Technical Data Sheet

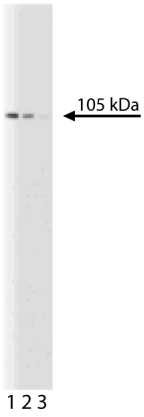
Purified Mouse Anti-Human MCM6

Product Information

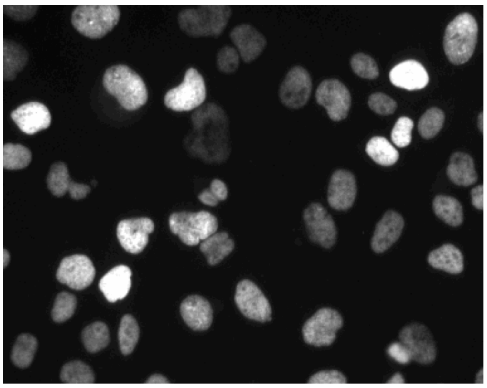
Material Number:	**611622**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	1/MCM6
Immunogen:	Human MCM6 aa. 670-792
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	105 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Mcm proteins are nuclear proteins that perform essential functions in the regulation of chromatin replication. They are primarily bound to chromatin during G1 and the beginning of S-phase, but are found in the nucleosol in postreplicative cells. Release of the bound Mcm proteins may be a mechanism for prevention of chromatin re-replication during S-phase. MCM6 is homologous to the yeast replication protein Mis5 and contains a DEFD-box motif and nucleotide-binding domain in the central region that are conserved in Mcm proteins. MCM6 forms a complex with MCM2, -4, and -7, which binds histone H3. In addition, the subcomplex of MCM4, -6, and -7 has helicase activity, which is mediated by the ATP-binding activity of MCM6 and the DNA-binding activity of MCM4. In *Xenopus*, formation of pre-replication complexes that include Mcm protein complexes requires phosphorylation, since intermediate phosphorylation of MCM4 correlates with Mcm protein complex formation and chromatin binding. Thus, Mcm proteins form heterocomplexes that participate in many different functions required for chromatin replication, such as DNA binding, helicase activity, and chromatin structure rearrangement.



Western blot analysis of MCM6 on HeLa lysate (Cat. No. 611449). *Lane 1: 1:1000, Lane 2: 1:2000, Lane 3: 1:4000 dilution of MCM6.*



Immunofluorescent staining of HeLa (ATCC CCL-2) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10 000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-MCM6 antibody. The second step reagent was Alexa Fluor® 555 goat anti mouse Ig (Invitrogen). Images were taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells and worked with both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).*

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Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
611449	HeLa Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

References

Holthoff HP, Hameister H, Knippers R. A novel human Mcm protein: homology to the yeast replication protein Mis5 and chromosomal location. *Genomics.* 1996; 37(1):131-134. (Biology)

Maiorano D, Lemaitre JM, Mechali M. Stepwise regulated chromatin assembly of MCM2-7 proteins. *J Biol Chem.* 2000; 275(12):8426-8431. (Biology)

Pereverzeva I, Whitmire E, Khan B, Coue M. Distinct phosphoisoforms of the Xenopus Mcm4 protein regulate the function of the Mcm complex. *Mol Cell Biol.* 2000; 20(10):3667-3676. (Biology)

You Z, Komamura Y, Ishimi Y. Biochemical analysis of the intrinsic Mcm4-Mcm6-mcm7 DNA helicase activity. *Mol Cell Biol.* 1999; 19(12):8003-8015. (Biology)