BOSTER
antibody and ELISA experts

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

Anti-Protein orai-3 ORAI3 Antibody

Catalog Number: A09399

About ORAI3

Antigen stimulation of immune cells triggers Ca++ entry through Ca++ release-activated Ca++ (CRAC) channels. ORAI3 is one of two mammalian homologs to ORAI1, a recently identified four-transmembrane spanning protein that is an essential component of CRAC. All three homologs have been shown to function as Ca++ plasma membrane channels gated through interactions with STIM1, the store-activated endoplasmic reticulum Ca++ sensor. However, ORAI3 channels failed to produce detectable Ca++ selective currents in cells co-transfected with ORAI3 and STIM1, indicating that ORAI3 channels undergo a lesser degree of depotentiation than ORAI1 or ORAI2. Na+ currents through ORAI1, 2 and 3 channels were equally inhibited by extracellular Ca++, indicating that each have similar affinities for Ca++ within the selectivity filter.

Overview

Product Name	Anti-Protein orai-3 ORAI3 Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Protein orai-3 ORAI3 Antibody (Catalog # A09399). Tested in ELISA, WB, ICC, IHC-P applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, IHC-P, ICC, WB
Clonality	Polyclonal
Formulation	ORAI3 Antibody is supplied in PBS containing 0.02% sodium azide.
Storage Instructions	ORAI3 antibody can be stored at 4°C for three months and -20°C, stable for up to one year. Avoid repeated freeze-thaw cycles. Antibodies should not be exposed to prolonged high temperatures.
Host	Rabbit
Uniprot ID	Q9BRQ5

Technical Details

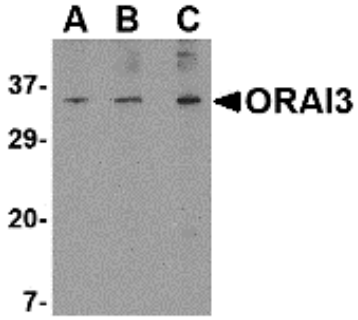
Immunogen	ORAI3 antibody was raised against a 15 amino acid synthetic peptide from near the amino terminus of human ORAI3. The immunogen is located within the first 50 amino acids of ORAI3.
Predicted Reactive Species	Bovine, Mouse, Pig
Cross Reactivity	This antibody is predicted to have no cross-reactivity to ORAI1 or ORAI2.
Isotype	IgG
Form	Liquid
Concentration	1 mg/mL

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

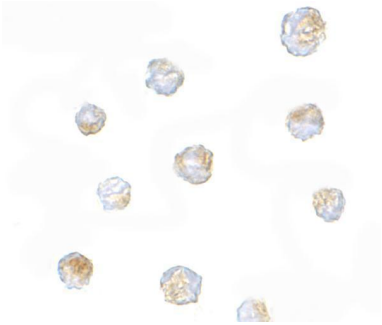
Purification	ORAI3 Antibody is affinity chromatography purified via peptide column.
Suggested Dilutions	ORAI3 antibody can be used for detection of ORAI3 by Western blot at 1 - 4 ug/mL. Antibody can also be used for immunocytochemistry starting at 10 ug/mL and Immunohistochemistry starting at 2 ug/mL Antibody validated: Western Blot in mouse samples; Immunohistochemistry in mouse samples and Immunocytochemistry in mouse samples. All other applications and species not yet tested. Optimal dilutions for each application should be determined by the researcher.

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

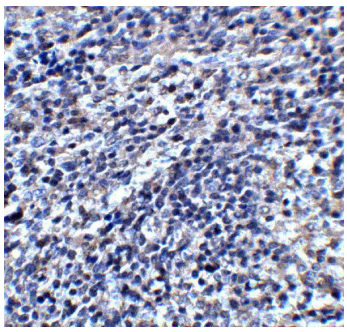
Anti-Protein orai-3 ORAI3 Antibody (A09399) Images



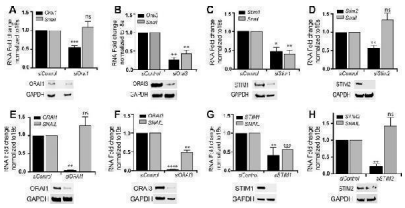
Western blot analysis of ORAI3 in A20 cell lysate with ORAI3 antibody at (A) 1, (B) 2 and (C) 4 ug/mL.



Immunocytochemistry of ORAI3 in A20 cells with ORAI3 antibody at 10 ug/mL.

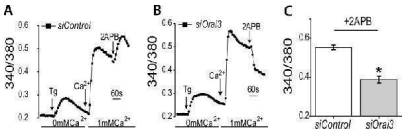


Immunohistochemistry of ORAI3 in mouse spleen tissue with ORAI3 antibody at 2 ug/mL.

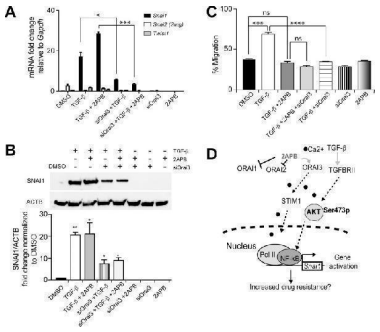


Orai3 and Stim1 silencing blocks TGF-beta induced SNAI1 transcription. NMuMG (A – D) or MDA-MB-231 (E – H) cells were transfected with indicated siRNAs for 96 h using a final concentration of 100 picomoles of siRNA. The cells were treated with TGF-beta for 2 h prior to RNA and protein isolation. RNA was converted to cDNA and RT-PCR performed to analyze both the gene knockdown efficiency for each gene, and Snai1 transcript levels. Western blots were performed against each protein to test efficiency of knockdown and normalized using GAPDH. All data are representative of at least 3 biological replicates (See for quantitation). Statistical analyses were performed with Graphpad Prism software. * = p -value ≤ 0.05, ** = p -value ≤ 0.01; *** = p -value ≤ 0.001.Index in PubMed under a CC BY license. PMID: 30034631

Orai3 silencing blocks 2APB dependent increase in SOCE. Calcium imaging was performed in control (A) and Orai3



knockdown (B) NMuMG cells. Analog plots of the fluorescence ratio (340/380) from an average of 40–60 cells are shown. (C) Quantification (mean ± SD) of fluorescence ratio (340/380). All data are representative of at least 3 biological replicates. Statistical analyses were performed with Graphpad Prism software. * = p -value ≤ 0.05.Index in PubMed under a CC BY license. PMID: 30034631



Orai3 silencing inhibits both cell migration and Snai1 transcription in response to TGF-beta. (A) NMuMG cells were treated as indicated with TGF-beta, TGF-beta+2APB or DMSO, in the presence or absence of siOrai3 and RNA isolated. RNA was converted to cDNA and analyzed by real-time PCR using primers specific to mouse Snai1, Snai2 or Twist1, and normalized to Gapdh. Data represent the average of 3 individual biological replicates. (B) Proteins isolated from the same cells as in (A) were evaluated for SNAI1 expression by immunoblotting. Antibody to ACTIN was used a loading control, and the blots are representative of at least 3 independent biological replicates. Blots were quantitated using the LiCOR imaging software and are represented as SNAI1/ACTB signal, after normalizing to DMSO control. Error bars represent SEM and statistical analyses were performed Graphpad PRISM. * = p -value ≤ 0.05, ** = p -value ≤ 0.01 relative to control. (C) Confluent NMuMG cells in a 6-well plate were serum starved for 4 h prior to treatment, and TGF-beta (for 8 h) and/or 2APB (for 24 h) were added to the wells prior to wounding using a sterile 200 ul tip. Three representative fields were marked and imaged immediately at time of (0 h) and a time period after (8 h) wounding as described in materials and methods. The images were captured using an Olympus IX71 microscope camera. All data are representative of at least 3 biological replicates. Statistical analyses were performed with Graphpad Prism software. * = p -value ≤ 0.05, ** = p -value ≤ 0.01; *** = p -value ≤ 0.001; **** = p -value ≤ 0.0001. (D) Model for ORAI3-mediated Snai1 upregulation. AKT (green oval) pathway can be activated by both calcium (black circles) and by TGF-beta signaling. 2APB prevents SOCE via ORAI1 and ORAI2, while increasing calcium influx through ORAI3. Activation of AKT triggers increased binding of p65 at the Snai1 promoter, leading to increased recruitment of Pol II and hence transcription of Snai1 .Index in PubMed under a CC BY license. PMID: 30034631

Anti-Protein orai-3 ORAI3 Antibody

For Research Use Only. Not for use in diagnostic procedures.