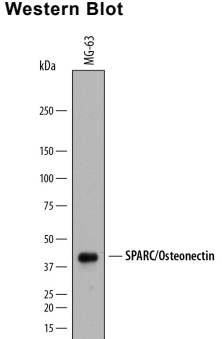


| DESCRIPTION               |   |
|---------------------------|---|
| <b>Species Reactivity</b> | Human   |
| <b>Specificity</b>        | Detects human SPARC/Osteonectin in direct ELISAs and Western blots. In direct ELISAs, approximately 25% cross-reactivity with recombinant mouse SPARC is observed, and less than 3% cross-reactivity with recombinant human SPARC L1 is observed. |
| <b>Source</b>             | Polyclonal Goat IgG   |
| <b>Purification</b>       | Antigen Affinity-purified   |
| <b>Immunogen</b>          | Mouse myeloma cell line NS0-derived recombinant human SPARC/Osteonectin<br>Ala18-Ile303<br>Accession # P09486   |
| <b>Endotoxin Level</b>    | <0.10 EU per 1 µg of the antibody by the LAL method.  |
| <b>Formulation</b>        | Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.<br>*Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.   |

| APPLICATIONS   |  |   |
|--|--|---|
| <b>Please Note:</b> Optimal dilutions should be determined by each laboratory for each application. <i>General Protocols</i> are available in the <i>Technical Information</i> section on our website. |  |   |
|  | Recommended Concentration  | Sample  |
| <b>Western Blot</b>  | 2 µg/mL  | See Below   |
| <b>Immunohistochemistry</b>  | 5-15 µg/mL   | See Below   |
| <b>Intracellular Staining by Flow Cytometry</b>  | 2.5 µg/10 <sup>6</sup> cells   | MG-63 human osteosarcoma cell line fixed with paraformaldehyde and permeabilized with saponin |
| <b>Simple Western</b>  | 20 µg/mL   | See Below   |
| <b>CyTOF-ready</b>   | Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation. |   |

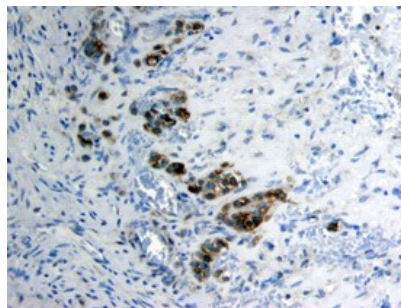
**DATA**

**Western Blot**



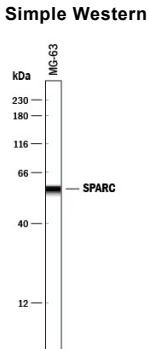
**Detection of Human SPARC by Western Blot.** Western blot shows lysates of MG-63 human osteosarcoma cell line. PVDF membrane was probed with 2 µg/mL of Goat Anti-Human SPARC Antigen Affinity-purified Polyclonal Antibody (Catalog # AF941) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). A specific band was detected for SPARC at approximately 43 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**Immunohistochemistry**




**SPARC/Osteonectin in Human Ovary.** SPARC/Osteonectin was detected in immersion fixed paraffin-embedded sections of human ovary using Goat Anti-Human SPARC/Osteonectin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF941) at 3 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). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

**Simple Western**



**Detection of Human SPARC by Simple Western™.** Simple Western lane view shows lysates of MG-63 human osteosarcoma cell line, loaded at 0.2 mg/mL. A specific band was detected for SPARC at approximately 57 kDa (as indicated) using 20 µg/mL of Goat Anti-Human SPARC Antigen Affinity-purified Polyclonal Antibody (Catalog # AF941) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



#### PREPARATION AND STORAGE

|                                |   |
|--------------------------------|---|
| <b>Reconstitution</b>          | Reconstitute at 0.2 mg/mL in sterile PBS.   |
| <b>Shipping</b>                | The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.<br>*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C  |
| <b>Stability &amp; Storage</b> | <p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul> |

#### 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 286 amino acid (aa), 43 kDa protein contains an N-terminal acidic region that binds calcium, a follistatin domain that contains Kazal-like sequences, and a C-terminal extracellular calcium (EC) binding domain with two EF-hand motifs (1-5). Crystal structure modeling 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 undergoing an endothelial to mesenchymal transition; its expression, however, mainly decreases the likelihood of metastasis and confers sensitivity to chemotherapy and radiation (4, 9-11). 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 (12). Mature human SPARC shows 92%, 92%, 97%, 99%, 96%, and 85% aa identity with mouse, rat, canine, bovine, porcine, and chick SPARC, respectively.

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