Technical Data Sheet

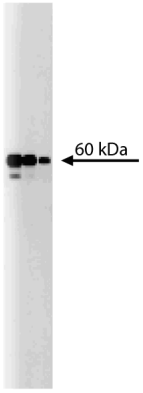
Purified Mouse Anti-Human Cyclin A

Product Information

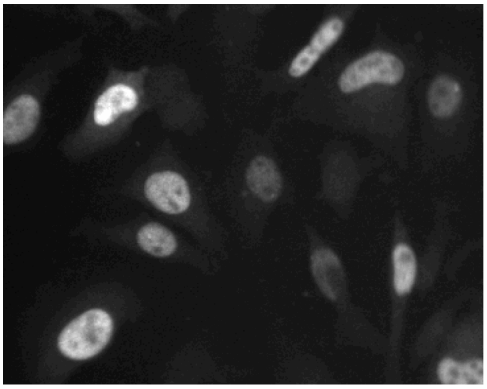
Material Number:	**611269**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	25/Cyclin A
Immunogen:	Human Cyclin A aa. 26-144
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	60 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Progression of the mammalian cell cycle is regulated by phosphorylation of many key proteins. Several classes of cyclins (A-E) act as regulatory subunits for cyclin-dependent kinases (cdks). These cyclin-cdk holoenzymes are essential for proper control of cell cycle progression. They phosphorylate and regulate a variety of substrates whose activity is required for cell cycle transitions. The temporal expression of cyclins is tightly regulated throughout the cell cycle by synthesis and degradation. Such regulation plays a critical role in controlling the enzymatic activity of the cdks. Cyclin A, one of the mitotic cyclins, activates Cdk2 near the start of S phase and is necessary for the initiation of DNA replication. In mammalian somatic cells, Cyclin A is required during S phase and passage through G2. The D and E type cyclins regulate passage through G1, while Cyclin B is a critical regulator of mitosis. It has been shown in a number of species that mutation or disruption of normal Cyclin A expression causes cells to arrest at G2. Cyclin A binds both the cdc2 (Cdk1) and Cdk2 kinases and may also have a role in mitotic dependence on S phase completion.



Western blot analysis of Cyclin A on a A431 lysate. *Lane 1: 1:250, lane 2: 1:500, lane 3: 1:1000 dilution of the Cyclin A 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-Cyclin A antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a BD Pathway™ 855 Bioimager system using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells using both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).*

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Preparation and Storage

Store undiluted at -20°C.

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

Application Notes

Application

Western blot	Routinely Tested
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/pharmingen/protocols/Western_Blotting.shtml

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)
611447	A431 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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

References

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Pines J. Cyclins and cyclin-dependent kinases: take your partners. *Trends Biochem Sci.* 1993; 18(6):195-197. (Biology)

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