

Anti-Syntaxin-17 (Human) mAb

CODE No.	M212-3
CLONALITY	Monoclonal
CLONE	2F8
ISOTYPE	Mouse IgG2a κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Human Syntaxin-17, recombinant protein
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

<u>Western blotting</u>	1 μ g/mL for chemiluminescence detection system
<u>Immunoprecipitation</u>	2 μ g/sample
<u>Immunocytochemistry</u>	Not recommended

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	Jurkat, A549, HeLa	NIH/3T3	PC12	CHO
Reactivity	+	-	-	-

Entrez Gene ID 55014 (Human)

REFERENCES
1) Hamasaki, M., *et al.*, *Nature* **495**, 389-393 (2013)
2) Itakura, E., *et al.*, *Cell* **151**, 1256-1269 (2012)

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RELATED PRODUCTS

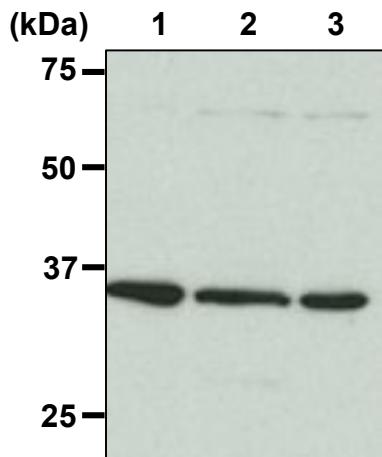
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 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 3 min. and centrifuge. Load 10 μ 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/cm² for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). 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 the 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, 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 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, A549 and HeLa)



Western blot analysis of Syntaxin-17

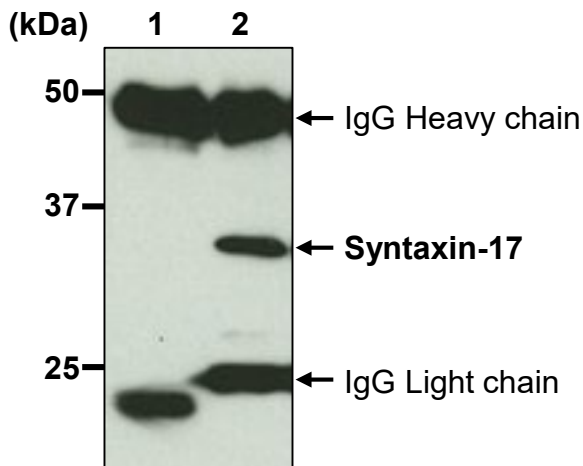
Lane 1: Jurkat
Lane 2: A549
Lane 3: HeLa

Immunoblotted with Anti-Syntaxin-17 (Human) mAb (M212-3)

Immunoprecipitation

- 1) Resuspend 1×10^7 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, then sonicate the cell suspension for 20 sec.
- 2) Centrifuge the tube at 12,000 x g for 10 min. at 4°C and transfer the supernatant to another tube.
- 3) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 300 µL of Extraction buffer with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at 4°C.
- 4) Wash the beads 1 time with 1 mL of Extraction buffer.
- 5) Add 300 µL of cell lysate (prepared sample from step 2)), then incubate with gentle agitation for 1 hr. at 4°C.
- 6) Wash the beads 4 times with 1 mL of Extraction buffer.
- 7) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 8) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 9) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 12) 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.)
- 13) Wash the membrane with PBS-T (5 min. x 3 times).
- 14) Incubate the membrane with the 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.
- 15) Wash the membrane with PBS-T (5 min. x 3 times)
- 16) Wipe excess buffer on the membrane, 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.
Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; HeLa)



Immunoprecipitation of Syntaxin-17 from HeLa

Lane 1: Mouse IgG2a (M076-3)

Lane 2: Anti-Syntaxin-17 (Human) mAb (M212-3)

Immunoblotted with M212-3