

Affinity-Purified Goat Anti-human MafF Antibody

ORDERING INFORMATION

Catalog Number: AF3917

Lot Number: WHZ01

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

Storage: -20° C

Specificity: human MafF

Immunogen: *E. coli*-derived rhMafF

Ig Type: goat IgG

Applications: Western blot
Immunohistochemistry

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. Large Mafs contain an N-terminal acidic domain important for transcriptional activation that is lacking in small Maf family members. Small Maf/Nrf2 heterodimers have been implicated in the regulation of antioxidant response element-dependent genes.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived full-length recombinant human MafF (rhMafF; aa 1 - 164; Accession # NP_036455). 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 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 MafF at 18 kDa using Western blot. The antibody does not cross-react with recombinant human MafG or MafK.

Applications

Western blot - An antibody concentration of 2.0 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

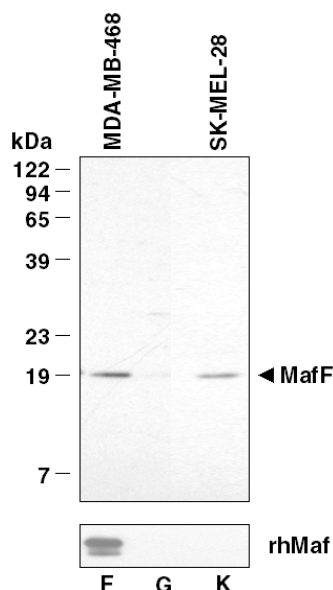
5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

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 overnight at 4° C in Antibody Solution containing 2.0 µg/mL goat anti-human MafF.
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-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with WesternGlo Chemiluminescent Detection Reagent (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 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.

Immunohistochemistry - This antibody will detect human MafF in cells and tissues. The working dilution is 15 µg/mL. For chromogenic detection of labeling, use R&D Systems Cell and Tissue Staining Kits (CTS series).

Optimal dilutions should be determined by the individual laboratory.



Detection of MafF with AF3917. 2 ng of rhMafF, rhMafG, and rhMafK (lower panel), and lysates from human MDA-MB-468 and SK-MEL-28 cells (upper panel) were resolved by SDS-PAGE. Following electrophoresis, proteins and lysates were transferred to an Immobilon-P membrane and immunoblotted with 2.0 µg/mL anti-MafF, as described in *Protocols for Immunoblotting*. Ten minute exposures to film are shown.

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