Datasheet for ABIN967391

anti-CDKN1B antibody

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Overview

Quantity:	0.1 mg
Target:	CDKN1B
Reactivity:	Human, Mouse
Host:	Mouse
Clonality:	Monoclonal
Application:	Western Blotting (WB), Immunoprecipitation (IP), BioImaging (BI)

Product Details

Brand:	BD Pharmingen™
Immunogen:	Mouse p27 [Kip1] (full-length) Recombinant Protein
Clone:	G173-524
Isotype:	IgG1
Cross-Reactivity:	Human
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results. 2. Please refer to us for technical protocols. 3. 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. 4. 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.

Product Details

	5. Triton is a trademark of the Dow Chemical Company.
Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Target Details

Target:	CDKN1B
Alternative Name:	p27 Kip1 (CDKN1B Products)
Background:	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. These include p15, p16, p18, p19, p21 and p27 [Kip1]. p27 [Kip1] has been shown to inhibit the activity of multiple cyclin-cdk complexes, including cyclin D-cdk4, cyclin E-cdk2 and cyclin A-cdk2. p27 [Kip1] is a 27 kD protein which shares N-terminal sequence homology with p21, and like p21, p27 [Kip1] contains a nuclear localization signal in its C-terminal region. IL-2 activation of T cells has been reported to lead to a decrease in p27 [Kip1] and entry into S phase. Removal of IL-2 from T cell cultures results in increased levels of p27 [Kip1] and cell quiescence.
Molecular Weight:	27 kDa
Pathways:	Cell Division Cycle, Fc-epsilon Receptor Signaling Pathway, EGFR Signaling Pathway, Neurotrophin Signaling Pathway, Positive Regulation of Peptide Hormone Secretion, Negative Regulation of Hormone Secretion, Sensory Perception of Sound, Mitotic G1-G1/S Phases, DNA Replication, Positive Regulation of Endopeptidase Activity, Synthesis of DNA, Autophagy

Application Details

Application Notes:	Bioimaging 1. Seed the cells in appropriate culture medium at ~10,000 cells per well in an 96-well Imaging Plate and culture overnight. 2. Remove the culture medium from the wells, and fix the cells by adding 100 myl of Fixation Buffer to each well. Incubate for 10 minutes at room temperature (RT). 3. Remove the fixative from the wells, and permeabilize the cells using either 90% methanol, or Triton™ X-100: a. Add 100 myl of -20°C 90% methanol to each well and incubate for 5 minutes at RT. OR b. Add 100 myl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.

Application Details

	4. Remove the permeabilization buffer, and wash the wells twice with 100 myl of 1× PBS. 5. Remove the PBS, and block the cells by adding 100 myl of to each well. Incubate for 30 minutes at RT. 6. Remove the blocking buffer and add 50 myl 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 myl of 1× PBS. 8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 myl 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 myl of 1× PBS. 10. Remove the PBS, and counter-stain the nuclei by adding 200 myl per well of 2 myg/ml Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging. 11. View and analyze the cells on an appropriate imaging instrument.
Comment:	Related Products: ABIN967389
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	0.5 mg/mL
Buffer:	Aqueous buffered solution containing ≤0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C
Storage Comment:	Store undiluted at 4°C.

Publications

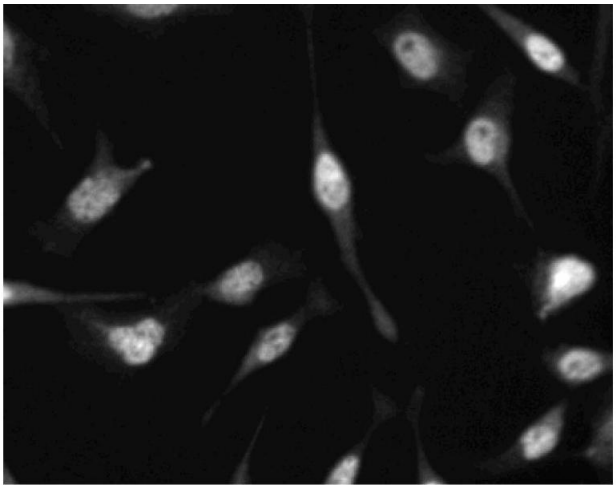
Product cited in:	Jan, Adolfsson, Allaman, Buccarello, Magistretti, Pfeifer, Muhs, Lashuel: "Abeta42 neurotoxicity is mediated by ongoing nucleated polymerization process rather than by discrete Abeta42 species." in: **The Journal of biological chemistry**, Vol. 286, Issue 10, pp. 8585-96, (2011) (PubMed). Deshmukh, Salehzadeh, Metayer-Coustard, Fahlman, Nair, Al-Khalili: "Post-transcriptional gene

Publications

silencing of ribosomal protein S6 kinase 1 restores insulin action in leucine-treated skeletal muscle." in: **Cellular and molecular life sciences : CMLS**, Vol. 66, Issue 8, pp. 1457-66, (2009) (PubMed).

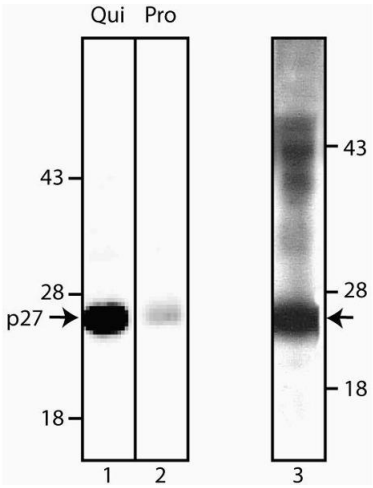
Images

Image 1.



Immunofluorescence

Image 2. Immunofluorescent staining of HeLa (ATCC CCL-2) cells. Cells were seeded in a 96 well imaging plate at ~10,000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-p27 [Kip1] antibody. The second step reagent was Alexa Fluor® 555 goat anti-mouse IgG (Invitrogen). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) cells and worked with both the Triton™ X-100 and alcohol perm protocols.



Western Blotting

Image 3. Immunoprecipitation/Western Blot analysis of p27 [Kip1]. Lanes 1 and 2, Equal amounts of protein (25 µg/lane) from BALB/c 3T3 cell lysates of quiescent (lane 1) and proliferating (lane 2) cells were seperated by SDS-PAGE and were probed with clone G173-524 (ABIN967391). Cells may be made quiescent by techniques such as serum starvation. The antibody identifies p27 [Kip1] as a 27 kDa band and demonstrates that the level of p27 [Kip1] is higher during cell quiescence than during cell proliferation. Lane 3, lysate from quiescent BALB/c 3T3 cells was immunoprecipitated with clone G173-524. The immune complex was seperated by SDS-PAGE and p27 [Kip1] was detected by western blot analysis with clone G173-524.

Please check the product details page for more images. Overall 4 images are available for ABIN967391.