Technical Data Sheet

Purified Mouse Anti-Human Cyclins D1, D2, D3

Product Information

Material Number:	**554203**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	G124-259
Immunogen:	Recombinant Human Cyclin D1, expressed in the Baculovirus Expression System (BEVS)
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	36 kDa (D1), 35 kDa (D2), 31/34 kDa doublet (D3)
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

Cyclins and cyclin-dependent kinases (cdks) are evolutionary 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 proteins that bind to cyclins, cdks, or cyclin-cdk complexes, e.g., p21 and p27[Kip1]. Specific substrates for cdk-cyclin complexes include nuclear lamins, histones, oncogenes (c-src, c-abl, SV40 large T-Ag), tumor suppressor genes (e.g., retinoblastoma protein [Rb] and p53), nucleolin, RNA polymerase II and others. It is thought that D-type cyclins are involved in regulating in the passage of mammalian cells through G1. The reduced molecular weights of D-type cyclins are as follows: cyclins are D1 (36 kDa), cyclin D2 (35 kDa) and cyclin D3 [31 and 34 kDa (doublet)]. G124-259 recognizes human cyclins D1 (36 kDa), D2 (35 kDa), and D3 (31 and 33 kDa). Recombinant full-length human cyclin D1, expressed in the Baculovirus Expression System (BEVS), was used as immunogen. Hybridomas were selected by ELISA and western blot reactivity. G124-259 was selected, as it reacted with D1, D2, and D3, apparently recognizing a common epitope among these three cyclin D proteins.

D-type cyclins are differentially expressed in distinct cell types. This differential expression appears to exist even among cell types which generally have high levels of proliferative proteins. For example, whereas cyclin D1 was readily detected in a human glioblastoma cell line (U118 MG), it was undetected in transformed primary human embryonic kidney cells (293). Cell types which have been documented to express high levels of a given D-type cyclin are suggested as positive controls. WI-38 human diploid fibroblasts (ATCC CCL 75) and U-118 (ATCC HTB 15) are suggested as positive controls for detecteing cyclin D1 and D3. Primary human peripheral blood T lymphocytes stimulated with phytohemagglutin in (PHA) and Raji human Burkitt lymphoma cells (ATCC CCL 86) are suggested as positive controls for detecting cyclin D2.

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

Intracellular staining (flow cytometry)	Routinely Tested
Immunoprecipitation	Tested During Development
Western blot	Tested During Development

Recommended Assay Procedure:

Applications include western blot analysis (1-2 µg/ml), immunoprecipitation (1-2 µg/1 x 10^6 cells) and flow cytometric analysis (0.06-1.0 µg/1 x 10^6 cells).

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

Gong J, Bhatia U, Traganos F, Darzynkiewicz Z. Expression of cyclins A, D2 and D3 in individual normal mitogen stimulated lymphocytes and in MOLT-4 leukemic cells analyzed by multiparameter flow cytometry. *Leukemia.* 1995; 9(5):893-899.(Clone-specific: Flow cytometry, Immunoprecipitation, Western blot)

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