

## Anti- IZUMO1 antibody, rat monoclonal (#125) , KO-Validated

73-045 100 µg

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

**Validation:** Specificity validated with knock-out mice.

**Reactivity:** Mouse and human. Not tested in other species.

**Immunogen:** Mouse sperm

**Isotype:** Rat IgG2a kappa

### Applications:

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

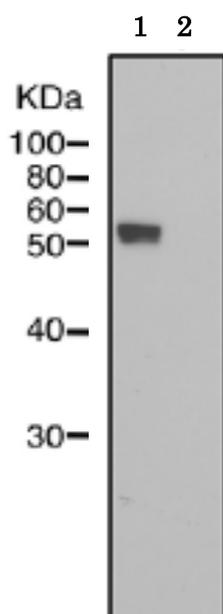
**Form:** Purified IgG 1 mg/ml in PBS, 50% glycerol. Filter-sterilized. Azide- and carrier- free.

**Background:** 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.

**Database Links:** UniProtKB [Q9D9J7](#) (mouse IZUMO1), UniProtKB [Q8IYV9](#) (human IZUMO1)

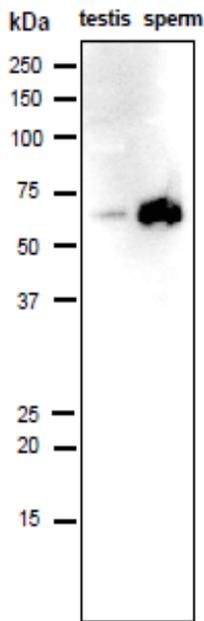


**Fig.1 Identification of IZUMO1 protein in sperm lysate of wild type mouse but not in that of IZUMO1 knock-out mouse by western blotting with anti-IZUMO1 monoclonal antibody #125).**

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.

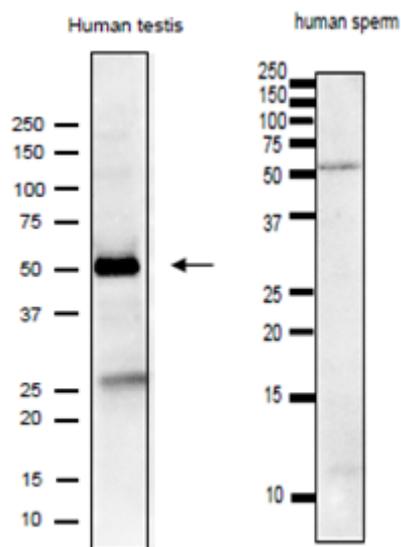
Lane 1; Sperm lysate from wild-type mouse

Lane 2; Sperm lysate from IZUMO1 knock-out mouse



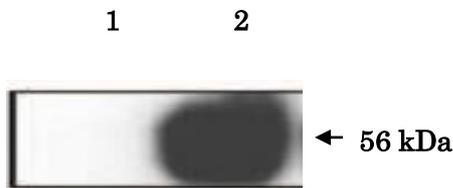
**Fig.2 Analysis of IZUMO1 protein in the lysates of mouse testis and sperm by western blotting with anti-IZUMO1 antibody (#125).**

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 Analysis of IZUMO1 protein in the lysate of human testis and sperm by western blotting with anti-IZUMO1 antibody (#125).**

Proteins in the lysates were separated on SDS-PAGE, electro-blotted to PVDF membrane (wet system) 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.4 Immunoprecipitation of IZUMO1 protein from lysate of mouse sperm by using anti-IZUMO1 antibody (#125).**

Mouse sperm lysate (1.5 mg protein in 1ml) solubilized with 1% Brij 97 was incubated with anti-IZUMO1 antibody (10  $\mu$ g) 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.5 Immunofluorescence staining 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  $\mu$ g/ml. The slide was then incubated with Alexa Fluor 488-conjugated anti-rat IgG. The stained cells were observed under a fluorescence microscope.

**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](#)