For Research Use Only.
Not for use in diagnostic procedures.

Anti-Strep-tag II mAb

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

APPLICATIONS-CONFIRMED

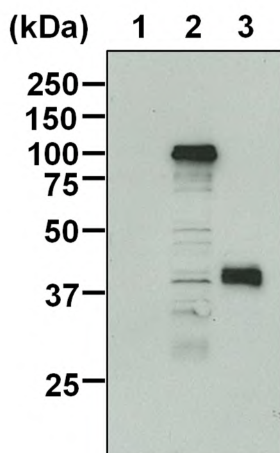
Western blotting	1 μg/mL
Immunoprecipitation	2 μg/sample
Immunocytochemistry	1 μg/mL

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 x 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 µL 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^2 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.



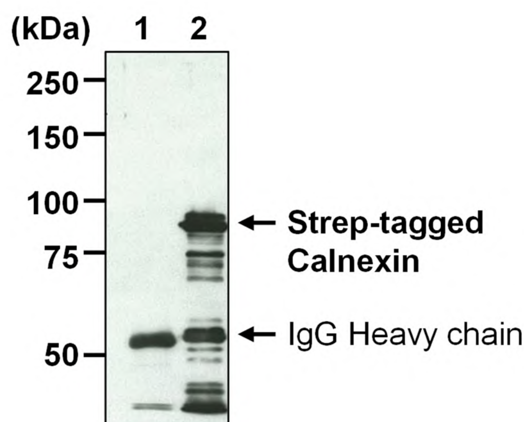
Western blotting analysis of Strep-tagged protein

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 x 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 µL of 50% protein A agarose beads slurry resuspended in 400 µL 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 µL 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 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
13) Load 10 µL 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^2 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.



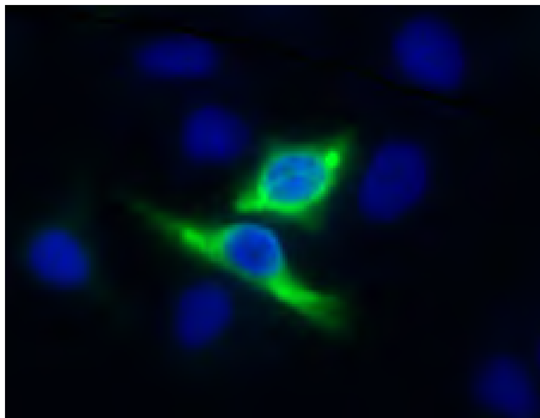
Immunoprecipitation of Strep-tagged protein

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_2 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_3/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_3/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