

Anti- IZUMO1 antibody, rat monoclonal (#125)

Product code	73-045
Size	100 µg
Storage	-20°C
Concentration	1.0 mg/ml
Buffer	PBS- with 50% glycerol
Purity	Purified IgG fraction with protein A from hybridoma cell culture medium.
Immunogen	Mouse sperm
Isotype	Rat IgG2aĸ
Reactivity	Mouse and human. Not tested in other species.
Special notes	N/A
Application	 Western blotting (1/1,000 dilution) Immunoprecipitation (1/100 dilution) Immunofluorescence staining (1/250 dilution)
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'.
Data Link	UniProtKB <u>Q9D9J7</u> (mouse IZUMO1), UniProtKB <u>Q8IYV9</u> (human IZUMO1)
Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC	
PROCEDURES. NOT FOR MILITARY USE.	



Data Images: 73-045 Anti- IZUMO1 antibody, rat monoclonal (#125)



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 (10µg) 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 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 (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) solubilyzed with 1% Brij 97 was incubated with anti-IZUMO1 antibody (10μ 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 μ 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.

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



4. Inoue N. et al. (2013). <u>Molecular dissection of IZUMO1</u>, a sperm protein essential for sperm-egg fusion. Development. 140: 3221-9. PubMed <u>23824580</u> WB, IF. Open access