abcam

ab28283

Anti-Cyclin D3/CCND3 antibody [DCS2.2]

KO Validated

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	Preservative: 0.08% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Full Length Protein corresponding to Human G1/S-specific cyclin-D3. Database link P30281
Clone number	DCS2.2
Purification technique	Affinity purification Protein A/G
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information G1/S-specific cyclin-D3

Function	Regulatory component of the cyclin D3-CDK4 (DC) complex that phosphorylates and inhibits members of the retinoblastoma (RB) protein family including RB1 and regulates the cell-cycle during G(1)/S transition (PubMed:8114739). Phosphorylation of RB1 allows dissociation of the transcription factor E2F from the RB/E2F complex and the subsequent transcription of E2F target genes which are responsible for the progression through the G(1) phase (PubMed:8114739). Hypophosphorylates RB1 in early G(1) phase (PubMed:8114739). Cyclin D-CDK4 complexes are major integrators of various mitogenenic and antimitogenic signals (PubMed:8114739). Component of the ternary complex, cyclin D3/CDK4/CDKN1B, required for nuclear translocation and activity of the cyclin D-CDK4 complex (PubMed:16782892). Shows transcriptional coactivator activity with ATF5 independently of CDK4 (PubMed:15358120).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

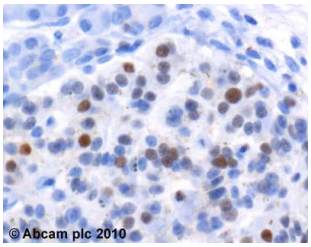
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

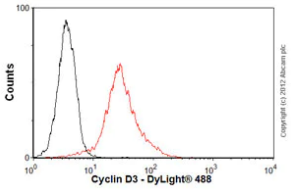
5 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cyclin D3/CCND3 antibody [DCS2.2] (ab28283)

Cyclin D3/CCND3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Cyclin D3/CCND3 antibody

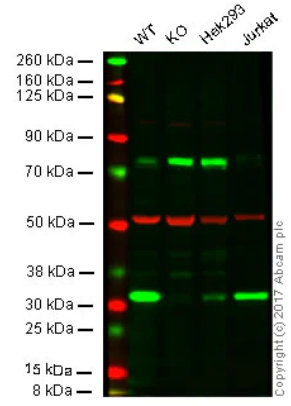
ab28283 (4µg/ml) staining Cyclin D3/CCND3 in human pancreas, using an automated system (DAKO Autostainer Plus). Using this protocol there is strong nuclear staining.
Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer citrate pH6.1 in a DAKO PT link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.



Flow Cytometry - Anti-Cyclin D3/CCND3 antibody [DCS2.2] (ab28283)

Cyclin D3/CCND3 Flow Cytometry staining using mouse Anti-Cyclin D3/CCND3 antibody

Overlay histogram showing HeLa cells stained with ab28283 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab28283, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was a goat anti-mouse DyLight® 488 (IgG; H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Western blot - Anti-Cyclin D3/CCND3 antibody [DCS2.2] (ab28283)

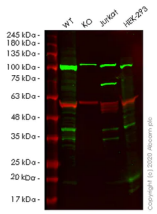
Cyclin D3/CCND3 Western blot staining using mouse Anti-Cyclin D3/CCND3 antibody

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: Cyclin D3/CCND3 (KO) knockout HAP1 whole cell lysate (20 µg)
Lane 3: HEK293 whole cell lysate (20 µg)
Lane 4: Jurkat whole cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab28283 observed at 33 kDa. Red - loading control, ab176560, observed at 50 kDa.
ab28283 was shown to specifically recognize CCND3 in wild-type HAP1 cells along with additional cross reactive bands. No bands was observed when CCND3 knockout samples were uexamined.

Wild-type and CCND3 knockout samples were subjected to SDS-PAGE. ab28283 and ab176560 (Rabbit anti alpha Tubulin loading control) were incubated overnight at 4°C at 1 ug/ml and 1/10,000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed ab216772 and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed ab216777 secondary antibodies at 1/20,000 dilution for 1 hour at room temperature before imaging.
All lanes:
Western blot - Anti-Cyclin D3/CCND3 antibody [DCS2.2] (ab28283)

Predicted band size: 33 kDa

Observed band size: 33 kDa



Western blot - Anti-Cyclin D3/CCND3 antibody [DCS2.2] (ab28283)

Cyclin D3/CCND3 Western blot staining using mouse Anti-Cyclin D3/CCND3 antibody

Lanes 1-4: Merged signal (red and green). Green - ab28283 observed at 35 kDa. Red - loading control ab52901.
ab28283 Anti-Cyclin D3/CCND3 antibody [DCS2.2] was shown to specifically react with Cyclin in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab264931 (knockout cell lysate ab257876) was used. Wild-type and Cyclin knockout samples were subjected to SDS-PAGE. ab28283 and Anti-beta Tubulin [EP1331Y] - Microtubule Marker (ab52901) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Cyclin D3/CCND3 antibody [DCS2.2] (ab28283) at 1/1000 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
CCND3 knockout HeLa cell lysate at 20 µg

Lane 3:
Jurkat cell lysate at 20 µg

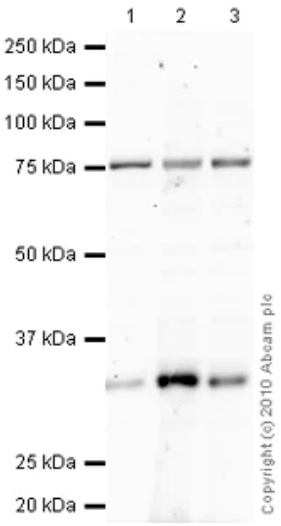
Lane 4:
HEK-293 cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) at 1/10000 dilution

Predicted band size: 33 kDa

Observed band size: 35 kDa



Western blot - Anti-Cyclin D3/CCND3 antibody [DCS2.2] (ab28283)

All lanes:
Western blot - Anti-Cyclin D3/CCND3 antibody [DCS2.2] (ab28283) at 1 µg/mL

Lane 1:
HeLa (Human epithelial carcinoma cell line) Nuclear Lysate at 10 µg

Lane 2:
Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate at 10 µg

Lane 3:
K562 (Human erythromyeloblastoid leukemia cell line) Whole Cell Lysate at 10 µg

Secondary

All lanes:
Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 33 kDa

Observed band size: 35 kDa, 75 kDa

Exposure time: 8min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.