Technical Data Sheet

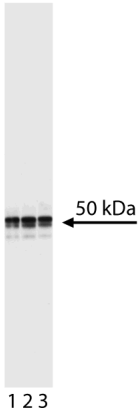
Purified Mouse Anti-Human Cyclin E

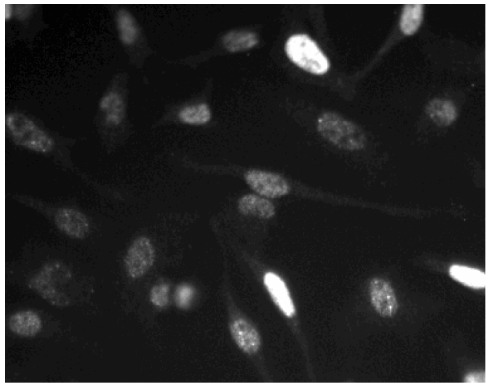
Product Information

Material Number:	**551160**
Reactivity:	QC Testing: Human
Target MW:	50 kDa
Component:	**51-1459GR**
Description:	Purified Mouse Anti-Human Cyclin E
Size:	50 µg (3 ea)
Clone Name:	HE12
Isotype:	Mouse IgG1
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.
Component:	**51-16516N**
Description:	HeLa Control Lysate
Size:	50 µg (1 ea)
Concentration:	1.0 mg/ml
Storage Buffer:	SDS-PAGE buffer (62mM Tris pH 6.8, 2% SDS, 0.9% b-mercaptoethanol, 0.003% bromophenol blue, 5% glycerol)

Description

Cyclins and cyclin-dependent kinases (cdks) are evolutionarily 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. 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]. Cyclin E is expressed in G1 and associates with cdk2 to form an active kinase where it plays an important role in the regulation of the G1/S restriction checkpoint in the cell cycle. Abberant expression of cyclin E has been reported to be associated with the oncogenic transformation of cells. This antibody has been reported not to cross-react with mouse cyclin E.





Left: Western blot analysis of Cyclin E. *Lysate from HeLa cells was probed with the anti-cyclin E antibody at concentrations of 1.0 µg/mL (lane 1), 0.5 µg/mL (lane 2), and 0.25 µg/mL (lane 3). Cyclin E is identified as a band of 50 kDa.* ***Right: 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 E antibody. The second step reagent was Alexa Fluor® 555 goat anti-mouse IgG (Invitrogen). 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 and worked with both the Triton™ X-100 and alcohol 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.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development
Flow cytometry	Reported

BD Biosciences

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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 or Triton™ X-100:
   a. Add 100 µl of -20°C 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
611449	HeLa Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(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)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal

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

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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. (Biology: Flow cytometry)

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Sherr CJ. Mammalian G1 cyclins. *Cell.* 1993; 73(6):1059-1065. (Biology)