

ORDERING INFORMATION

Catalog Number: AF5778

Lot Number: CCJF01

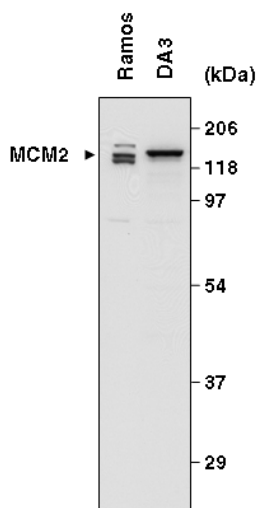
Size: 100 µg

Specificity: human/mouse MCM2

Immunogen: *E. coli*-derived rhMCM2
(aa 143 - 296)

Ig Type: goat IgG

Applications: Western blot
Immunohistochemistry



Detection of MCM2 with AF5778

Lysates from human Ramos and mouse DA3 cells were resolved by SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with 1.0 µg/mL anti-MCM2, as described in *Protocols for Immunoblotting*.

Background

MCM2 (Minichromosome maintenance protein 2; also BM28) is a member of the MCM family of proteins. Although its predicted MW is 102 kDa, it runs anomalously at 125 kDa in SDS-PAGE. MCM2 forms a large, 450 - 600 kDa prereplication complex with MCM3 through 7 plus HBO1, and associates with the ORC to initiate DNA synthesis. Human MCM2 is 904 amino acids (aa) in length (Accession # P49736). It contains one C4-type zinc finger region (aa 329 - 355) and a P-loop NTPase region (aa 485 - 659) and at least 12 Ser/Thr phosphorylation sites in the N-terminal one-third of the molecule. MCM2 has several potential isoforms. There is an alternate start site at Met10 with or without a 47 aa substitution for aa 109 - 157, a deletion of aa 31 - 126, a Gly substitution for aa 113 - 132 coupled with a 69 aa insertion after Lys476, and a seven aa substitution for aa 1 - 137. Over aa 143 - 296, human MCM2 shares 99% aa identity with mouse MCM2.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant mouse MCM2 (rhMCM2; aa 143 - 296; Accession # P49736). 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 and mouse MCM2 in Western blot with an approximate molecular weight of 120 kDa.

Applications

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Protocols for Immunoblotting

| Blotting Buffer | Blocking Solution | Antibody Solution |
|--------------------|--------------------|--------------------|
| 25 mM Tris, pH 7.4 | 5% nonfat dry milk | 5% nonfat dry milk |
| 0.15 M NaCl | in Blotting Buffer | in Blotting Buffer |
| 0.1% Tween® 20 | Adjust pH to 7.4 | Adjust pH to 7.4 |

- Transfer the electrophoresed proteins to a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
- Incubate the membrane 1 hour at room temperature in Antibody Solution containing 1.0 µg/mL goat anti-MCM2.
- Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
- 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).
- Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
- 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.

Immunohistochemistry - This antibody will detect MCM2 in cells and tissues. The working dilution is 3 µg/mL. Antigen retrieval is recommended.

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