

## Anti-Strep-tag II mAb

<b>CODE No.</b>	M211-3
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	4F1
<b>ISOTYPE</b>	Mouse IgG2a $\kappa$
<b>QUANTITY</b>	100 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>IMMUNOGEN</b>	KLH-conjugated synthetic peptide, WSHPQFEK
<b>FORMULATION</b>	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

### APPLICATIONS-CONFIRMED

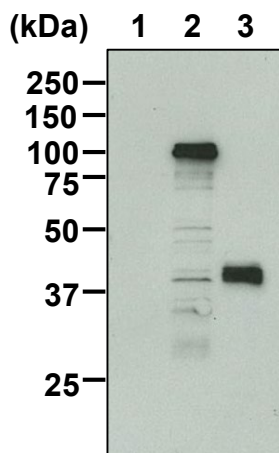
<u>Western blotting</u>	1 $\mu$ g/mL
<u>Immunoprecipitation</u>	2 $\mu$ g/sample
<u>Immunocytochemistry</u>	1 $\mu$ g/mL

For more information, please visit our website at <https://ruo.mbl.co.jp/>.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

### **SDS-PAGE & Western blotting**

- 1) Wash  $1 \times 10^7$  cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Boil the samples for 2 min. and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% Methanol). See the manufacturer's manual for precise transfer procedure.
- 5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 8) Wash the membrane with PBS-T (5 min. x 3 times).
- 9) Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 10) Wash the membrane with PBS-T (5 min. x 3 times).
- 11) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 12) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.



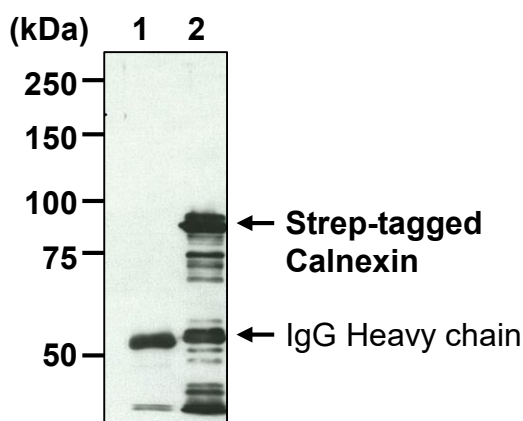
#### ***Western blotting analysis of Strep-tagged protein***

- Lane 1: HEK293T
- Lane 2: Strep-tagged Calnexin/HEK293T
- Lane 3: Strep-tagged Sox2/HEK293T

Immunoblotted with Anti-Strep-tag II mAb (M211-3)

### **Immunoprecipitation**

- 1) Resuspend  $1 \times 10^7$  cells with 1 mL of ice-cold Extraction buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors, and then sonicate the cell suspension for 15 sec.
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Mix 20  $\mu$ L of 50% protein A agarose beads slurry resuspended in 400  $\mu$ L of Extraction buffer with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at 4°C.
- 4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 5) Resuspend the beads with 1 mL of Extraction buffer.
- 6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 7) Add 250  $\mu$ L of cell lysate (prepared sample from step 2), and then incubate with gentle agitation for 1 hr. at 4°C.
- 8) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 9) Resuspend the beads with 1 mL of Extraction buffer.
- 10) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 11) Repeat steps 8)-10) 4 times.
- 12) Resuspend the beads in 20  $\mu$ L of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 13) Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
- 14) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% Methanol). See the manufacturer's manual for precise transfer procedure.
- 15) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 16) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 17) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 18) Wash the membrane with PBS-T (5 min. x 3 times).
- 19) Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 20) Wash the membrane with PBS-T (5 min. x 3 times)
- 21) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 22) Expose to an X-ray film in a dark room for 30 sec. Develop the film as usual. The condition for exposure and development may vary.



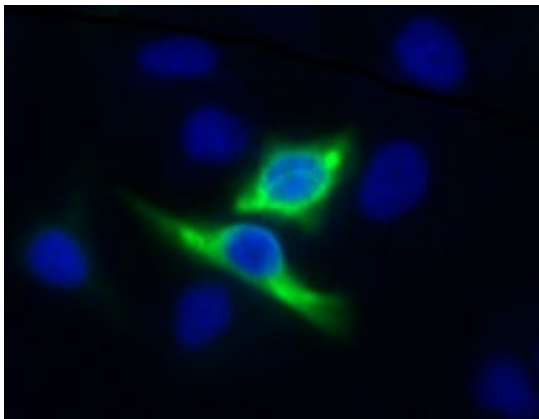
### ***Immunoprecipitation of Strep-tagged protein***

Cells: Strep-tagged Calnexin/HEK293T  
Lane 1: Mouse IgG2a (M076-3)  
Lane 2: Anti-Strep-tag II mAb (M211-3)

Immunoblotted with M211-3

### **Immunocytochemistry**

- 1) Spread the cells on a glass slide, then incubate in a CO<sub>2</sub> incubator for one night.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Wash the slide 2 times with PBS.
- 4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
- 5) Wash the slide 2 times with PBS.
- 6) Permeabilize the cells with 0.2% Triton X-100/PBS for 10 min. at room temperature.
- 7) Wash the slide 2 times with PBS.
- 8) Tip off PBS and add 200 µL of the primary antibody diluted with 2% FCS/0.09% NaN<sub>3</sub>/PBS as suggested in the **APPLICATIONS** onto the cells. Incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)  
\*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
- 9) Wash the slide 2 times with PBS.
- 10) Add 200 µL of 1:500 Alexa Fluor® 488 Goat Anti-mouse IgG (Thermo Fisher Scientific; code no. A11001) diluted with 2% FCS/0.09% NaN<sub>3</sub>/PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
- 11) Wash the slide 2 times with PBS.
- 12) Counterstain with DAPI for 5 min. at room temperature.
- 13) Wash the glass slide 2 times with PBS.
- 14) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
- 15) Promptly add mounting medium onto the slide, then put a cover slip on it.



### ***Immunocytochemical detection of Strep-tagged protein***

Cells: Strep-tagged Calnexin/HeLa  
Green: Anti-Strep-tag II mAb (M211-3)  
Blue: DAPI