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 cdk's (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, cdk's, or cyclin-cdk complexes, e.g., p21 and p27[Kip1]. Specific substrates for cdk-cyclin complexes include nuclear lamins, histones, oncoproteins (e.g., 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 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 detecting cyclin D1 and D3. Primary human peripheral blood T lymphocytes stimulated with phytohemagglutinin in (PHA) and Raji human Burkitt lymphoma cells (ATCC CCL 86) are suggested as positive controls for detecting cyclin D2.

Western blot analysis of cyclin D1, D2, D3 on A431 human epidermoid carcinoma cell line. A431 (ATCC CRL-1555) cell lysate was stained with Purified Mouse Anti-Human Cyclins D1, D2, D3 (Cat. No. 554203) at 2, 1, 0.5, and 0.25 µg/test (Lanes 1-4, respectively), followed by HRP Goat Anti-Mouse Ig (Cat. No. 554002).
Preparation and Storage
Store undiluted at 4°C.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

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<th>Application</th>
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<tr>
<td>Western blot</td>
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<td>Intracellular staining (flow cytometry)</td>
<td>Tested During Development</td>
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<tr>
<td>Immunoprecipitation</td>
<td>Tested During Development</td>
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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).

Suggested Companion Products

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<tr>
<th>Catalog Number</th>
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<tbody>
<tr>
<td>554002</td>
<td>HRP Goat Anti-Mouse Ig</td>
<td>1 mL</td>
<td>(none)</td>
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Product Notices
1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

References
Sherr CJ. Mammalian G1 cyclins. Cef. 1993; 73(6):1059-1065. (Biology)
Xiong Y, Zhang H, Beach D. D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. Cef. 1992; 71(3):505-514. (Biology)