

Affinity-purified Goat Anti-human/mouse EBF-3 Antibody

ORDERING INFORMATION

Catalog Number: AF5166

Lot Number: CAPY01

Size: 100 µg

Storage: -20° C

Specificity: human/mouse EBF-3

Immunogen: *E. coli*-derived recombinant mouse EBF-3 (rmEBF-3; aa 399 - 504)

Ig Type: affinity-purified goat IgG

Application: Western blot

Background

EBF-3 (Early B cell Factor 3; also OE2 and COE3) is a 65 kDa (predicted) member of the COE family of transcription factors. It has limited expression, being identified principally in cerebellar Purkinje cells, olfactory neurons, and differentiating mantle layer neurons. Mouse EBF-3 is 596 amino acids (aa) in length it contains one DNA-binding region with an embedded zinc-finger motif (aa 51 - 235), a dimerization segment between aa 371 - 431, and a Pro/Ser-rich transactivation domain (aa 464 - 555). EBF-3 either homodimerizes, or heterodimerizes with EBF-2 or -1. There are at least two potential splice variants, one that shows a nine aa deletion between aa 252 - 260, and a second that shows the same deletion coupled to a one aa substitution for aa 521 - 557. Over aa 399 - 504, mouse EBF-3 shows more than 99% aa identity to rat and human EBF-3.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived, recombinant mouse EBF-3 (rmEBF-3, aa 399 - 504; Accession # O08791). Polyclonal antibody was affinity-purified on a column derivatized with rmEBF-3 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% Na₃N.

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 endogenous human and mouse EBF-3 in Western blots.

Application

Western blot - An antibody concentration of 2.0 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer

25 mM Tris, pH 7.5
0.15 M NaCl
0.05% Tween® 20

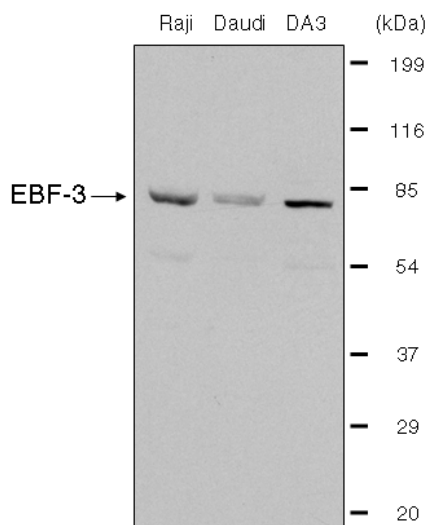
Blocking Solution

5% nonfat dry milk
in Blotting Buffer
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 1 hour at room temperature in Blocking Solution containing 2.0 µg/mL goat anti-EBF-3 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 1:2000 dilution of HRP-conjugated donkey anti-goat Ig (R&D Systems, Catalog # HAF019).
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 - A single plate (150 mm) of exponentially growing cells is washed twice in cold PBS. 1 mL of boiling 1% SDS lysis buffer (1% SDS, 10 mM Tris-HCL, pH 7.4, 1 mM sodium ortho-vanadate) is added to the plate. The plate is then scraped and the lysis is collected, sonicated and quantified. 30 µg of cellular protein is added to an equal amount of 2x SDS loading buffer. Samples are then boiled for 5 minutes and run on a SDS-PAGE gel.

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



30 µg of whole cell extracts from exponentially growing human Raji, Daudi and mouse DA3 cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 2.0 µg/mL goat anti-EBF-3 antibody.