Cyclin D1 / bcl-1 Ab-2 (DCS-11)

Mouse Monoclonal Antibody

Cat. #MS-211-P0, -P1, or -P (0.1ml, 0.5ml, or 1.0ml at 200μg/ml) (Purified Ab with BSA and Azide)
Cat. #MS-211-P1ABX or -PABX (0.1ml or 0.2ml at 1.0mg/ml) (Purified Ab without BSA and Azide)

Please note this data sheet has been changed effective December 6, 2011

Description: Cyclin D1or PRAD-1 or bcl-1 is one of the key cell cycle regulators, and functions in association with cdk4 and/or cdk6 by phosphorylating the Rb protein. It is a putative proto-oncogene overexpressed in a wide variety of human neoplasms including mantle cell lymphomas (MCL)

Comments: Ab-2 is highly specific to cyclin D1 and shows no cross-reaction with cyclin D2 or D3. It supports the cdks-associated kinase activity (e.g. using pRB as a substrate.

Mol. Wt. of Antigen: 36kDa

Epitope: Not determined

Species Reactivity: Human, Mouse, and Rat. Others not known.

Clone Designation: DCS-11

Ig Isotype: IgG_{2a}

Immunogen: Human recombinant full length cyclin D1 protein

Applications and Suggested Dilutions:

- Kinase Assay

The optimal dilution for a specific application should be determined by the investigator.

Cellular Localization: Nuclear

Supplied As:
200μg/ml antibody purified from the ascites fluid by Protein A chromatography. Prepared in 10mM PBS, pH 7.4, with 0.2% BSA and 0.09% sodium azide. Also available without BSA and azide at 1mg/ml.

Storage and Stability:
Ab with sodium azide is stable for 24 months when stored at 2-8°C. Antibody WITHOUT sodium azide is stable for 36 months when stored at below 0°C.

Key References:

1. Lukas J, *et. al.* Molecular and Cellular Biology, 1995, 15(5):2600-11.
2. Lukas J; et al. Oncogene, 1995,10:2125-34.
3. Lukas J; etal. Oncogene, 1994, 9(3):707-18.

Limitations and Warranty:
Our products are intended FOR RESEARCH USE ONLY and are not approved for clinical diagnosis, drug use or therapeutic procedures. No products are to be construed as a recommendation for use in violation of any patents. We make no representations, warranties or assurances as to the accuracy or completeness of information provided on our data sheets and website. Our warranty is limited to the actual price paid for the product. Lab Vision is not liable for any property damage, personal injury, time or effort or economic loss caused by our products.

Material Safety Data:
This product is not licensed or approved for administration to humans or to animals other than the experimental animals. Standard Laboratory Practices should be followed when handling this material. The chemical, physical, and toxicological properties of this material have not been thoroughly investigated. Appropriate measures should be taken to avoid skin and eye contact, inhalation, and ingestion. The material contains 0.09% sodium azide as a preservative. Although the quantity of azide is very small, appropriate care should be taken when handling this material as indicated above. The National Institute of Occupational Safety and Health has issued a bulletin citing the potential explosion hazard due to the reaction of sodium azide with copper, lead, brass, or solder in the plumbing systems. Sodium azide forms hydrazoic acid in acidic conditions and should be discarded in a large volume of running water to avoid deposits forming in metal drainage pipes.

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PROTOCOL FOR CYCLIN D1-ASSOCIATED *in vitro* KINASE ASSAY

1) Scrape (or suspend) the cells in IP buffer (0.5 ml per 6 cm dish or 1 ml per 10 cm dish)
IP buffer (Matsushime et al., Mol. Cell. Biol. 14, 2066-2076, 1994):
50 mM HEPES, pH 7.5
150 mM NaCl
1 mM EDTA
2.5 mM EGTA
10% glycerol
1 mM DTT
0.1% Tween-20
10 mM β-glycerophosphate
1 mM NaF
0.1 mM sodium orthovanadate
2 μg/ml aprotinin
5 μg/ml leupeptin
0.1 mM PMSF

2) Freeze quickly on powdered dry ice or dip briefly into liquid nitrogen, thaw, and incubate for 1 hr on ice with occasional vortexing.

3) Clarify the lysate by centrifugation (Eppendorf centrifuge, full speed, 10 min at 4°C).

4) Pre-clear the lysates by rotating aliquots containing about 1mg of total extracted protein with protein A(G)-Sepharose (20 μl of swollen beads) for 1 hr at 4°C.

5) Precipitate cyclin D1-cdk complexes with protein G-Sepharose (10 μl) pre-coated with saturating amount of antibody (approx. 10 μg of immunoglobulin) against cyclin D1 (DCS-11) or control antibody (DCS-6 or another IgG_{2a}) for 3-6 hrs at 4°C. Use 10 μl of swollen beads per 200 μg of extracted protein.

6) Wash the beads 4 times in IP buffer and 2 times in 50 mM HEPES, pH 7.5 containing 1 mM DTT.

7) Suspend 10 μl of the beads in 30 μl of **Kinase Assay Buffer:**
50 mM HEPES, pH 7.5
10 mM $MgCl_2$
1mM DTT
2.5 mM EGTA
10 mM β-glycerophosphate containing substrate protein: GST-Rb pocket (aa 379-928); 1 μg per assay.
(One minute pre-incubation at 30°C optional)

8) Add 10 μl of ATP to achieve a final concentration of 50 μM and 10 μCi of (γ-^{32}P) ATP (6000 Ci/mmol).

9) Incubate for 30 minutes at 30°C with occasional gentle re-suspending the beads.

10) Stop the reaction by adding 30μl of Laemmli sample buffer, boil for 5 min.

11) Load the whole reaction on 8% SDS-PAGE and analyze after drying the gel by autoradiography or using phosphorimager.

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