BACKGROUND
POSH (plenty of SH3s) was encoded by sh3rf1 gene, and contains an N-terminus RING-finger, four SH3 domains, and a region implicated in binding of the Rho GTPase Rac. It interacts with GTP form of Rac1 but not with the GDP-bound Rac1. POSH also acts as a scaffold protein by interacting with JIPs (JNK-interacing protein) for a JNK pathway protein complex, and plays an important role in the activation of the c-Jun N-terminal kinase signaling pathway. It is also reported that POSH recruits activated Rac1 to the plasma membrane to control the formation of cytoplasmic dilation of the leading process and neuronal migration.

DESCRIPTION
Target  POSH
Host Species  Rabbit
Clonality  Polyclonal
Immunogen  Recombinant fragment corresponding to amino acids 253-363 of mouse POSH
Purification  Purified from rabbit serum
Quantity  100 µL
Storage  This antibody is stable for one year from the date of purchase when stored at -20°C
Storage Buffer  PBS containing 50% Glycerol (pH 7.2). No preservatives is contained.

APPLICATIONS
Western blot  1:1000
Immunoprecipitation  Not tested
Immunocytochemistry  See REFERENCES
Flow cytometry  Not tested

DATA

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Western blot analysis
Lane 1: Immunogen protein

Immunogen protein
REFERENCES
POSH localizes activated Rac1 to control the formation of cytoplasmic dilation of the leading process and neuronal migration.
PROTOCOLS

Western blot

1. Harvest $1 \times 10^7$ cells, wash 3 times with PBS and suspend them in 0.5 mL of extraction buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40), then sonicate briefly (up to 15 seconds). After centrifuged, mix the supernatant with equal volume of Laemmli’s sample buffer.

   For recombinant protein samples: Mix the samples with equal volume of Laemmli’s sample buffer.

2. Boil the samples for 3 minutes and centrifuge. Load 20 µL of the sample per lane and carry out electrophoresis.

3. Blot the protein to a PVDF membrane at 1 mA/cm² for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol).

4. Incubate the membrane in 10% skimmed milk (in PBS, pH 7.2) for at least 1 hour at room temperature or overnight at 4°C.

5. Wash the membrane with PBS-T (0.05% Tween-20 in PBS) for 3 times at least 5 minutes each time on the shaker.

6. Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the APPLICATIONS for 1 hour at room temperature.

7. Wash the membrane with PBS-T for 3 times at least 10 minutes each time.

8. Incubate the membrane with the HRP-conjugated anti-Rabbit IgG antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.

9. Wash the membrane with PBS-T for 3 times at least 10 minutes each time.

10. Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane and seal it in plastic wrap.

11. Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.