BD Pharmingen™

Bioimaging Certified Reagent

Technical Data Sheet

Purified Mouse Anti-Human Cdc25C

Product Information

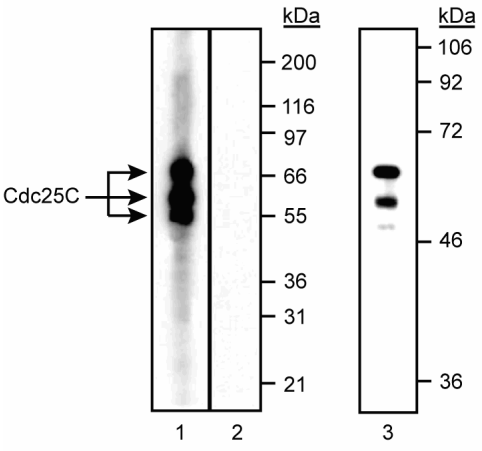
Material Number:	**556576**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	C2-2
Immunogen:	Human Cdc25C
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	60-63 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

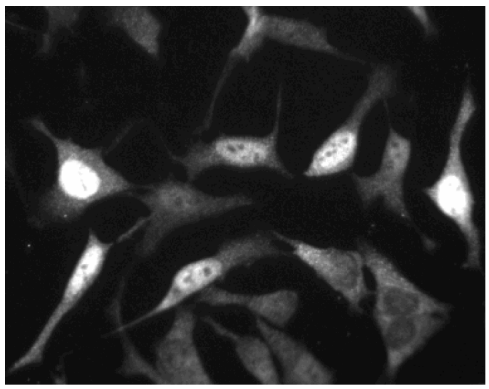
Description

Cyclins and cyclin-dependent kinases (cdks) are evolutionarily conserved proteins that are essential for cell-cycle control in eukaryotes. Cyclins contain a conserved amino acid sequence motif, the cyclin box, through which they bind to cdks to form active 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 regulatory proteins including p21 and p27Kip1. Cdc25A, Cdc25B and Cdc25C are a family of phosphatases that activate cyclin-cdk complexes at different checkpoints of the cell cycle. Cdc25C appears to be active during G2 where it controls entry into mitosis by dephosphorylating and thus activating the cdk1 component of cyclin B1-cdk1 complexes. In response to DNA damage, Cdc25C becomes phosphorylated by the recently identified kinases Chk1 and c-TAK1 creating a binding site for 14-3-3 proteins which inhibits the phosphatase activity of Cdc25 proteins. Thus Cdc25C serves a dual purpose during the cell cycle via its ability to promote G2/M transition in normally cycling cells as well as by providing a cell cycle checkpoint in response to DNA damage.

Multiple electrophoretic forms of Cdc25C have been reported and are thought to result from differences in the phosphorylation state of the Cdc25C protein. Full length, recombinant human Cdc25C was used as immunogen. HeLa human cervical carcinoma (ATCC CCL-2) and A549 human lung carcinoma (ATCC CCL-185) cells are suggested as positive controls for the western blot application.

This antibody is routinely tested by Western blot analysis and immunofluorescent imaging. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.





Left: Western blot analysis of Cdc25C in human and mouse cell lysates. *Lanes 1 and 2, A549 human lung carcinoma cells were probed with clone C2-2 at 1-2 µg/ml (lane 1) or with an isotype control (lane 2). Lane 3, Rous Sarcoma Virsu-transformed mouse 3T3 cells. The C2-2 antibody identifies Cdc25C isoforms ranging between 60-63 kDa in both human and mouse cell types.* ***Right: Immunofluorescent staining of A549 cells.*** *Cells were seeded in a 96 well imaging plate (Ca. No. 353219) at ~ 10 000 cells per well. After overnight incubation, cells were stained using the Triton X100 fix/perm protocol (see Recommended Assay Procedure) and the anti-Cdc25C antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). Images were taken on a Pathway 850 imager using a 20x objective. This antibody also stained HeLa and U2OS cells and worked with both the Triton X100 and Methanol fix/perm protocols (see Recommended Assay Procedure).*

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit bdbiosciences.com/how_to_order/

BD

BD Biosciences

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

Application Notes

Application

Bioimaging	Routinely Tested
Western blot	Routinely Tested

Recommended Assay Procedure:

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
611449	HeLa Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Igs	1.0 ml	(none)
554001	FITC Goat Anti-Mouse Igs	0.5 mg	Polyclonal

Product Notices

1. 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.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Peng CY, Graves PR, Ogg S, et al. C-TAK1 protein kinase phosphorylates human Cdc25C on serine 216 and promotes 14-3-3 protein binding. *Cell Growth Differ.* 1998; 9(3):197-201.(Biology)

Peng CY, Graves PR, Thoma RS, Wu Z, Shaw AS, Piwnica-Worms H. Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. *Science.* 1997; 277(5331):1501-1505.(Biology)

Sadhu K, Reed SI, Richardson H, Russell P. Human homolog of fission yeast cdc25 mitotic inducer is predominantly expressed in G2. *Proc Natl Acad Sci U S A.* 1990; 87(13):5139-5143.(Biology)

Sanchez Y, Wong C, Thoma RS, et al. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. *Science.* 1997; 277(5331):1497-1501.(Biology)

Strausfeld U, Labbe JC, Fesquet D, et al. Dephosphorylation and activation of a p34cdc2/cyclin B complex in vitro by human CDC25 protein. *Nature.* 1991; 351(6323):242-245.(Biology)