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RiboCluster Profiler™

Anti-SFPQ (PSF) mAb

CODE No. RN014MW

CLONALITY Monoclonal
CLONE C23
ISOTYPE Mouse IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN KLH-conjugated synthetic peptide, CAGYGRGREEYEGPNKKPRF (corresponding to amino acid residues 681-699 of mouse Sfpq)
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
Immunohistochemistry 1 µg/mL
Heat treatment for paraffin embedded section: Microwave oven; 100°C for 20 min. in 10 mM citrate buffer (pH 6.2)
Immunocytochemistry 0.5 µg/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, Jurkat, HL-60	MEF, WR19L, C2C12	Rat1	CHO
Reactivity	+	+	+	+

Entrez Gene ID 6421 (Human), 71514 (Mouse), 252855 (Rat), 100761355 (Hamster)

REFERENCES

1) Imamura, K., *et al.*, *Mol. Cell* **53**, 393-406 (2014)
2) Hirose, T., *et al.*, *Mol. Biol. Cell.* **25**, 169-183 (2014)
3) Elzbieta, K., *et al.*, *Mol. Cell. Biol.* **32**, 4585-4594 (2012)
4) Nakagawa, S. and Hirose, T., *Cell Mol. Life Sci.* **69**, 3027–3036 (2012)
5) Nakagawa, S., *et al.*, *J. Cell Biol.* **193**, 31-39 (2011)
6) Kuwahara, S., *et al.*, *Biol. Reprod.* **75**, 352-359 (2006)
7) Fox, A. H., *et al.*, *Curr. Biol.* **12**, 13-25 (2002)

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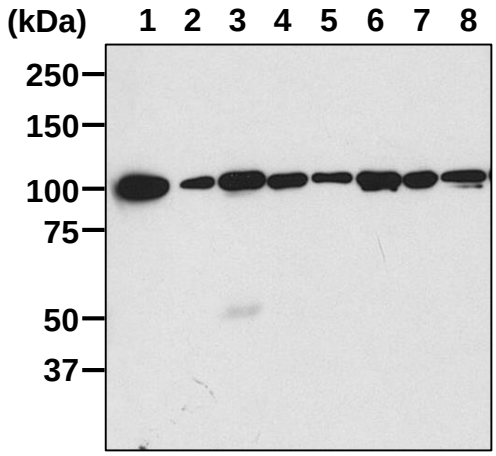
SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspend them in 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 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% 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) for 1 hr. at room temperature.
6) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3].
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).
9) Incubate the membrane with the 1:10,000 of 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).
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; HeLa, Jurkat, HL-60, MEF, WR19L, Rat1, C2C12 and CHO)



Western blotting analysis of SFPQ (PSF)

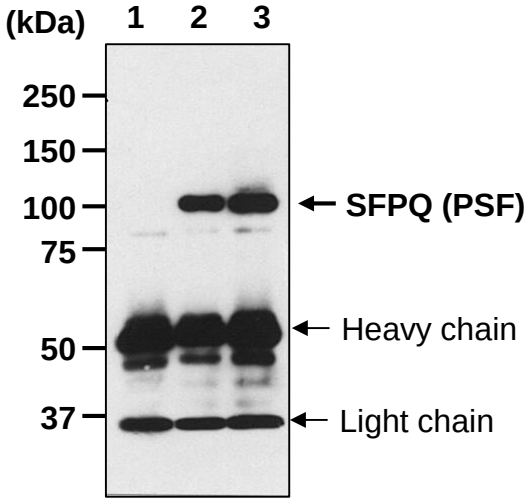
Lane 1: HeLa
Lane 2: Jurkat
Lane 3: HL-60
Lane 4: MEF
Lane 5: WR19L
Lane 6: Rat1
Lane 7: C2C12
Lane 8: CHO

Immunoblotted with Anti-SFPQ (PSF) mAb (MBL; code no. RN014MW)

Immunoprecipitation

1) Wash 1 x 10^7 cells 3 times with PBS and suspend them with 1 mL of Extraction buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors, then sonicate briefly (up to 15 sec.).
2) Incubate the tube for 15 min. on ice.
3) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
4) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 400 µL of IP buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40] with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
5) Wash the beads 3 times with 1 mL of IP buffer.
6) Add 250 µL of cell lysate (prepared sample from step 3), then incubate with gentle agitation overnight at 4°C.
7) Wash the beads 6 times with 1 mL of Extraction buffer.
8) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
9) Load 10 µL of the sample per lane in a 1 mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
10) 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.
11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
13) Incubate the membrane with 1 µg/mL of Anti-SFPQ (PSF) mAb (MBL; code no. RN014MW) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
14) Wash the membrane with PBS-T (5 min. x 3).
15) Incubate the membrane with 1:10,000 of 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.
16) Wash the membrane with PBS-T (5 min. x 3).
17) 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 a paper towel, and seal it in a 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 SFPQ (PSF) from HeLa cells

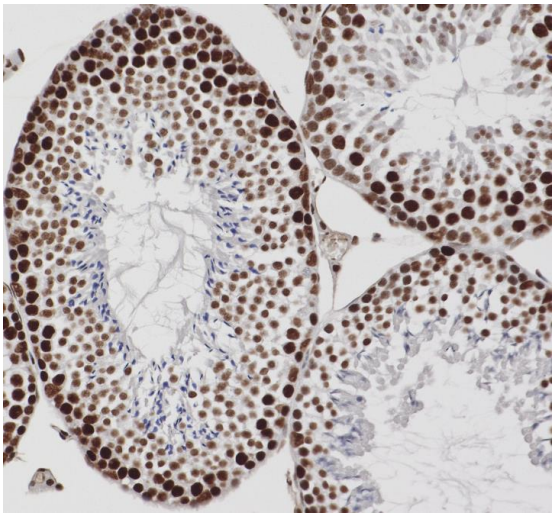
Lane 1: IP with 5 µg of Mouse IgG2a (isotype control) (M076-3)
Lane 2: IP with 2 µg of Anti-SFPQ (PSF) mAb (RN014MW)
Lane 3: IP with 5 µg of Anti-SFPQ (PSF) mAb (RN014MW)

Immunoblotted with RN014MW

Immunohistochemical staining for formalin fixed paraffin-embedded section

1) Deparaffinize the sections with Xylene 3 times for 5 min. each.
2) Wash the slides with Ethanol 3 times for 5 min. each.
3) Wash the slides with PBS 3 times for 5 min. each.
4) Remove the slides from PBS and heat-treated with 10 mM Citrate buffer (pH 6.2) for 20 min. at 100°C using microwave oven.
5) Let the slides cool down at room temperature in the Citrate buffer.
6) Remove the slides from the Citrate buffer and block endogenous peroxidase with 3% H_2O_2 in PBS for 10 min.
7) Wash the slides with PBS twice for 5 min. each.
8) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 min. at room temperature (20~25°C) to block non-specific staining. Do not wash.
9) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS** for 1 hr. at 4°C. (The concentration of antibody will depend on the conditions.)
10) Wash the slides 3 times in PBS for 5 min. each.
11) Wipe gently around each section and cover tissues with Histostar (Ms + Rb) (MBL; code no. 8469). Incubate for 1 hr. at room temperature.
12) Wash the slides 3 times in PBS for 5 min. each.
13) Visualize by reacting for 10 min. with Histostar DAB (MBL; code no. 8469) at room temperature. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
14) Wash the slides in water for 5 min.
15) Counter stain in hematoxylin for 1 min., wash the slides 3 times in water for 5 min. each, and then immerse the slides in PBS for 5 min.
16) Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each. Now ready for mounting.

(Positive control for Immunohistochemistry; Mouse testis)



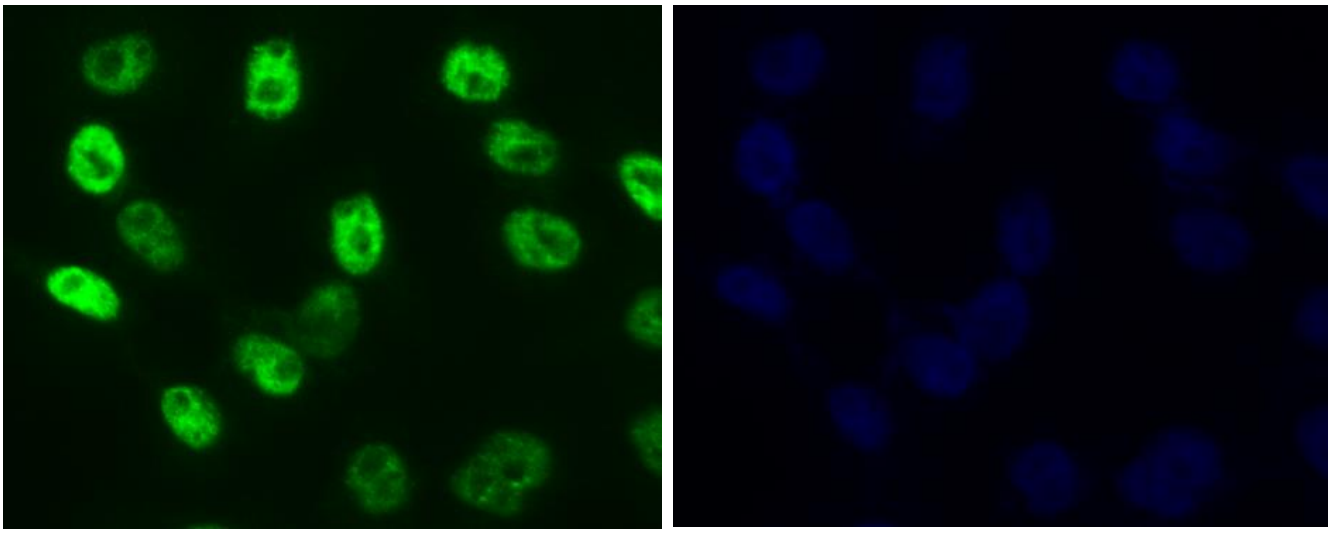
Immunohistochemical detection of SFPQ (PSF) in mouse testis

Brown: Anti-SFPQ (PSF) mAb (MBL; code no. RN014MW)
Blue: Hematoxylin

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator overnight.
2) Remove the culture supernatant by careful aspiration.
3) Wash the slide twice with PBS.
4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
5) Wash the slide 3 times with PBS.
6) Permeabilize the cells with 0.2% Triton X-100/PBS for 10 min. at room temperature.
7) Wash the slide 3 times with PBS.
8) Tip off PBS and add the primary antibody diluted with 2% fetal calf serum (FCS)/PBS as suggested in the **APPLICATIONS** onto the cells. Incubate for 30 min. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide 3 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 PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
11) Wash the slide 3 times with PBS.
12) Wipe excess liquid from the slide but take care not to touch the cells. Never leave the cells to dry.
13) Counterstain with DAPI for 5 min. at room temperature.
14) Wash the slide 1 time with PBS.
15) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)



Immunocytochemical detection of SFPQ (PSF) in HeLa cells

Green: Anti-SFPQ (PSF) mAb (MBL; code no. RN014MW)
Blue: DAPI