

## ORAI Detection Set

CATALOG No.: PSI-1819

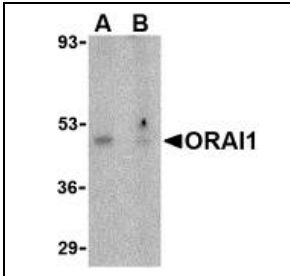
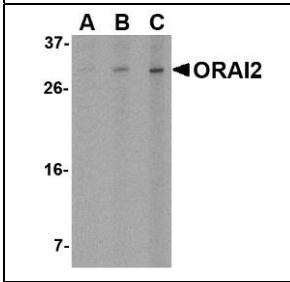
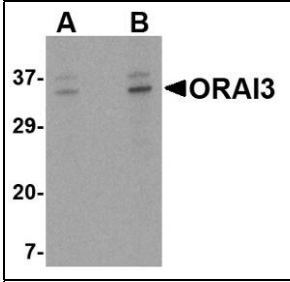
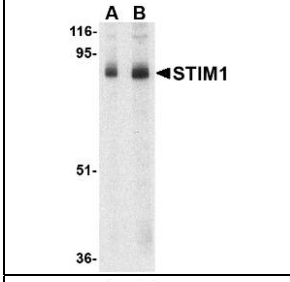
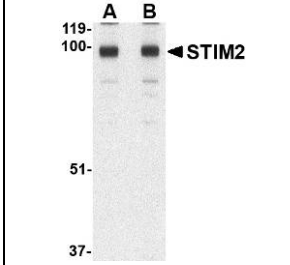
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### BACKGROUND:

Antigen stimulation of immune cells triggers  $Ca^{++}$  entry through  $Ca^{++}$  release-activated  $Ca^{++}$  (CRAC) channels (reviewed in 1,2). The ORAI family is a recently identified set of proteins that are essential components of these CRAC channels. A missense mutation in the ORAI1 protein in humans is the cause of one form of hereditary severe combined immune deficiency (SCID) which results in ablated T-cell  $Ca^{++}$  entry (3). It has been suggested that ORAI1 functions as a highly selective  $Ca^{++}$  plasma membrane channel that is gated through interactions with the stromal interaction molecule 1 (STIM1), the store-activated endoplasmic reticulum  $Ca^{++}$  sensor (4). Like ORAI1, ORAI2 also functions as a highly selective  $Ca^{++}$  plasma membrane channel that is gated through interactions with STIM1, although at a lesser efficacy than ORAI1 (5,6). Although ORAI3 can also function as  $Ca^{++}$  plasma membrane channel, 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 (6).  $Na^{+}$  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 (6). STIM1, in its function as a  $Ca^{++}$  sensor and an activator of CRAC channels, migrates to the plasma membrane from endoplasmic reticulum-like sites which act as cellular  $Ca^{++}$  stores (4,7,8). A related molecule, STIM2, inhibits the STIM1-mediated store-operated  $Ca^{++}$  entry, and can form complexes with STIM1, suggesting these two proteins may play a coordinated role in controlling  $Ca^{++}$  entry (9). The ORAI antibodies are predicted to have no cross-reactivity to the other ORAI proteins. Similarly, the STIM antibodies will not cross-react with the other STIM protein.

### KIT CONTENTS:

ORAI1 antibody, **Catalog No. 4041 (50µg)**.  
 ORAI2 antibody, **Catalog No. 4111 (50µg)**.  
 ORAI3 antibody (CT), **Catalog No. 4215 (50µg)**.  
 STIM1 antibody, **Catalog No. 4119 (50µg)**.  
 STIM2 antibody (CT), **Catalog No. 4123 (50µg)**.

	<p>Western blot analysis of ORAI1 in human ovary tissue lysate with ORAI1 antibody at 1 µg/ml in the (A) absence or (B) presence of blocking peptide.</p>
	<p>Western blot analysis of ORAI2 in Jurkat cell lysate with ORAI2 antibody at (A) 1, (B) 2 and (C) 4 µg/ml.</p>
	<p>Western blot analysis of ORAI3 in A20 cell lysate with ORAI3 antibody at (A) 2 and (B) 4 µg/ml.</p>
	<p>Western blot analysis of STIM1 in mouse thymus tissue lysate with STIM1 antibody at (A) 1 and (B) 2 µg/ml.</p>
	<p>Western blot analysis of STIM2 in A-20 cell lysate with STIM2 antibody at (A) 0.5 and (B) 1 µg/ml.</p>



**SOURCE:**

Rabbit polyclonal antibodies were raised against peptides corresponding to amino acid sequences from each of the corresponding proteins.

**APPLICATION:**

These polyclonal antibodies can be used for detection of ORAI1, ORAI2, ORAI3, STIM1 and STIM2 by immunoblot at 0.5 – 4 µg/ml. **For research use only.**

**STORAGE:**

Antibodies are supplied as affinity chromatography purified IgG in PBS containing 0.02% sodium azide. Antibodies should be stored at -20°C.

**POSITIVE CONTROLS:**

ORAI1 antibody: Human Ovary Tissue Lysate,  
**Catalog No. 1316.**  
ORAI2 antibody: Jurkat Cell Lysate, **Catalog No. 1205.**  
ORAI3 antibody: A-20 Cell Lysate, **Catalog No. 1288.**  
STIM1 antibody: Mouse Thymus Tissue Lysate,  
**Catalog No. 1409.**  
STIM2 antibody: A-20 Cell Lysate, **Catalog No. 1288.**

**RELATED PRODUCTS:**

ORAI1 peptide, **Catalog No. 4041P.**  
ORAI2 peptide, **Catalog No. 4111P.**  
ORAI3 peptide (CT), **Catalog No. 4215P.**  
STIM1 peptide, **Catalog No. 4119P.**  
STIM2 peptide (CT), **Catalog No. 4123P.**

**REFERENCES:**

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2. Luik RM and Lewis RS. New insights into the molecular mechanisms of store-operated Ca<sup>2+</sup> signaling in T cells. *Trends Mol. Med.* 2007; 13:103-7.
3. Feske S, Gwack Y, Prakriya M, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 2006; 441:179-85.
4. Soboloff J, Spassova MA, Dziadek MA, et al. Calcium signals mediated by STIM and Orai proteins – a new paradigm in inter-organelle communication. *Biochim. Biophys. Acta.* 2006; 1763:1161-8.
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7. Zhang SL, Yu Y, Roos J, et al. STIM1 is a Ca<sup>2+</sup> sensor that activates CRAC channels and migrates from the Ca<sup>2+</sup> store to the plasma membrane. *Nature* 2005; 437:902-5.
8. Spassova MA, Soboloff J, He L-P, et al. STIM1 has a plasma membrane role in the activation of store-operated Ca<sup>2+</sup> channels. *Proc. Natl. Acad. Sci. USA* 2006; 103:4040-5.
9. Soboloff J, Spassova MA, Hewavitharana T, et al. STIM2 is an inhibitor of STIM1-mediated store-operated Ca<sup>2+</sup> entry. *Curr. Biol.* 2006; 16:1465-70.



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#### **WESTERN BLOT PROTOCOL:**

- 1) Load 20 to 25 micrograms of whole cell lysate per lane in an SDS-PAGE mini gel.
- 2) Run at 20 mA per gel until the dye front is close to the bottom.
- 3) Transfer the proteins to a nitrocellulose membrane at 250 mA in transfer buffer for 1-4 h, depending on the size of the target protein.
- 4) Incubate the blot with blocking buffer (5% non-fat dry milk in TBS) overnight at 4°C or 2 hr at room temperature (RT).
- 5) Incubate the blot with primary antibody (diluted 1:250 to 1:1000 in blocking buffer) for 1 hr in blocking buffer at RT.
- 6) Wash the blot 3 x 10 min in washing buffer (TBS containing 0.1% Tween 20) with shaking.
- 7) Incubate blot with anti-rabbit IgG-HRP conjugate (diluted 1:10,000 -1:20,000 in blocking buffer) for 1 h in blocking buffer at RT.
- 8) Wash 3 x 10 min in washing buffer with shaking.
- 9) Drain washing buffer, add ECL solution and develop for 1 min.
- 10) Expose to X-ray film for 1 to 30 min.

#### **MATERIALS NEEDED:**

Nitrocellulose membrane  
Non-fat dry milk  
Tween-20  
Antibody detection kit

##### TBS:

- 125 mM NaCl
- 25 mM Tris pH 8.0
- 0.1% Tween 20

##### SDS/Running Buffer:

- 25 mM Tris
- 192 mM Glycine
- 0.1% SDS

##### Transfer Buffer:

- 20 mM Tris
- 150 mM Glycine
- 20% methanol
- 0.038% SDS