

Monoclonal Antibodies to Osteopontin



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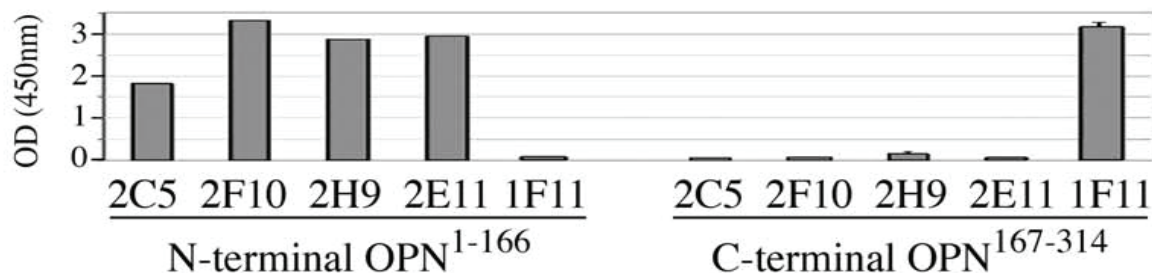
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YOUR PARTNER IN ANTIBODY DEVELOPMENT

Osteopontin (OPN) is a well-characterized secreted protein found in the circulation and is emerging as a potential biomarker for many cancers. Maine Biotechnology Services, Inc.(MBS), in collaboration with Maine Medical Center Research Institute (MMCRI) and the University of Southern Maine (USM), developed five anti-human OPN monoclonal Antibodies.

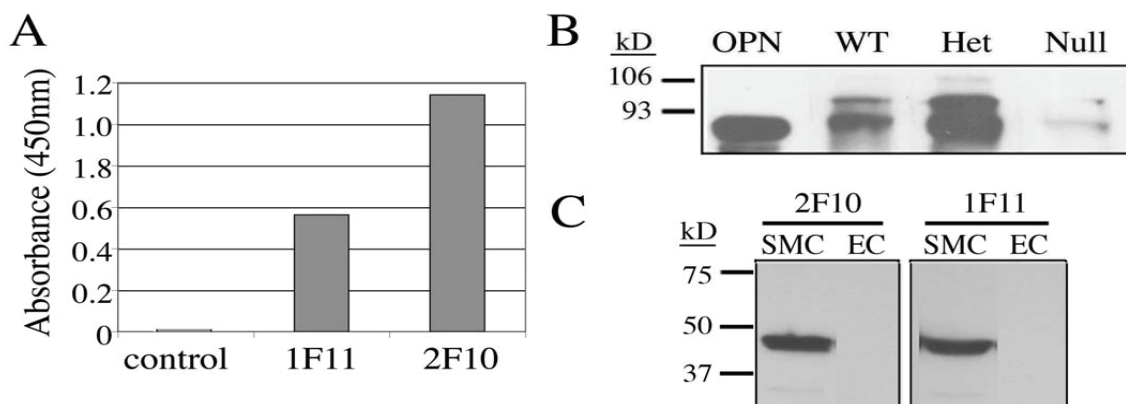
ELISA Data

- All Five clones recognize full length OPN.
- Four of the five clones recognize the N terminal of OPN and one recognizes the C terminal of OPN.



An ELISA was performed using the human N-terminal (aa-1-166) or C-terminal (aa167-314) human recombinant fragments. 2C5, 2H9, 2F10, and 2E11 recognize OPN epitopes on the N-terminal fragment, while 1F11 recognizes an epitope on the C-terminal fragment All 5 clones recognize fl-OPN. (1)

Recognition of Native OPN



A) Human milk OPN was used in ELISA, and 1F11 and 2F10 binding compared to an irrelevant antibody (MAb 1E3, control). B) Whole kidney lysates were collected from wild type (WT) mice, OPN heterozygous mice (Het), and OPN null mutant mice (Null). Lysates were loaded equally and analyzed by SDS-PAGE followed by immunoblotting using 1F11. Recombinant OPN was used as a control. C) Human aortic smooth muscle cells (SMC) were transduced with activated Notch1 receptor (2) to increase OPN expression, and compared to human umbilical vein endothelial cells (EC). Both 2F10 and 1F11 recognize OPN in SMC.

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(1)Christensen B, Nielsen MS, Haselmann KF, Petersen TE, Sorensen ES: Posttranslationally modified residues of native human osteopontin are located in clusters: identification of 36 phosphorylation and five O-glycosylation sites and their biological implications. *Biochem J* 2005, 390(Pt 1):285-292.

(2) Havrda MC, Johnson MJ, O'Neill CF, Liaw L: A novel mechanism of transcriptional repression of p27kip1 through Notch/HRT2 signaling in vascular smooth muscle cells. *Thromb Haemost* 2006, 96(3):361-370