Technical Data Sheet

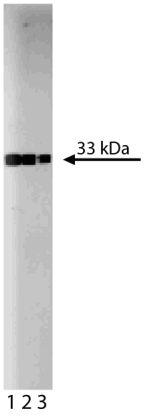
Purified Mouse Anti-Cdk2

Product Information

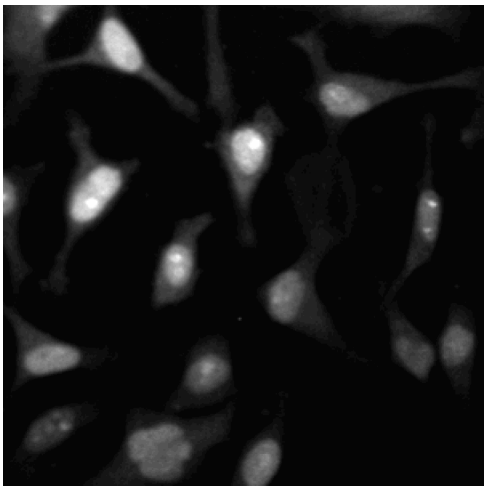
Material Number:	**610146**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	55/Cdk2
Immunogen:	Human Cdk2 aa. 109-298
Isotype:	Mouse IgG2a
Reactivity:	QC Testing: Human Tested in Development: Dog, Mouse, Rat
Target MW:	33 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Cyclin-dependent kinase 2 (Cdk2) is a member of a family of cdc2-related cell cycle protein kinases. Cdk2 shares 60% identity with cdc2 and its activity is regulated by phosphorylation in a similar fashion. Cdk2 is expressed earlier in the cell cycle than is cdc2. Like p34 [cdc2], p33 [cdk2] associates with Cyclin A in human cells. However, kinase activity associated with Cyclin A-Cdk2 is present in S phase, whereas, the kinase activity associated with Cyclin A-cdc2 is found only in G2. Cdk2 can also complex with cyclins E, D1, and D3. It is not known if the D cyclins can form active complexes with Cdk2. Cyclin E-Cdk2 kinase is active in the G1 and S phases of the cell cycle and is important (as is Cyclin A-Cdk2) for the progression from G1 to S phase. The levels of Cyclin A-Cdk2 are maximal at the G1/S transition and both Cdk2 and Cyclin A associate with DNA in the initiation complex during replication. The Rb protein has been identified as a substrate for Cdk2-Cyclin E and/or Cdk2-Cyclin A in vivo. This observation is supported by further evidence which shows that Cdk2 is activated and specifically localized to the nucleus during late G1, S phase, and G2.



Western blot analysis of Cdk2 on a Jurkat lysate. *Lane 1: 1:2500, lane 2: 1:5000, lane 3: 1:10000 dilution of the Cdk2 antibody.*



Immunofluorescent staining of HeLa (ATCC CCL-2) 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-Cdk2 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). Images were taken on a BD Pathway™ 855 bioimaging system using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells and worked with both the Triton™ X-100 and alcohol fix/perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Immunohistochemistry	Tested During Development
Immunoprecipitation	Tested During Development
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. 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.
2. 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).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. 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.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. 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 minutes at RT.
6. 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 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. 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 minutes before imaging.
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/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/monoclonal_anti.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611451	Jurkat Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Triton is a trademark of the Dow Chemical Company.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. 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.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Hinds PW, Mittnacht S, Dulic V, Arnold A, Reed SI, Weinberg RA. Regulation of retinoblastoma protein functions by ectopic expression of human cyclins. *Cell.* 1992; 70(6):993-1006. (Biology)

Mal A, Chattopadhyay D, Ghosh MK, Poon RY, Hunter T, Harter ML. p21 and retinoblastoma protein control the absence of DNA replication in terminally differentiated muscle cells. *J Cell Biol.* 2000; 149(2):281-292. (Biology: Immunoprecipitation, In vitro kinase assay, Western blot)

Mohapatra S, Agrawal D, Pledger WJ. p27Kip1 regulates T cell proliferation. *J Biol Chem.* 2001; 276(24):21976-21983. (Biology: Western blot)

Porter DC, Zhang N, Danes C, et al. Tumor-specific proteolytic processing of cyclin E generates hyperactive lower-molecular-weight forms. *Mol Cell Biol.* 2001; 21(18):6254-6269. (Biology: Western blot)

Saitoh H, Pizzi MD, Wang J. Perturbation of SUMOlation enzyme Ubc9 by distinct domain within nucleoporin RanBP2/Nup358. *J Biol Chem.* 2002; 277(7):4755-4763. (Biology: Immunofluorescence)