

Affinity-Purified Sheep Anti-human SPRY1 Antibody

ORDERING INFORMATION

Catalog Number: AF6097

Lot Number: CAZH02

Size: 100 µg

Storage: -20° C

Specificity: human SPRY1

Immunogen: *E. coli*-derived rhSPRY1
(aa 1 - 178)

Ig Type: sheep IgG

Application: Western blot

Background

SPRY1 (sprouty homolog 1) is a 34 - 38 kDa member of the sprouty family of proteins. It is widely expressed, being found in multiple embryonic and adult tissues. SPRY1 is considered a negative regulator of cellular signaling. In particular, it appears to both inhibit MAP kinase signaling following RTK activation, and block TCR signaling following antigen activation. It interacts with a number of molecules, including PLC γ 1, LAT, CBL, caveolin-1 and SPRY2. Human SPRY1 is 319 amino acids (aa) in length and contains one CBL-TKB binding site (aa 51 - 57) that is phosphorylated at Tyr53, a Ser-rich region (aa 112 - 131), and a Cys-rich domain (aa 181 - 306) that mediates intracellular translocation. In addition, SPRY1 undergoes serine phosphorylation, ubiquitination and palmitoylation, the latter which induces SPRY1 to associate with cell membranes.

Preparation

Sheep antibodies were raised against purified, *E. coli*-derived recombinant human SPRY1 (rhSPRY1; aa 1 - 178; Accession # O43609). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein.

Formulation

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

Reconstitution

Reconstitute in PBS containing 0.02% NaN₃.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody detects endogenous human SPRY1 at approximately 37 kDa using Western blots.

Application

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

Protocols for Immunoblotting

Blotting Buffer

25 mM Tris, pH 7.4
0.15 M NaCl
0.1% Tween® 20

Blocking Solution

5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

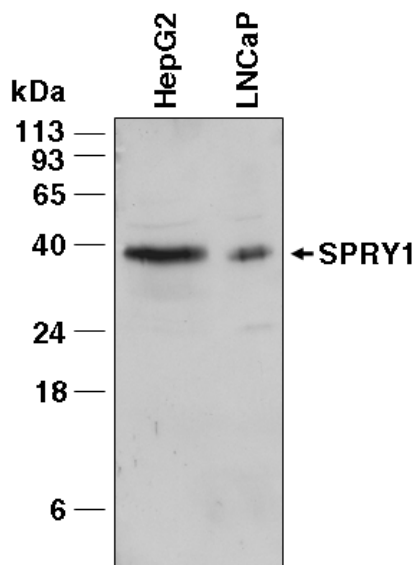
Antibody Solution

5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane for 1 hour at room temperature in Antibody Solution containing 1.0 µg/mL sheep anti-human SPRY1.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-sheep IgG (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at 2 x 10⁶ - 1 x 10⁷ cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each.

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



Detection of SPRY1 with AF6097.

Lysates from human HepG2 and LNCaP cells were resolved by SDS-PAGE. Following electrophoresis, proteins were transferred to an Immobilon-P membrane and immunoblotted with 1.0 µg/mL anti-SPRY1, as described in *Protocols for Immunoblotting*.