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

My select sampler set

Anti-Syntaxin-17 (Human) pAb

CODE No. PM076MS

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 20 µL

SOURCE Purified IgG from rabbit serum
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

Western blotting	1:1,000 for chemiluminescence detection system
Immunoprecipitation	2.5 µL/sample
Immunocytochemistry	1:2,000

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	Jurkat, A549, HeLa	NIH/3T3	NRK	Not tested
Reactivity	+	-	-	

Entrez Gene ID 55014 (Human)

REFERENCE

1) Itakura, E., *et al., Cell* **151**, 1256–1269 (2012)

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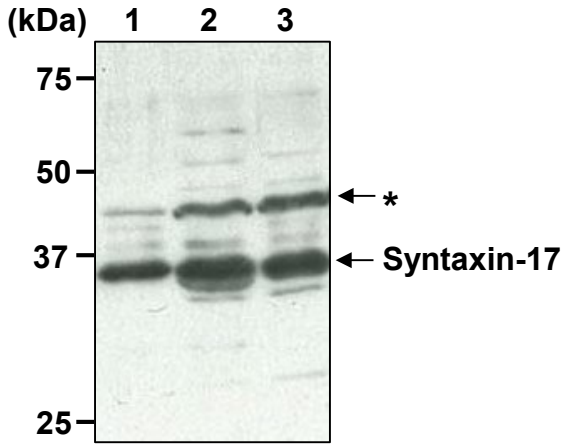
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Wash 1 x 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 5 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% 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) 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.)
7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
8) Incubate the membrane with the 1:10,000 anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. x 3 times)
10) 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.
11) 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)



* Non-specific band

Western blot analysis of Syntaxin-17

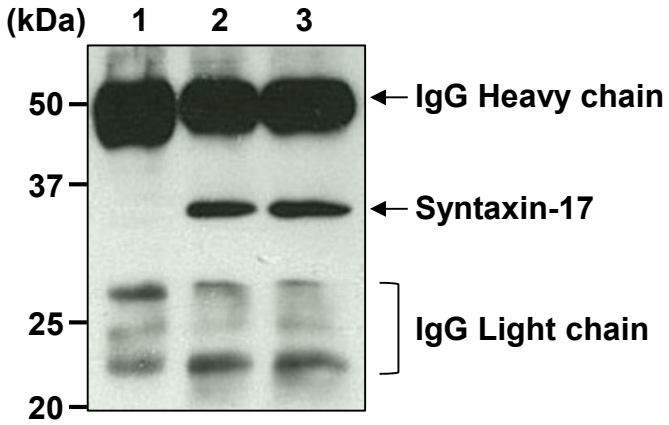
Lane 1: Jurkat
Lane 2: A549
Lane 3: HeLa

Immunoblotted with Anti-Syntaxin-17 (Human) pAb (PM076)

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, 1% NP-40) containing appropriate protease inhibitors.
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 400 µL of IP buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 30 min. at 4°C.
4) Wash the beads 1 time with 1 mL of IP 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 5 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/cm^2 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) 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.)
12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
13) Incubate the membrane with the 1:10,000 anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
14) Wash the membrane with PBS-T (5 min. x 3 times)
15) 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; Jurkat)



Immunoprecipitation of Syntaxin-17 from Jurkat

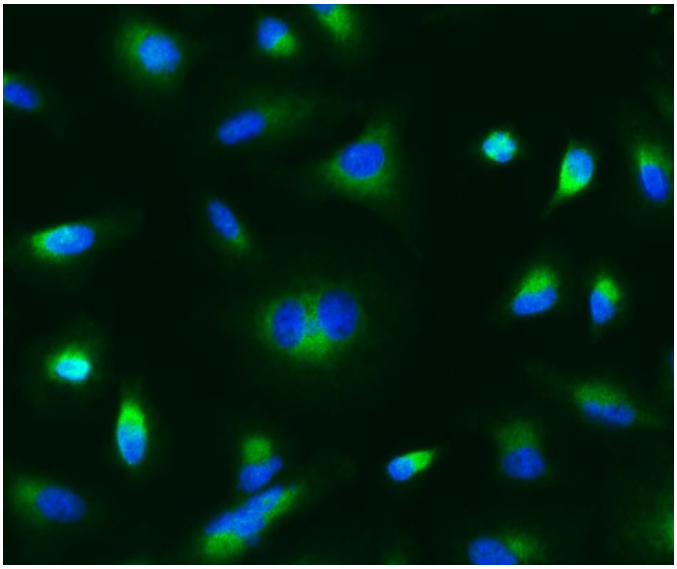
Lane 1: IP with 1 µL of Normal Rabbit IgG (MBL; code no. PM035)
Lane 2: IP with 2.5 µL of Anti-Syntaxin-17 (Human) pAb (PM076)
Lane 3: IP with 5 µL of Anti-Syntaxin-17 (Human) pAb (PM076)

Immunoblotted with PM076

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 100 µg/mL of Digitonin/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 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.)
9) Wash the slide 2 times with PBS.
10) Add 200 µL of 1:500 Alexa Fluor® 488 Goat Anti-rabbit IgG (Invitrogen; code no. A11008) diluted with 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 minutes 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.

(Positive control for Immunocytochemistry; A549)



Immunocytochemical detection of Syntaxin-17 in A549

Green: Anti-Syntaxin-17 (Human) pAb (PM076)
Blue: DAPI