Technical Data Sheet

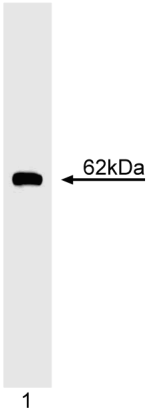
Purified Mouse Anti-Cyclin B1

Product Information

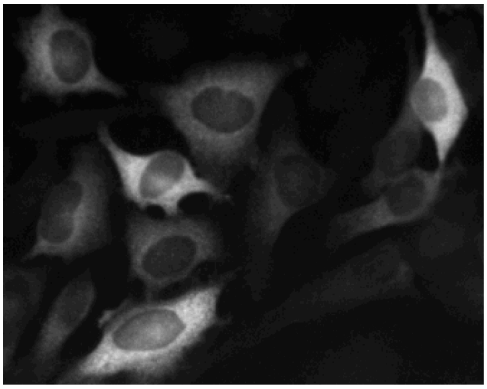
Material Number:	**554178**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	GNS-11
Immunogen:	Recombinant Human Cyclin B1
Isotype:	Mouse IgG2a
Reactivity:	QC Testing: Human Reported: Mouse
Target MW:	62 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

During the cell cycle, most eukaryotic cells double in mass, replicate their DNA and then distribute identical copies of their genome to progeny cells during mitosis. An internal biochemical clock ensures that all the necessary events occur in proper sequence and at the appropriate time. Cell in M phase contain a dominant regulatory factor known as maturation promoting factor (MPF). MPF triggers a variety of enzymatic and ultrastructural changes that are necessary for cell division. In higher eukaryotes, these mitosis-specific alterations include disassembly of the nuclear envelope, packaging of the DNA into chromosomes and assembly of the mitotic spindle. Purified MPF is a regulator of a protein kinase cascade and is evolutionarily conserved in all eukaryotic cells ranging from yeast to man. MPF consists predominantly of two polypeptides, cyclin B1 and p34, and contains protein kinase activity itself. It is the major M-phase-specific histone H1 kinase, but also phosphorylates a variety of other substrates including lamins, nucleolin, RNA polymerase II, retinoblastoma protein, SV40 large T antigen, p53, and the oncogenes c-src and c-abl. Cyclin B1 migrates at a reduced molecular weight of 62 kDa on SDS-PAGE.



Western blot analysis of Cyclin B1. *Lysate from Jurkat T cells was probed with the anti-Cyclin B1 antibody at 2 µg/ml. Cyclin B1 is identified as an ~62 kDa band.*



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-Cyclin B1 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). Images were taken on a BD Pathway™ 855 Bioimager system 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).*

Preparation and Storage

Store undiluted at 4°C.

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

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development
Flow cytometry	Reported
Immunoprecipitation	Reported

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
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611451	Jurkat Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(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. Triton is a trademark of the Dow Chemical Company.

References

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Gong J, Traganos F, Darzynkiewicz Z. Simultaneous analysis of cell cycle kinetics at two different DNA ploidy levels based on DNA content and cyclin B measurements. *Cancer Res.* 1993; 53(21):5096-5099. (Clone-specific: Flow cytometry, Immunofluorescence)

Hoffmann I, Clarke PR, Marcote MJ, Karsenti E, Draetta G. Phosphorylation and activation of human cdc25-C by cdc2--cyclin B and its involvement in the self-amplification of MPF at mitosis. *EMBO J.* 1993; 12(1):53-63. (Biology)

Jessus C, Beach D. Oscillation of MPF is accompanied by periodic association between cdc25 and cdc2-cyclin B. *Cell.* 1992; 68(2):323-332. (Biology)

Keyomarsi K, Pardee AB. Redundant cyclin overexpression and gene amplification in breast cancer cells. *Proc Natl Acad Sci U S A.* 1993; 90(3):1112-1116. (Clone-specific: Western blot)

Pines J, Hunter T. Human cyclins A and B1 are differentially located in the cell and undergo cell cycle-dependent nuclear transport. *J Cell Biol.* 1991; 115(1):1-17. (Clone-specific: Immunofluorescence)

Zhang H, Xiong Y, Beach D. Proliferating cell nuclear antigen and p21 are components of multiple cell cycle kinase complexes. *Mol Biol Cell.* 1993; 4(9):897-906. (Clone-specific: Immunoprecipitation)