Datasheet for ABIN968618

anti-MCM6 antibody (AA 670-792)

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Overview

Quantity:	50 µg
Target:	MCM6
Binding Specificity:	AA 670-792
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This MCM6 antibody is un-conjugated
Application:	Western Blotting (WB), BioImaging (BI)

Product Details

Immunogen:	Human MCM6 aa. 670-792
Clone:	1-MCM6
Isotype:	IgG1
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results. 2. Please refer to us 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.

Product Details

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

Target Details

Target:	MCM6
Alternative Name:	MCM6 (MCM6 Products)
Background:	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.
Molecular Weight:	105 kDa
Pathways:	DNA Damage Repair, Mitotic G1-G1/S Phases, DNA Replication, Synthesis of DNA

Application Details

Application Notes:	Bioimaging 1. Seed the cells in appropriate culture medium at ~10,000 cells per well in an 96-well Imaging Plate and culture overnight. 2. Remove the culture medium from the wells, and fix the cells by adding 100 myl of Fixation Buffer to each well. Incubate for 10 minutes at room temperature (RT). 3. Remove the fixative from the wells, and permeabilize the cells using either 90% methanol, or Triton™ X-100: a. Add 100 myl of -20°C 90% methanol to each well and incubate for 5 minutes

Application Details

	at RT. OR b. Add 100 myl 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 myl of 1× PBS. 5. Remove the PBS, and block the cells by adding 100 myl of to each well. Incubate for 30 minutes at RT. 6. Remove the blocking buffer and add 50 myl 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 myl of 1× PBS. 8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 myl 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 myl of 1× PBS. 10. Remove the PBS, and counter-stain the nuclei by adding 200 myl per well of 2 myg/ml Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging. 11. View and analyze the cells on an appropriate imaging instrument.
Comment:	Related Products: ABIN968535
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	250 µg/mL
Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C
Storage Comment:	Store undiluted at -20°C.

Publications

Product cited in:	Maiorano, Lemaître, Méchali: "Stepwise regulated chromatin assembly of MCM2-7 proteins." in: **The Journal of biological chemistry**, Vol. 275, Issue 12, pp. 8426-31, (2000) (PubMed). Pereverzeva, Whitmire, Khan, Coué: "Distinct phosphoisoforms of the Xenopus Mcm4 protein regulate the function of the Mcm complex." in: **Molecular and cellular biology**, Vol. 20, Issue 10,

pp. 3667-76, (2000) (PubMed).

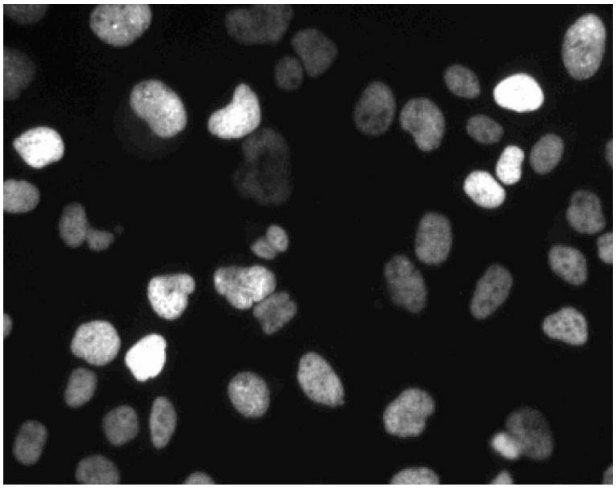
You, Komamura, Ishimi: "Biochemical analysis of the intrinsic Mcm4-Mcm6-mcm7 DNA helicase activity." in: **Molecular and cellular biology**, Vol. 19, Issue 12, pp. 8003-15, (2000) (PubMed).

Holthoff, Hameister, Knippers: "A novel human Mcm protein: homology to the yeast replication protein Mis5 and chromosomal location." in: **Genomics**, Vol. 37, Issue 1, pp. 131-4, (1997) (PubMed).

Images

Western Blotting

Image 1. Western blot analysis of MCM6 on HeLa lysate (ABIN968535). Lane 1: 1:1000, Lane 2: 1:2000, Lane 3: 1:4000 dilution of MCM6.



Immunofluorescence

Image 2. Immunofluorescent staining of HeLa (ATCC CCL-2) cells. Cells were seeded in a 96 well imaging plate 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.

Western Blotting

Image 3.