

ORDERING INFORMATION

Catalog Number: MAB3809

Clone: 381923

Lot Number: YHE02

Size: 100 µg (sufficient for 50 mL of blotting solution)

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

Storage: -20° C

Specificity: human/mouse MafK

Immunogen: *E. coli*-derived rhMafK

Ig class: mouse IgG_{2b}

Recommended Application:
Western blot

Background

Maf family members form a unique subclass of basic-leucine zipper (bZIP) transcription factors. Maf proteins are subdivided into two groupings: large, including c-Maf, Nrl, MafA, and MafB; and small, including MafF, MafG, and MafK, also known as p18. Large Mafs contain an N-terminal acidic domain important for transcriptional activation that is lacking in small Maf family members. Heterodimers of MafK and p45 NF-E2, another bZIP factor, promote globin gene expression and erythroid differentiation in erythroleukemia cells.

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived full-length recombinant human MafK (rhMafK; aa 1 - 156; Accession # O60675). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

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

Reconstitution

Reconstitute with sterile PBS. If 0.2 mL of PBS is used, the antibody concentration will be 500 µg/mL.

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.**

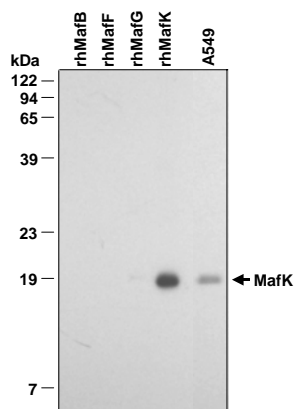
Specificity

This antibody detects endogenous human and mouse MafK at 18 kDa using Western blot. The antibody does not cross-react with recombinant human MafB or MafF. Prolonged film exposures may slightly detect recombinant human MafG.

Application

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

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



Detection of MafK with MAB3809.

Recombinant human MafB, MafF, MafG, and MafK, and lysates of human A549 cells were resolved by SDS-PAGE. Following electrophoresis, proteins were transferred to an Immobilon-P membrane and immunoblotted with 2.0 µg/mL anti-MafK, as described in *Protocols for Immunoblotting*. A one minute exposure to film is shown.

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Protocols for Immunoblotting

<u>Blotting Buffer</u>	<u>Blocking Solution</u>	<u>Antibody Solution</u>
25 mM Tris, pH 7.4	2% nonfat dry milk in Blotting Buffer	2% nonfat dry milk in Blotting Buffer
0.15 M NaCl		
0.1% Tween [®] 20	Adjust pH to 7.4	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 overnight at 4° C in Antibody Solution containing 2.0 µg/mL anti-human/mouse MafK.
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 goat anti-mouse IgG (R&D Systems, Catalog # HAF007).
5. Wash the membrane for 1 hour with 5 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 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 2×10^5 - 1×10^7 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.