

Technical Data Sheet

Purified Mouse Anti-Cyclin B1

Product Information

Material Number:	554177
Size:	0.25 mg
Concentration:	0.5 mg/ml
Clone:	GNS-1
Immunogen:	Human Cyclin B1 Recombinant Protein
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Reported: Hamster, Mouse
Target MW:	62 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

Cyclins and cyclin-dependent kinases (cdks) are evolutionarily conserved proteins that are essential for cell-cycle control in eukaryotes. Cyclins (regulatory subunits) bind to cdks (catalytic subunits) to form complexes that regulate the progression of the cell cycle. The main cyclin-cdks complexes formed in vertebrate cells are cyclin D-cdk4 (G0/G1), cyclin E-cdk2 (G1/S), cyclin A-cdk2 (S) and cyclin B1-cdk1 (G2/M). These complexes are regulated by activating and inhibitory phosphorylation events, as well as by interactions with small regulatory proteins, such as p21 and p27 [Kip1]. Cyclin B1 is a mitotic cyclin, where expression is normally low in G0/G1, increases in S and is maximal during the G2/M phase. Cyclin B1 is rapidly degraded at the end of mitosis, and is required for cells to exit from mitosis. This antibody has been reported to react to hamster and mouse cyclin B1. In addition, the GNS-1 antibody has been reported to recognize an epitope between amino acids 1-21 of human cyclin B1.

Preparation and Storage

Store undiluted at 4°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
Flow cytometry	Reported
Fluorescence microscopy	Reported
Immunohistochemistry	Reported
Immunoprecipitation	Reported

Recommended Assay Procedure:

Bioimaging

- 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.
- 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).
- Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
 - 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.
- Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
- 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 min at RT.
- 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 hr at RT.
- Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
- Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in dark for 1 hr at RT.
- Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
- 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 min before imaging.

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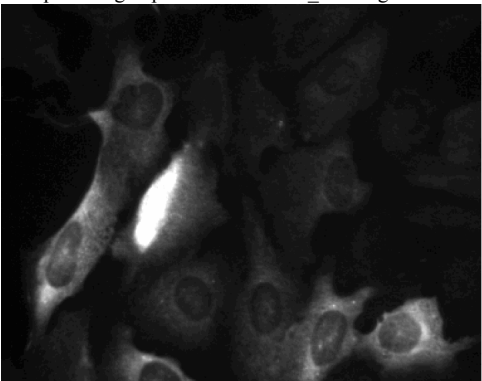
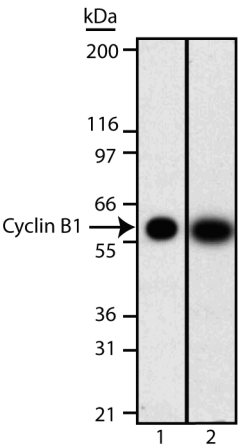
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11. View and analyze the cells on an appropriate imaging instrument.

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

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



Left Figure: Western blot analysis of cyclin B1. Lane 1: K562 human leukemia cell lysate. Lane 2: 293 human embryonic kidney cell lysate. Anti-human cyclin B1 (Cat. No. 554176) identifies cyclin B1 as an ~62 kDa band. **Right Figure: Cyclin B1 staining of U-2 OS (ATCC HTB-96) 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 Alexa Fluor® 488 goat anti mouse Ig (Invitrogen). Images were taken on a BD Pathway™ 855 Bioimager system using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and HeLa (CCL-2) cells and worked with both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

- Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 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.
- 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.
- Triton is a trademark of the Dow Chemical Company.

References

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