

## DESCRIPTION

<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human Osteopontin in direct ELISAs and Western blots. In direct ELISAs and Western blots, this antibody does not cross-react with recombinant mouse Osteopontin.
<b>Source</b>	Monoclonal Mouse IgG <sub>2A</sub> Clone # 223126
<b>Purification</b>	Protein A or G purified from hybridoma culture supernatant
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant human Osteopontin Ile17-Asn300 Accession # NP_000573.1
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

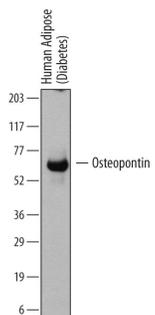
## APPLICATIONS

**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
<b>Western Blot</b>	2 µg/mL	See Below
<b>Simple Western</b>	100 µg/mL	See Below

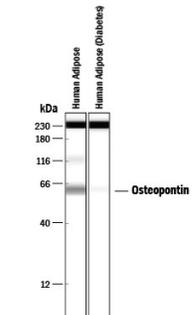
## DATA

**Western Blot**



**Detection of Human Osteopontin/OPN by Western Blot.**  
Western blot shows lysates of human adipose (diabetes) tissue. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human Osteopontin/OPN Monoclonal Antibody (Catalog # MAB14331) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for Osteopontin/OPN at approximately 60 to 65 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**Simple Western**



**Detection of Human Osteopontin/OPN by Simple Western™.**  
Simple Western lane view shows lysates of human adipose tissue and human adipose (diabetes) tissue, loaded at 0.2 mg/mL. A specific band was detected for Osteopontin/OPN at approximately 63 kDa (as indicated) using 100 µg/mL of Mouse Anti-Human Osteopontin/OPN Monoclonal Antibody (Catalog # MAB14331). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

\*Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.5 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. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <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**

Osteopontin (OPN, previously also referred to as transformation-associated secreted phosphoprotein, bone sialoprotein I, 2ar, 2B7, early T lymphocyte activation 1 protein, minopotin, calcium oxalate crystal growth inhibitor protein), is a secreted, highly acidic, calcium-binding, RGD-containing, phosphorylated glycoprotein originally isolated from bone matrix (1). Subsequently, OPN has been found in kidney, placenta, blood vessels and various tumor tissues. Many cell types (including macrophages, osteoclasts, activated T-cells, fibroblasts, epithelial cells, vascular smooth muscle cells, and natural killer cells) can express OPN in response to activation by cytokines, growth factors or inflammatory mediators. Elevated expression of OPN has also been associated with numerous pathobiological conditions such as atherosclerotic plaques, renal tubulointerstitial fibrosis, granuloma formations in tuberculosis and silicosis, neointimal formation associated with balloon catheterization, metastasizing tumors, and cerebral ischemia. Human OPN cDNA encodes a 314 amino acid (aa) residue precursor protein with a 16 aa residue predicted signal peptide that is cleaved to yield a 298 aa residue mature protein with an integrin binding sequence (RGD), and N- and O-glycosylation sites. By alternative splicing, at least three human OPN isoforms exist. OPN has been shown to bind to different cell types through RGD-mediated interaction with the integrins  $\alpha_v\beta_1$ ,  $\alpha_v\beta_3$ ,  $\alpha_v\beta_5$ , and non-RGD-mediated interaction with CD44 and the integrins  $\alpha_8\beta_1$  or  $\alpha_9\beta_1$ . OPN exists both as a component of extracellular matrix and as a soluble molecule. Functionally, OPN is chemotactic for macrophages, smooth muscle cells, endothelial cells and glial cells. OPN has also been shown to inhibit nitric oxide production and cytotoxicity by activated macrophages. Human, mouse, rat, pig and bovine OPN share from approximately 40% - 80% amino acid sequence identity. Osteopontin is a substrate for proteolytic cleavage by thrombin, enterokinase, MMP-3 and MMP-7. The functions of OPN in a variety of cell types were shown to be modified as a result of proteolytic cleavage (2, 3).

**References:**

1. Ann. N.Y. Acad. Sci. (1995) **760**, Apr. 21.
2. Senger, D.R. *et al.* (1996) *Biochim. Biophys. Acta.* **1314**:13.
3. Agnihotri, R. *et al.* (2001) *J. Biol. Chem.* **276**:28261.