Affinity-purified Sheep Anti-human JunB Antibody

ORDERING INFORMATION

Catalog Number: AF4456

Lot Number: ZTL01

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS and 5% trehalose

Storage: -20° C

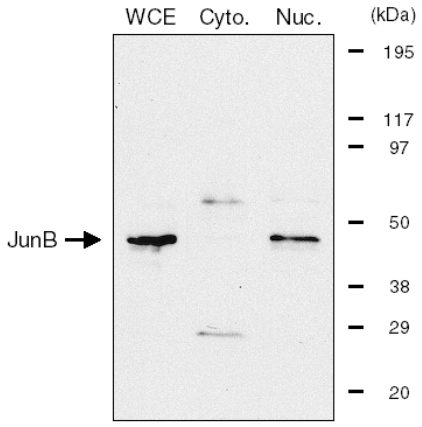
Reconstitution: sterile PBS and 0.02% NaN_3

Specificity: human JunB

Immunogen: *E. coli*-derived recombinant human JunB (rhJunB; aa 1 - 347)

Ig Type: affinity-purified sheep IgG

Application: Western blot



Sheep anti-human JunB figure legend: 30 µg of whole cell (WCE), 20 µg of cytoplasmic (Cyto), and 10 µg of nuclear (Nuc) extracts from exponentially growing HeLa cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 1.0 µg/ml sheep anti-JunB antibody.

Background

JunB is a member of the bzip Jun family of transcription factors. JunB binds as a homo or heterodimer, with other members of the Jun/Fos family, to the DNA element TGA[CG]TCA. Knock out mice indicate that JunB is involved in erythroid differentiation and hematopoietic stem cell viability.

Preparation

Sheep antibodies were raised against purified, *E. coli*-derived, recombinant human JunB (rhJunB; aa 1 - 347; Accession # NM_002229). Polyclonal antibody was affinity-purified on a column derivatized with rhJunB and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) containing 0.02% trehalose.

Reconstitution

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

Storage

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 human JunB.

Application

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	2% 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-JunB 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 1:5000 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 WesternGlo™ Chemiluminescent detection reagents (R&D Systems, Catalog # AR004) or equivalent.

Cell lysates for Western blottings - To prepare lysates, resuspend cells in Cytoplasmic buffer (10mM Hepes, pH 7.9, 10mM KCl, 0.1mM EDTA, 0.1mM EGTA, 1.0mM DTT, protease inhibitors (Sigma P8340)). Incubate the cells on ice for 15 minutes, followed by addition of 10% NP40 to a final concentration of 0.5%, vortex for 10 seconds. Cells are spun down at 800g for 5 minutes supernatant is removed and used as cytoplamsic fraction; the pellet is resuspended in Nuclear buffer (20mM Hepes, pH7.9, 0.4M KCl, 0.1mM EDTA, 0.1mM EGTA, 1.0 mM DTT, 20% glycerol, protease inhibitors). The resuspended pellet is incubated on ice for 30 minutes with 10 seconds vortexing every 10 minutes. Cells are spun at 13000g for 30 minutes. Supernatant is removed to a new tube and quantified for protein. Samples are diluted with 2X SDS sample buffer to the desired concentration then heated in a boiling water bath for 5 minutes and loaded onto polyacrylamide gels. Whole Cell Extracts (WCE) were prepared by RIPA buffer extraction.

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

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