

ORDERING INFORMATION

Catalog Number: AF6005

Lot Number: CDET01

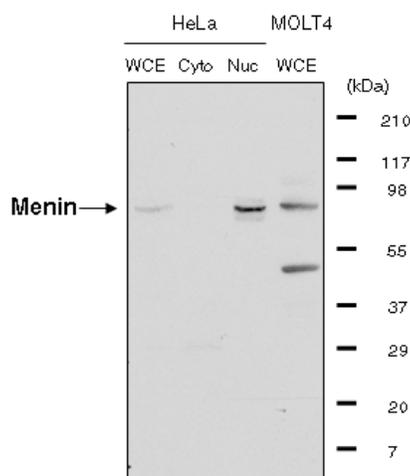
Size: 100 µg

Specificity: human Menin

Immunogen: *E. coli*-derived rhMenin
(aa 555 - 615)

Ig Type: goat IgG

Application: Western blot



Detection of Menin with AF6005.

30 µg of whole cell extract, 20 µg of cytoplasmic extract and 10 µg of nuclear extract from HeLa cells and 30 µg of MOLT4 whole cell extracts were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 2.0 µg/mL goat anti-Menin antibody, as described in *Protocols for Immunoblotting*.

Background

Menin (Multiple endocrine neoplasia type 1) is a 75 - 78 kDa tumor-suppressor protein that has no apparent structural relationship to any known protein family. It is ubiquitously expressed and interacts with other molecules, including NFκB, FANCD2 and ASK. Menin appears to block gene transcription, promote DNA repair, and inhibit cell proliferation. Human Menin is 615 amino acids (aa) in length. Consistent with its unique molecular taxonomy, it contains no definitive structural module(s). However, it is known to be phosphorylated on Ser399 and Thr599. There are a number of potential splice variants. Individually or in combination, there are deletions of aa 89 - 95, 149 - 153, 171 - 173, 189 - 223, and 423 - 426. There is also a 50 aa substitution for aa 68 - 615, a 66 aa substitution for aa 383 - 615, and a two aa substitution for aa 507 - 536. Over aa 555 - 615, human and mouse Menin are completely identical in aa sequence.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant human Menin (rhMenin; aa 555 - 615; Accession # O00255). 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 Menin in Western blot with an approximate molecular weight of 90 kDa.

Application

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 a PVDF membrane 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 Menin.
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 Antibody Solution containing a 1:2000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
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.