse SPARC Antigen Affinity-purified Polyclonal Antibody (Catalog # AF942) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to endoplasmic reticulum. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Intracellular Staining by Flow Cytometry
Detection of SPARC in Ba/lb/C-3T3 Mouse Cell Line by Flow Cytometry. Ba/lb/C-3T3 mouse fibroblast cell line was stained with Goat Anti-Mouse SPARC Polyclonal Antibody (Catalog # AF942, filled histogram) or Goat IgG control antibody (Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated anti-Goat IgG (Catalog # F0107). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC006). Staining was performed using our Staining Intracellular Molecules protocol.
PREPARATION AND STORAGE

Reconstitution
Reconstitute at 0.2 mg/mL in sterile PBS.

Shipping
The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C

Stability & Storage
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

SPARC, an acronym for “secreted protein, acidic and rich in cysteine”, is also known as osteonectin or BM-40 (1-5). It is the founding member of a family of secreted matricellular proteins with similar domain structure. The 302 amino acid (aa), 43 kDa protein contains a 17 aa signal sequence, an N-terminal acidic region that binds calcium, a follistatin domain containing Kazal-like sequences, and a C-terminal extracellular calcium (EC) binding domain with two EF-hand motifs (1-5). Crystal structure shows that residues implicated in cell binding, inhibition of cell spreading and disassembly of focal adhesions cluster on one face of SPARC, while a collagen binding epitope and an N-glycosylation site are opposite this face (6). SPARC is produced by fibroblasts, capillary endothelial cells, platelets, and macrophages, especially in areas of tissue morphogenesis and remodeling (3, 7). SPARC shows context-specific effects, but generally inhibits adhesion, spreading and proliferation, and promotes collagen matrix formation (3-5). For endothelial cells, SPARC disrupts focal adhesions and binds and sequesters PDGF and VEGF (3-5). SPARC is abundantly expressed in bone, where it promotes osteoblast differentiation and inhibits adipogenesis (5, 8). SPARC is potentially cleaved by metalloproteinases, producing an angiogenic peptide that includes the copper-binding sequence KGHK (7). Paradoxically, SPARC is highly expressed in many tumor types, yet expression mainly decreases the likelihood of metastasis and confers sensitivity to chemotherapy and radiation (4, 9, 10). Stabilin-1, which is expressed on alternately activated macrophages, is the first SPARC receptor to be identified. It binds the SPARC EC domain and mediates endocytosis for degradation (11). Mature mouse SPARC shows 97%, 92%, 92%, 92%, and 83% aa identity with rat, human, dog, cow, and chick SPARC, respectively.

References: