abcam

Product datasheet

# Anti-Cyclin D3/CCND3 antibody [DCS2.2] ab28283

敲除验证

30 References 5 图像

## 概述

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|---|---|
| **产品名称** | Anti-Cyclin D3/CCND3抗体[DCS2.2] |
| **描述** | 小鼠单克隆抗体[DCS2.2] to Cyclin D3/CCND3 |
| **宿主** | Mouse |
| **经测试应用** | **适用于:** Flow Cyt, WB, IHC-P |
| **种属反应性** | **与反应:** Human<br>**预测可用于:** Mouse, Rat |
| **免疫原** | Recombinant full length protein corresponding to Human Cyclin D3/CCND3.<br>Sequence:<br>MELLCCEGTRHAPRAGPDPRLLGDQRVLQSLLRLEERYVPRA<br>SYFQCVQR EIKPHMRKML<br>AYWMLEVCEEQRCEEEVFPLAMNYLDRYLSCVPTRKAQ<br>LQLLGAVCMLLASKLRETTPLT<br>IEKLCIYTDHAVSPRQLRDWEVLVLG<br>KLKWDLAAVIAHDFLAFILHRLSLPRDRQALVKK<br>HAQTFLALCATDYT<br>FAMYPPSMIATGSIGAAVQGLGACSMSGDELTELLAGITGTE<br>VDCL RA<br>CQEQIEAALRESLREASQTSSSPAPKAPRGSSSQGPSQTSTP<br>TDVTAIHL<br><br>Database link: P30281<br>Run BLAST with Run BLAST with |
| **阳性对照** | WB: HeLa, K562, HAP1, HEK-293T and Jurkat cell lysates. Flow Cyt: HeLa cells. IHC-P: Human pancreas tissue. |
| **常规说明** | The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.<br>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As |

## 性能

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|---|---|
| **形式** | Liquid |
| **存放说明** | Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles. |
| **存储溶液** | Preservative: 0.08% Sodium azide<br>Constituent: PBS |
| **纯度** | Protein A/G purified |
| **克隆** | 单克隆 |
| **克隆编号** | DCS2.2 |
| **同种型** | IgG1 |

## 应用

**The Abpromise guarantee** **Abpromise™承诺保证使用ab28283于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

| 应用 | Ab评论 | 说明 |
|---|---|---|
| **Flow Cyt** | | Use 1µg for $10^6$ cells.<br>**ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody. |
| **WB** | | Use at an assay dependent concentration. Predicted molecular weight: 35 kDa. |
| **IHC-P** | | Use at an assay dependent concentration. |

## 靶标

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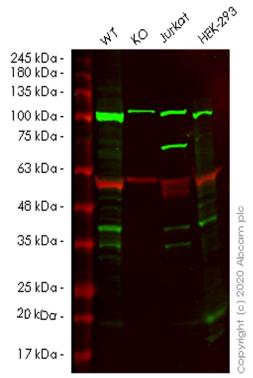
|---|---|
| **功能** | Regulatory component of the cyclin D3-CDK4 (DC) complex that phosphorylates and inhibits members of the retinoblastoma (RB) protein family including RB1 and regulates the cell-cycle during G(1)/S transition. Phosphorylation of RB1 allows dissociation of the transcription factor E2F from the RB/E2F complex and the subsequent transcription of E2F target genes which are responsible for the progression through the G(1) phase. Hypophosphorylates RB1 in early G(1) phase. Cyclin D-CDK4 complexes are major integrators of various mitogenenic and antimitogenic signals. Also substrate for SMAD3, phosphorylating SMAD3 in a cell-cycle-dependent manner and repressing its transcriptional activity. Component of the ternary complex, cyclin D3/CDK4/CDKN1B, required for nuclear translocation and activity of the cyclin D-CDK4 complex. |
| **序列相似性** | Belongs to the cyclin family. Cyclin D subfamily.<br>Contains 1 cyclin N-terminal domain. |
| **细胞定位** | Nucleus. Cytoplasm. Membrane. Cyclin D-CDK4 complexes accumulate at the nuclear membrane and are then translocated to the nucleus through interaction with KIP/CIP family members. |

## 图片




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

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

**Lane 1 :** Wild-type HeLa cell lysate
**Lane 2 :** CCND3 knockout HeLa cell lysate
**Lane 3 :** Jurkat cell lysate
**Lane 4 :** HEK-293 cell lysate

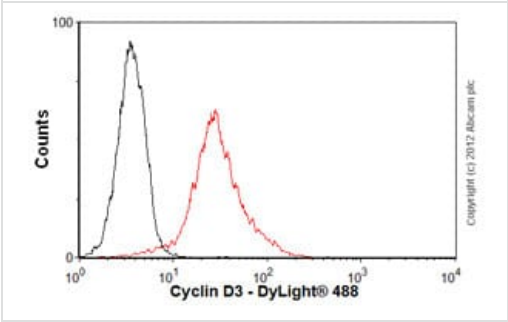
Lysates/proteins at 20 µg per lane.

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

**Predicted band size:** 35 kDa
**Observed band size:** 35 kDa

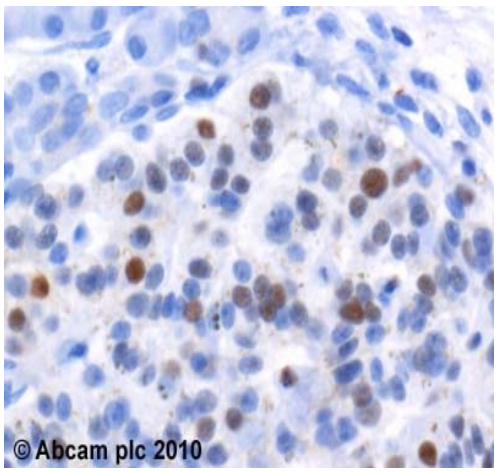
**Lanes 1-4:** Merged signal (red and green). Green - ab28283 observed at 35 kDa. Red - loading control **ab52901**.

ab28283 Anti-Cyclin D3/CCND3 antibody [DCS2.2] was shown to specifically react with Cyclin D3 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 D3 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.



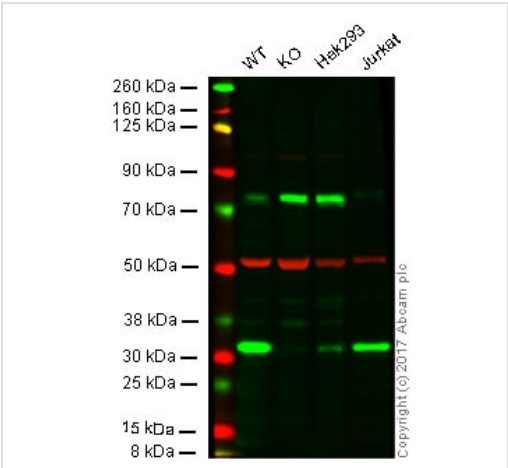

Flow Cytometry - Anti-Cyclin D3/CCND3 antibody [DCS2.2] (ab28283)

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/1x$10^6$ cells) for 30 min at 22ºC. The secondary antibody used was a goat **anti-mouse DyLight® 488** (IgG; H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x$10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed.




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

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.

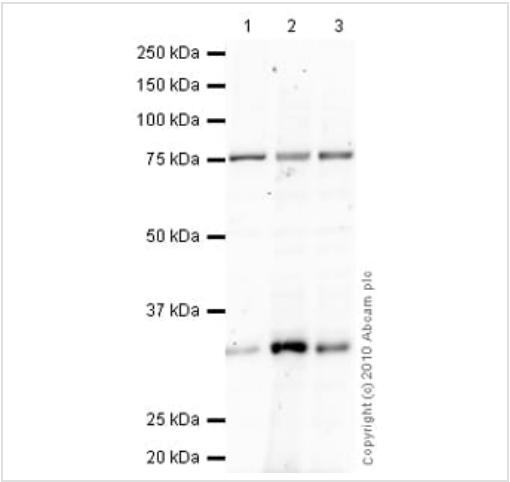



Western blot - Anti-Cyclin D3/CCND3 antibody [DCS2.2] (ab28283)

**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.




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

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

**Lane 1 :** HeLa (Human epithelial carcinoma cell line) Nuclear Lysate
**Lane 2 :** Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
**Lane 3 :** K562 (Human erythromyeloblastoid leukemia cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

**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:** 35 kDa
**Observed band size:** 35 kDa
**Additional bands at:** 75 kDa. We are unsure as to the identity of these extra bands.

**Exposure time:** 8 minutes



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