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Product Information

Monoclonal Anti-Orai1, clone ORAI1-89
produced in mouse, purified immunoglobulin

Catalog Number **SAB4200273**

Product Description

Monoclonal Anti-Orai1 (mouse IgG1 isotype) is derived from the hybridoma ORAI1-89 produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide located near the C-terminus of human Orai1 (GeneID: 84876), conjugated to KLH. The corresponding sequence differs by 2 amino acids in rat and mouse. The isotype is determined by ELISA using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor.

Monoclonal Anti-Orai1 recognizes human Orai1. The antibody may be used in various immunochemical techniques including immunoblotting (glycosylated Orai1 ~ 50 kDa and non-glycosylated Orai1 ~ 30 kDa), immunoprecipitation and immunofluorescence. Detection of the Orai1 band by immunoblotting is specifically inhibited by the immunizing peptide.

Orai1, also called CRACM1, is an evolutionary conserved plasma membrane protein essential for store-operated calcium entry in T cells and fibroblasts. Store-operated calcium entry is mediated by Ca^{2+} release-activated Ca^{2+} (CRAC) channels, following 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.[1,2] Three mammalian homologs of *Drosophila* Orai (dOrai) have been identified: Orai1, 2 and 3. All three are widely expressed at the mRNA level and all are incorporated into the plasma membrane.[3] 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, E^{106} and E^{190}, diminish Ca^{2+} influx and promote changes in ion selectivity, providing strong evidence that Orai is a pore subunit of the CRAC channel.[4-6] Orai1 co-localizes with STIM1 (stromal interaction molecule 1) near the plasma membrane after store depletion.[3] STIM1 is a single-pass transmembrane protein required for the activation of store-operated Ca^{2+} influx. STIM1 is localized predominantly in the ER membrane. It contains an N-terminal EF hand located in the ER lumen and appears to function as a sensor of ER 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.[3] Overexpression of STIM1 and Orai1 together markedly increases the CRAC current (I-CRAC).[7]

A point mutation in Orai1 is responsible for the genetic defect in store-operated 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.[1]

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a 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 at –20°C 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 dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of 0.5-1.0 µg/mL is recommended using whole extracts of HEK-293T cells over-expressing human Orai1.

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

References
1. Feske, S., et al., *Nature*, **441**, 179-185 (2006).
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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).

ST,RC,KAA,PHC 05/11-1