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 Molecular Weight:	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 (catlytic 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.

This antibody is routinely tested by Western blot analysis and immunofluorescent imaging. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

Application Notes

Application

Bioimaging	Routinely Tested
Western blot	Routinely Tested
Flow cytometry	Reported
Fluorescence microscopy	Reported
Immunohistochemistry	Reported
Immunoprecipitation	Reported

Recommended Assay Procedure:

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

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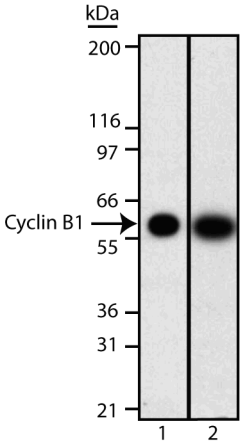

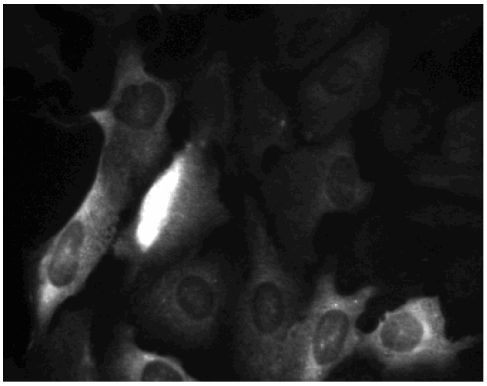

Left: Western blot analysis of cyclin B1. *Lane 1, K562 human leukemia cell lysate. Lane 2, 293 human embryonic kidney cell lysate. Anti-cyclin B1 (Cat. No. 554177) identifies cyclin B1 as an ~62 kDa band.* ***Right: Immunofluorescent staining of U2OS cells.*** *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol (see Recommended Assay Procedure) and the anti-Cyclin B1 antibody. The second step reagent was Alexa Fluor® 488 goat anti mouse Ig (Invitrogen). Images were taken on a Pathway 850 imager using a 20x objective. This antibody also stained A549 and HeLa cells and worked with both the Triton X100 and Methanol fix/perm protocols (see Recommended Assay Procedure).*

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. 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.

References

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