

Anti- IZUMO1 antibody, rabbit polyclonal

73-042 100 μ 1

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. Post-translational modification; Processing of N-terminal signal peptide with 21 amino acids. N-Glucosylation and phosphorylation.

Expression: This gene has expression in 4 organs: <u>EMAPA:18202</u>: epidermis, <u>EMAPA:16105</u>: heart, <u>MA:0000412</u>: seminiferous tubule, <u>MA:0000411</u>: testis

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

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

- [A] C+KSLEKDYLPGHLDA
- [B] C+TQVPKEKATDSRQQ
- [C] C+ATTESSISLQPLQ

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

Form: Rabbit antiserum added with 0.1% sodium azide.

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

Database Links: UniProtKB <u>Q8IYV9</u> (human IZUMO1), UniProtKB <u>Q9D9J7</u> (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 <u>15759005</u>

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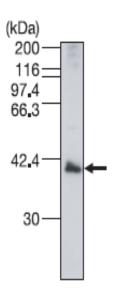


Fig.1 Identification of IZUMO1 protein in the lysate of human sperm by western blotting with anti-IZUMO1 antibody.

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

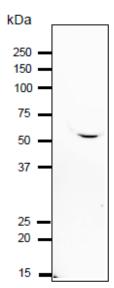


Fig.2 Analysis of IZUMO1 protein in the lysates of mouse sperm by western blotting with polyclonal anti- IZUMO1 antibody.

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

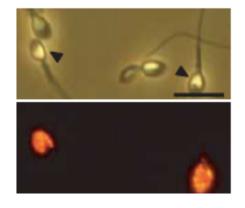


Fig.3 Immunostaining of IZUMO1 in human sperm using polyclonal anti-IZUMO1 antibody.

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).



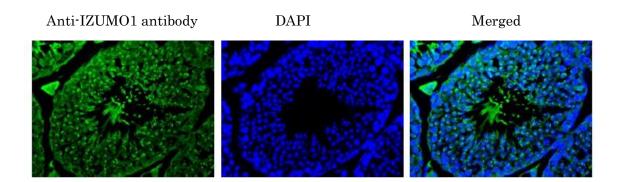


Fig.4. Immunohistochemistry of mouse testis using anti-IZUMO1 antibody.

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% 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 1.0μ g/mL DAPI in TBS 10 min