Anti- IZUMO1 antibody, rat monoclonal (#125)

73-045 100 µg

Key words: Acrosome reaction, Membrane fusion, Protein trafficking, IZUMO1, Sperm–egg fusion

Function: Essential sperm cell-surface protein required for fertilization by acting as a ligand for FOLR4/JUNO receptor on egg. The IZUMO1:FOLR4/JUNO interaction is a necessary adhesion event between sperm and egg that is required for fertilization but is not sufficient for cell fusion. The ligand-receptor interaction probably does not act as a membrane 'fusogen'

Molecular mass: 44,885 Da with 307 amino acids (mouse). Post-translational modification; Processing of N-terminal signal peptide with 21 amino acids. N-Glycosylation and phosphorylation.

Expression; Testis (low level) and sperm (high level). Not expressed in other organs.

Applications:

1. Western blotting (1/1,000 dilution))
2. Immunoprecipitation (1/100 dilution)
3. Immunofluorescence staining (1/250 dilution))

Immunogen: Mouse sperm

Reactivity: Mouse. Not tested with other species.

Isotype: Rat IgG

Form: Purified IgG 1 mg/ml in PBS, 50% glycerol. Filter-sterilized. Azide- and carrier- free.

Storage: Shipped at 4℃or at -20℃. Upon arrival, spin-down, aliquot and store at -20℃.

Database Links: UniProtKB Q9D9J7 (mouse IZUMO1)

Reference: This antibody was used in the following publications.

1. Inoue N. et al. (2010) Identification and disruption of sperm-specific angiotensin converting enzyme-3 (ACE3) in mouse. PLoS One. 2010 Apr 22;5(4):e10301. PubMed 20421979 **WB, IP, IF.** Open access
2. Ikawa M. et al. (2011). Calsperin is a testis-specific chaperone required for sperm fertility. J Biol Chem. 286: 5639-46. PubMed 21131354 **WB.** Open access
3. Satouh Y. et al. (2012) Visualization of the moment of mouse sperm-egg fusion and dynamic localization of IZUMO1. J Cell Sci. 125: 4985-90. PubMed 22946049 **WB.** Open access
4. Inoue N. et al. (2013). Molecular dissection of **IZUMO1**, a sperm protein essential for sperm-egg fusion. Development. 140: 3221-9. PubMed 23824580 **WB, IF.** Open access

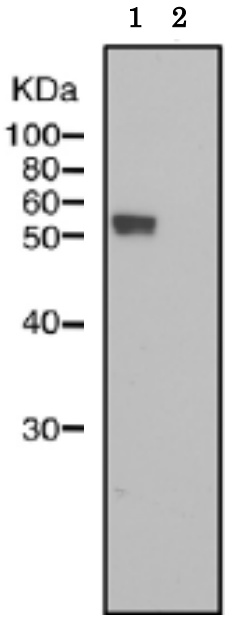

Fig.1 Identification of IZUMO1 protein in sperm lysate of wild type mouse but not in that of IZUMO1 knock-out mouse by western blotting with anti-IZUMO1 monoclonal antibody (#125).

Proteins in the lysates (10 μg) were separated on SDS-PAGE, electro-blotted to PVDF membrane and incubated with anti-IZUMO1 antibody (#125) at 1/1,000 dilution.

Lane 1; Sperm lysate from wild-type mouse

Lane 2; Sperm lysate from IZUMO1 knock-out mouse

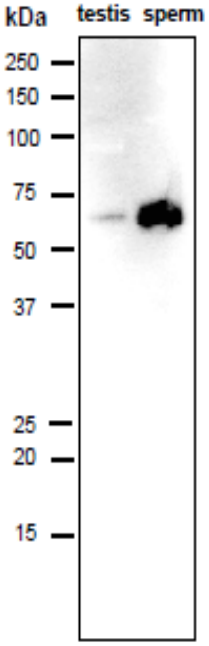

Fig.2 Analysis of IZUMO1 protein in the lysates of mouse testis and sperm by western blotting with anti-IZUMO1 antibody (#125).

Proteins in the lysates were separated on SDS-PAGE (10~20% gradient gel) electro-blotted to PVDF membrane and incubated with anti-IZUMO1 antibody (#125) at 1/1,000 dilution. As the second antibody, goat anti-rat IgG antibody conjugated with HRP (Abcam; ab97057) was used at 1/10,000.

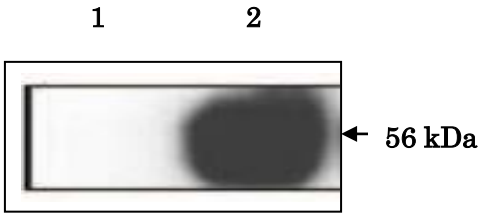

Fig.3 Immunoprecipitation of IZUMO1 protein from lysate of mouse sperm by using anti-IZUMO1 antibody (#125).

Mouse sperm lysate (1.5 mg protein in 1ml) solubilyzed with 1% Brij 97 was incubated with anti-IZUMO1 antibody (10 μg) and protein G beads. Proteins eluted from the beads were analyzed by western blotting using the same antibody.

Lane 1; Negative control without the antibody

Lane2; Anti-IZUMO1 antibody (#125)

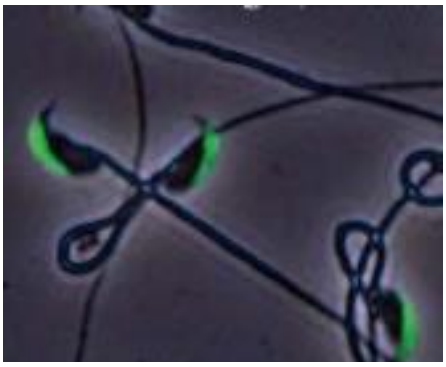

Fig.4 Immunofluorescence stainig of IZUMO1 protein in mouse spermatozoa with anti-IZUMO1 antibody (#125).

Spermatozoa was spotted onto slide, air dried, fixed with ice-cold 100% ethanol and rinsed with PBS. The slide was incubated with anti-IZUMO1 antibody (#125) at 4 μg/ml. The slide was then incubated with Alexa Fluor 488-conjugated anti-rat IgG. The stained cells were observed under a fluorescence microscope.