

## Anti- IZUMO1 antibody, rabbit serum

73-042      100 µl

**Shipping and Storage:** Shipped at 4°C or -20°C and store at -20°C.

**Immunogen:** KLH-conjugated synthetic peptides corresponding to the following three regions of human IZUMO 1.

[A] C+KSLEKDYLPGHLDA

[B] C+TQVPKEKATDSRQQ

[C] C+ATTESSISLQPLQ

**Form:** Rabbit antiserum added with 0.1% sodium azide.

**Reactivity:** Human and mouse. Not tested with other species.

### Applications:

1. Western blotting (1/1,000 dilution)
2. Immunofluorescence staining (1/100~1/300 dilution)
3. Immunohistochemistry (1/100 dilution)
4. Inhibition of sperm fusion with egg

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

**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'

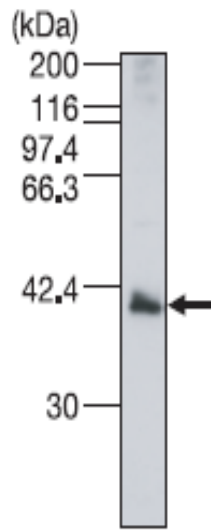
**Molecular mass:** 44,885 Da with 307 amino acids. Post-translational modification; Processing of N-terminal signal peptide with 21 amino acids. N-Glycosylation and phosphorylation.

**Expression:** This gene has expression in 4 organs : [EMAPA:18202](#): epidermis , [EMAPA:16105](#): heart , [MA:0000412](#): seminiferous tubule , [MA:0000411](#): testis

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

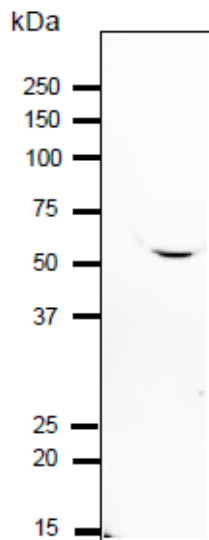
**Reference:** This antibody was described and used in the following publication.

Inoue N. et al. (2005) The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. Nature. 434:234-8. PubMed [15759005](#)



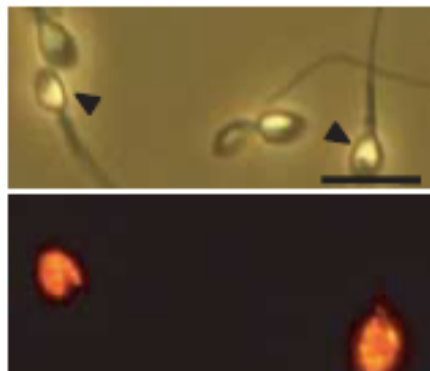
**Fig.1 Identification of IZUMO1 protein in the lysate of human sperm by western blotting with anti- IZUMO1 antibody.**

Proteins in the lysate (20  $\mu$ g) was separated on SDS-PAGE, blotted to PVDF membrane and reacted with anti-human IZUMO1 antibody at 1/1,000 dilution



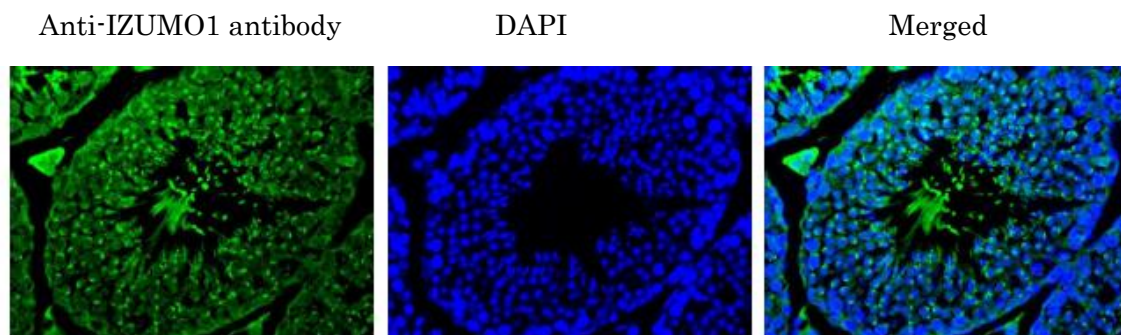
**Fig.2 Analysis of IZUMO1 protein in the lysates of mouse sperm by western blotting with polyclonal anti- IZUMO1 antibody.**

Proteins in the lysates (10  $\mu$ g) was separated on SDS-PAGE (10~20% gradient gel), blotted to PVDF membrane and reacted with the polyclonal anti-IZUMO1 antibody at 1/1,000 dilution



**Fig.3 Immunostaining of IZUMO1 in human sperm using polyclonal anti-IZUMO1 antibody.**

Human sperm on slide was incubated with polyclonal anti-IZUMO1 antibody at 1/100 dilution and reacted with a second antibody, Alexa Fluor 594-conjugated anti-rabbit IgG antibody at 1/1,000 (lower panel).



**Fig.4. Immunohistochemistry of mouse testis using anti-IZUMO1 antibody.**

Formalin-fixed and paraffin-embedded mouse testis

Deparaffinization by LemosolRA (#122-03991, Wako, Osaka)

Rehydration 100% EtOH, 95%, 90%, 70%, DW

Antigen retrieval Histo/Zyme (Cat.# k046; Diagnostic BioSystems)

Washing PBST (0.25% triton X-100/PBS-)

Blocking 10 % FBS / PBST 30 min

1st antibody 1/100 dilution in PBS- 4°C O/N

Washing PBS-

2nd antibody 1,000 dilution, 60 min (Alexa Flour-488 goat anti-rabbit IgG (H&L), #1166843 ; Molecular Probes)

Washing PBS- 5 min, 3 times

DAPI 1.0  $\mu$ g/mL DAPI in TBS 10 min