

## Anti-IGSF8 antibody, rabbit polyclonal

Product code	73-038
Size	100 μl
Storage	-20°C
Concentration	0.5 mg/ml
Buffer	PBS- with 50% glycerol, 0.05 % sodium azide
Purity	Purified IgG fraction with protein A from rabbit antiserum.
Immunogen	Full-length mouse IGSF8 with Flag tag
Isotype	Rabbit IgG
Reactivity	Mouse.
	Likely to react with rat and human due to high sequence homology.
Special notes	Validation: Specificity validated with knock-out mouse (Fig.1)
Application	1. Western blotting (1/500~1/1,000 dilution))
	2. Immunofluorescence and immunochemical staining (1/100 dilution).
	3. Immunohistochemical staining (1/100)
Background	IGSF8 may play a key role in diverse functions ascribed to CD81 and CD9 such
	as oocytes fertilization or hepatitis C virus function. May regulate proliferation
	and differentiation of keratinocytes. May be a negative regulator of cell motility:
	suppresses T-cell mobility coordinately with CD81, associates with CD82 to
	suppress prostate cancer cell migration, regulates epidermoid cell reaggregation
	and motility on laminin-5 with CD9 and CD81 as key linkers. May also play a
	role on integrin-dependent morphology and motility functions. May participate in the regulation of neurite outgrowth and maintenance of the neural network
	in the adult brain.
	Molecular mass: 65,011 Da with 611 amino acids
	Molecular mass, 69,011 Ba with 011 ammo acids
Data Link	uniprot/Q8R366 mouse IGSF8 Gene ID140559 mouse IGSF8
Please note: All products are FOR RESEARCH USE ONLY NOT FOR USE IN DIAGNOSTIC	

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Data Images: 73-038 Anti-IGSF8 antibody, rabbit polyclonal

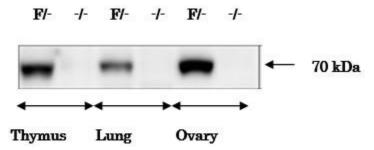


Fig 1. Analysis of IGSF8 protein in various tissues of Igff8-targeted mice by western blotting with anti-IGFS8 antibody.

Lysates of tissues (30  $\mu$ g) were analyzed by western blotting using the antibody at 1/500 dilution. "F" and "– "stand for floxed and knock-out alleles, respectively.

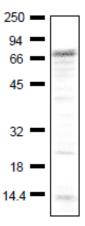


Fig.2 Detection of endogenous level of IGSF8 in crude extract of NIH3T3 cells by using anti-IGSF1 antibody.

Protiens in 40  $\mu g$  of the cell extract were separated by 12.5% SDSD-PAGE and electro-blotted at 15v, over night (wet system).

Blocking, 1hr, room temp.

1st antibody 1/1000 dilution

2nd, Goat polyclonal secondary antibody to rabbit IgG-H&L (HRP), ab97051

Positions marker proteins are shown in kDa on the left



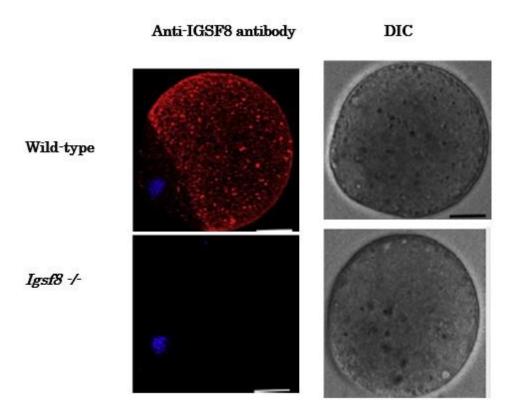


Fig.3 Immunofluorescence staining of IGSF8 protein in eggs of wild-type mouse and *Igsf8* knockout mouse with anti-IGSF8 antibody.

Zona-free eggs were fixed in PBS containing 0.5% (v/v) polyvinylpyrrolidone and 4% (v/v) paraformaldehyde. The anti-IGSF8 antibody was used at 1/100

dilution and as the second antibody, Alexa-Fuor 546 labeled anti-rabbit IgG was used (red). Then the DNA was stained with Hoechst 33342 (blue). "DIC" is picture of Differential Interference Contrast microscopy.

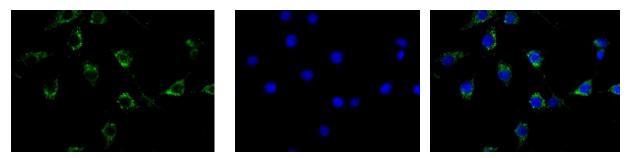


Fig.4. Immunofluorescence staining of IGSF8 protein in NIH3T3 cells with anti-IGSF8 antibody. NIH3T3 cell were fixed in 4% (v/v) paraformaldehyde. The anti-IGSF8 antibody was used at 1/100 dilution and as the second antibody, Alexa-Fluor 488 labeled anti-rabbit IgG was used (green) at 1/1,000 dilution. DNA was stained with DAPI (blue).



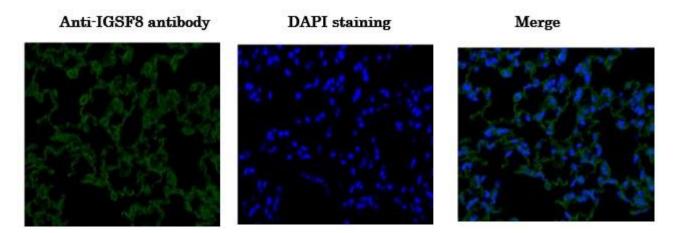


Fig.5 Immunohistochemical staining of IGSF8 protein in mouse lung tissue section using anti-IGSF1 antibody.

4% PFA fixed section of mouse lung tissue

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

Rehydration

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

Washing; PBST (0.25% triton X-100/PBS-) Blocking; 1 % BSA / PBST 60 min

1st antibody; 1/100 dilution in PBS-4°C overnight

Washing; PBS-

2<sup>nd</sup> antibody (Alexa-Fluor 488 labeled anti-rabbit IgG (green)); 1/1,000 dilution, 60 min

Washing; PBS-, 5 min 3 times

DAPI; 1.0 µg/mL DAPI in TBS 10 min

Washing; PBS-

Mount: ImmunoSelect Antifading Mounting Medium (SCR-38447; Dianova)

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

1. Inoue N. et al Tetraspanin-interacting protein IGSF8 is dispensable for mouse fertility. <u>Fertil</u> Steril. 2012 98(2):465-70.