

# Product Information

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## Anti-ORAI1

produced in rabbit, affinity isolated antibody

Catalog Number **O8264**

### Product Description

Anti-ORAI1 is produced in rabbit using as immunogen a synthetic peptide corresponding to amino acids 288-301 of human ORAI1 (GenelD: 84876), conjugated to KLH. The corresponding sequence differs by 2 amino acids in rat and mouse. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-ORAI1 recognizes human ORAI1. The antibody may be used in several applications including immunoblotting (glycosylated ORAI1 ~50 kDa, and non-glycosylated ORAI1 ~35 kDa) and immunofluorescence. Detection of the ORAI1 bands by immunoblotting is specifically inhibited by the immunizing peptide.

ORAI1, also called CRACM1, is an evolutionarily conserved plasma membrane protein essential for store-operated calcium entry in T cells and fibroblasts. Store-operated calcium entry is mediated by  $\text{Ca}^{2+}$  release-activated  $\text{Ca}^{2+}$  (CRAC) channels, following  $\text{Ca}^{2+}$  depletion from the endoplasmic reticulum (ER) stores, caused by stimulation of immune cells. This process is crucial for gene transcription, proliferation and cytokine release.<sup>1,2</sup> Three mammalian homologs of *Drosophila* Orai (dOrai) have been identified: Orai1, Orai2, and Orai3. All three are widely expressed at the mRNA level and all are incorporated into the plasma membrane.<sup>3</sup> ORAI1 is predicted to contain four transmembrane domains with its N- and C-termini in the cell cytoplasm. Mutations in two highly conserved glutamate residues, E106 and E190, diminish  $\text{Ca}^{2+}$  influx and promote changes in ion selectivity, providing strong evidence that Orai is a pore subunit of the CRAC channel.<sup>4-6</sup> ORAI1 co-localizes with STIM1 (stromal interaction molecule 1) near the plasma membrane after store depletion.<sup>3</sup> STIM1 is a single-pass transmembrane protein required for the activation of store-operated  $\text{Ca}^{2+}$  influx. STIM1 is localized predominantly in the membrane of the ER. It contains an N-terminal EF hand located in the ER lumen and appears to function as a sensor of ER  $\text{Ca}^{2+}$  levels. Upon store depletion, STIM1 redistributes into discrete spots (punctae) that move towards and accumulate in the cell periphery, possibly to activate ORAI1 that is located in the plasma membrane.<sup>3</sup> Overexpression of STIM1 and ORAI1

together markedly increases the CRAC current (I-CRAC).<sup>7</sup> A point mutation in ORAI1 is responsible for the genetic defect in store-operated  $\text{Ca}^{2+}$  entry and I-CRAC in cells of the hereditary severe combined immune deficiency (SCID) syndrome patients. Expression of wild-type ORAI1 in SCID T cells restored store-operated calcium ion influx and the I-CRAC.<sup>1</sup>

### Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as preservative.

Antibody concentration: ~1.0 mg/mL

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

### Product Profile

**Immunoblotting:** a working concentration of 1-2 µg/mL is recommended using a whole extract of human HEK-293T cells expressing ORAI1.

**Immunofluorescence:** a working concentration of 10-20 µg/mL is recommended using human Jurkat cells.

**Note:** In order to obtain the best results in using various techniques and preparations, we recommend determining optimal working dilutions by titration.

### References

1. Feske, S., et al., *Nature*, **441**, 179-185 (2006).
2. Vig, M., et al., *Science*, **312**, 1220-1223 (2006).

3. Gwack, Y., et al., *J. Biol. Chem.*, **282**, 16232-16243 (2007).
4. Yeromin, A.V., et al., *Nature*, **443**, 226-229 (2006).
5. Prakriya, M., et al., *Nature*, **443**, 230-233 (2006).
6. Vig, M., et al., *Curr. Biol.*, **16**, 1-7 (2006).
7. Mercer, J.C., et al., *J. Biol. Chem.*, **281**, 24979-24990 (2006).

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