

Affinity-Purified Sheep Anti-human/mouse/rat JAB1 Antibody

ORDERING INFORMATION

Catalog Number: AF6390

Lot Number: CEHQ01

Size: 100 µg

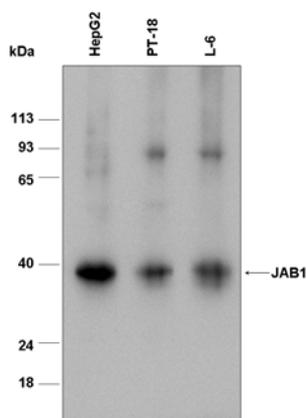
Storage: -20° C

Specificity: human, mouse and rat JAB1

Immunogen: *E. coli*-derived recombinant human JAB1 (aa 158 - 334)

Ig Type: sheep IgG

Application: Western blot



Detection of JAB1 with AF6390.

Lysates from human HepG2, mouse PT-18 and rat L-6 cells were resolved by SDS-PAGE. Following electrophoresis, proteins were transferred to an Immobilon-P membrane and immunoblotted with 1 µg/mL sheep anti-JAB1, as described in *Protocols for Immunoblotting*.

Background

JAB1 (Jun activation domain binding protein-1), also called COPS5 or CSN5 (COP9 signalosome-5), is a 334 amino acid (aa), 38 kDa protein that is a subunit of the COP9 signalosome complex. It mediates cytosolic translocation of the cell cycle inhibitory protein, CDKN1B/p27Kip1, for degradation, thus promoting proliferation. It is widely expressed, especially in proliferating cells and up-regulates granulopoiesis when overexpressed. Within the region used as an immunogen, human JAB1 shares 99% aa identity with mouse and rat JAB1. A 379 aa isoform contains 93 alternate aa in place of the N-terminal 48 aa of the 334 aa form.

Preparation

Sheep antibodies were raised against purified *E. coli*-derived, recombinant human JAB1 (rhJAB1; aa 158 - 334; Accession # Q92905). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

Formulation

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

Reconstitution

Reconstitute the antibody 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

The antibody detects endogenous human, mouse and rat JAB1 at 38 kDa using Western blots.

Application

Western blot - An antibody concentration of 1 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 2° - 8° C in Antibody Solution containing 1 µg/mL sheep anti-human/mouse/rat JAB1.
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 2×10^6 - 1×10^7 cells per mL.

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