Human Osteopontin Rabbit Polyclonal Antibody

Cat# CY-P1035

100 µg (0.5 mg/mL x 200 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
	WB, IHC	H	44-75 kDa	Rabbit IgG

Background

Osteopontin (OPN, also designated Bone Sialoprotein 1, Urinary Stone Protein, spp-1, eta-1, nephropontin, uropontin) is an acidic member of the small integrinbinding ligand N-linked glycoprotein (SIBLING) family of extracellular matrix proteins/cytokines that undergoes extensive posttranslational modification, including phosphorylation, glycosylation, and cleavage, yielding molecular mass variants ranging in size from 25 to 75 kDa. The result is a versatile protein(s) with multiple functions arising from its role as a mediator of cell-cell and cell-extracellular matrix (ECM) communication that encompass both normal and tumorigenic developmental processes, immunological responses during inflammation and wound healing, and biomineralization.

OPN contains a hydrophobic leader sequence characteristic of a secreted protein, a potential calcium phosphate apatite binding region of consecutive asparagine residues, a cell attachment RGD sequence, a thrombin cleavage site, and two glutamines that are recognized substrates for transglutaminase supported multimer formation.

Specificity/Sensitivity: Human Osteopontin Antibody detects endogenous levels of Osteopontin.

Source/Purification: Polyclonal antibody is produced by immunizing rabbit with a synthetic peptide, KSKKFRRPDIQYPDATDE (KLH coupled), corresponding to 170-187 a.a. of human Osteopontin isoform a. IgG is purified by peptide-affinity chromatography.

Recommended Antibody Dilutions: Western blotting: 0.5-1 µg/mL. Immunohistochemistry: 1 -3 µg/mL

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key: WB: Western **IP:** Immunoprecipitation **IHC:** Immunohistochemistry **IC:** Immunocytochemistry **F:** Flow cytometry **E:** ELISA **FP:** Fluorescence Polarization assay
Species Cross-Reactivity Key: H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Mi:** mink **C:** chicken **X:** *Xenopus* **Z:** zebra fish **All:** all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

General References:

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4. Craig AM. Et al. 1989. Osteopontin, a transformationassociated cell adhesion phosphoprotein, is induced by 12-O-tetradecanoylphorbol 13-acetate in mouse epidermis. J Biol Chem 264:9682-9689.

5. Singh, R.P., et al. 1990. Definition of a specific interaction between the early T lymphocyte activation 1 (Eta-1) protein and murine macrophages in vitro and its effect upon macrophages in vivo. J. Exp. Med. 171: 1931-1942.

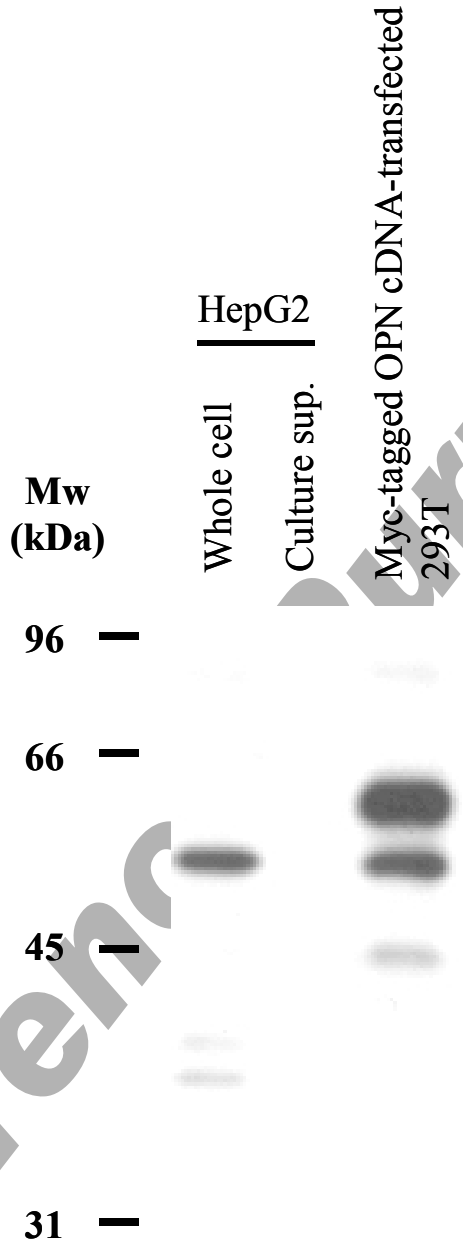
6. Prince, C.W., et al. 1991. Osteopontin, a substrate for transglutaminase and factor XIII activity. Biochem. Biophys. Res. Comm. 177: 1205-1210.

7. Ashkar, S et al. 2000. Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity. Science 287: 860-864

8. Diao, H et al. 2004. Osteopontin as a mediator of NKT cell function in T cell-mediated liver diseases. Immunity 21: 539-550.

9. Nomiyama, T. et al. 2007. Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. J. Clin. Invest. 117: 2877-2888.

Fig.1 Western blot analysis of Human Osteopontin expression



Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100%).
10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent for 20 mL, add 2 mL 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µL Tween-20 (100%).
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS, aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per plate of 10 cm2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.

Related Products

*** Human S100A4 (p9Ka) Rabbit Polyclonal antibody Cat# CY-P1026**
*** Human S100A12 (EN-RAGE) Rabbit Polyclonal antibody Cat# CY-P1027**
*** Human S100P Rabbit Polyclonal antibody Cat# CY-P1028**
*** Human S100A10 Rabbit Polyclonal antibody Cat# CY-P1033**

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