Affinity-purified Sheep Anti-human/mouse/rat TSC1 Antibody

ORDERING INFORMATION

Catalog Number: AF4379

Lot Number: ZRJ01

Size: 100 µg

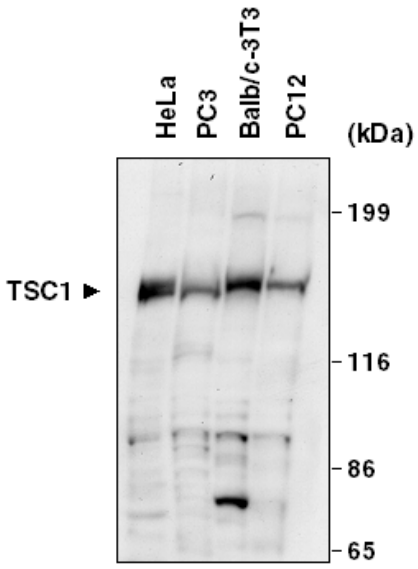
Storage: -20° C

Specificity: human, mouse, and rat TSC1

Immunogen: *E. coli*-derived recombinant human TSC1 (rhTSC1; aa 156 - 300)

Ig Type: affinity-purified sheep IgG

Applications: Western blot
Flow cytometry



Extracts from 1.5 x 10^5 exponentially growing HeLa, PC3 (human), Balb/c-3T3 (mouse), and PC12 (rat) cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 1.0 µg/mL sheep anti-TSC1 antibody.

Background

TSC1 (Tuberous sclerosis 1), or hamartin, is a tumor suppressor which interacts with tumor suppressor TSC2 (tuberin) to form a cytoplasmic heterodimer. Mutations in either hamartin or tuberin are responsible for tuberous sclerosis (TSC), an autosomal dominant disease characterized by renal dysfunction, seizures, developmental delays, benign hamartomas and low grade neoplasms predominantly affecting the CNS, kidney, lung, skin, and heart. The TSC1/TSC2 complex suppresses cell growth by inhibiting mTOR, with TSC1 acting to inhibit the ubiquitination of TSC2, leading to increased cellular levels of TSC2 and thus enhancing its catalytic activity as a GTPase-activating protein for Rheb. TSC1 and TSC2 are also involved in the G2/M transition of the cell cycle through their interactions with CDK1 and cyclin B1. TSC1 has also been shown to interact with F-actin and ERM (Ezrin-Radixin-Moesin) proteins, implying a role in the modulation of cell adhesion and morphology.

Preparation

Sheep antibodies were raised against purified, *E. coli*-derived, recombinant human TSC1 (rhTSC1; aa 156 - 300; Accession # NM_000368). Polyclonal antibody was affinity-purified on a column derivatized with rhTSC1 and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) containing 5% trehalose.

Reconstitution

Reconstitute the antibody with 100 µL of sterile PBS containing 0.02% NaN_3.

Storage

Lyophilized samples are stable for 12 months from date of receipt when stored at -20° C to -70° C. The reconstituted antibody should be aliquoted and stored at -20° C **in a manual defrost freezer** for 12 months without detectable loss of activity**. Avoid repeated freeze/thaw cycles.**

Specificity

The antibody detects endogenous human, mouse, and rat TSC1 in Western blots.

Applications

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution
25 mM Tris, pH 7.5	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer
0.05% Tween® 20	Adjust pH to 7.5

1. Transfer the electrophoresed proteins onto a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 2° - 8° C in Blocking Solution containing 1.0 µg/ml sheep anti-TSC1 antibody.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Blocking Solution containing a 1:2,000 dilution of HRP-conjugated donkey anti-sheep Ig (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with Chemiluminescent detection reagents.

Cell lysates for Western blottings - To prepare total cell lysates, solubilize cells in 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) and sonicate with a probe sonicator using 3 - 4 bursts of 5 - 10 seconds each. Heat extracts in a boiling water bath for 5 minutes and load onto polyacrylamide gels. Samples may be diluted with 1X SDS sample buffer to the desired concentration.

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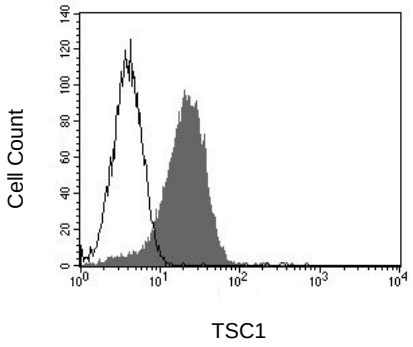
Flow cytometry - This antibody was tested for flow cytometry using Jurkat cells. Dilute this antibody to 25 μg/mL and add 10 μL of the diluted solution to 1 - 5 x 10^5 cells in a total reaction volume not exceeding 200 μL. The binding of unlabeled antibodies may be visualized by adding a secondary developing reagent such as anti-sheep IgG conjugated to a fluorochrome.



Jurkat cells were stained with anti-TSC1 (R&D Systems, Cat. # AF4379, filled histogram) or control antibody (R&D Systems, Cat. # 5-001-A, open histogram) followed by NL557-conjugated donkey anti-sheep IgG (R&D Systems, Cat. # NL010).

Optimal dilutions should be determined by each laboratory for each application.