abcam

Product datasheet

Anti-Cdc34 antibody [EPR16808] ab204515

Recombinant RabMAb

10 Images

Overview

Product name	Anti-Cdc34 antibody [EPR16808]
Description	Rabbit monoclonal [EPR16808] to Cdc34
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, IP, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Recombinant protein fragment human Cdc34; 293, K562, Jurkat, C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates; Mouse brain, heart and kidney lysates; Rat heart lysate. ICC/IF: Neuro-2a and HeLa cells. Flow Cyt (intra): Jurkat and HeLa cells. IP: K562 whole cell lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16808

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab204515 in the following tested applications.

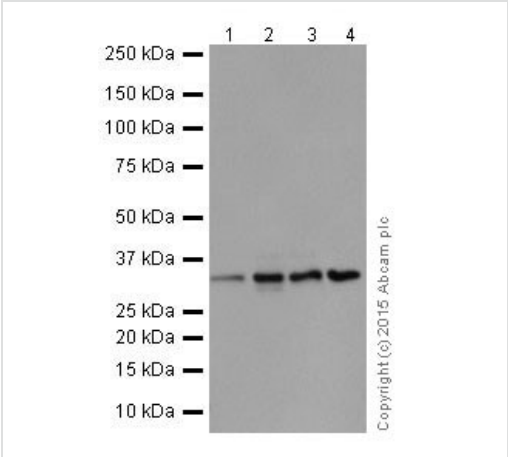
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/100.
ICC/IF		1/50.
IP		1/30.
WB		1/1000. Detects a band of approximately 35 kDa (predicted molecular weight: 26 kDa).

Target

Function	Accepts ubiquitin from the E1 complex and catalyzes its covalent attachment to other proteins. In vitro catalyzes 'Lys-48'-linked polyubiquitination. Cooperates with the E2 UBCH5C and the SCF(FBXW11) E3 ligase complex for the polyubiquitination of NFKBIA leading to its subsequent proteasomal degradation. Performs ubiquitin chain elongation building ubiquitin chains from the UBE2D3-primed NFKBIA-linked ubiquitin. UBE2D3 acts as an initiator E2, priming the phosphorylated NFKBIA target at positions 'Lys-21' and/or 'Lys-22' with a monoubiquitin. Cooperates with the SCF(SKP2) E3 ligase complex to regulate cell proliferation through ubiquitination and degradation of MYBL2 and KIP1. Involved in ubiquitin conjugation and degradation of CREM isoform ICERIIgamma and ATF15 resulting in abrogation of ICERIIgamma- and ATF5-mediated repression of cAMP-induced transcription during both meiotic and mitotic cell cycles. Involved in the regulation of the cell cycle G2/M phase throught its targeting of the WEE1 kinase for ubiquitination and degradation. Also involved in the degradation of beta-catenin. Is target of human herpes virus 1 protein ICP0, leading to ICP0-dependent dynamic interaction with proteasomes.
Tissue specificity	Expressed in testes during spermatogenesis to regulate repression of cAMP-induced transcription.
Pathway	Protein modification; protein ubiquitination.
Sequence similarities	Belongs to the ubiquitin-conjugating enzyme family.
Domain	The C-terminal acidic tail is required for nuclear localization and is involved in the binding to SCF E3 ligase complexes, and more specifically with the CUL1 subunit.
Post-translational modifications	Autoubiquitinated. Autoubiquitination is promoted by the human herpes virus 1 protein ICP0 and leads to degradation by the Ubiquitin-proteasomal pathway. Phosphorylated by CK2. Phosphorylation of the C-terminal tail by CK2 controles the nuclear localization.
Cellular localization	Cytoplasm. Nucleus. The phosphorylation of the C-terminal tail plays an important role in mediating nuclear localization. Colocalizes with beta-tubulin on mitotic spindles in anaphase.

Images



Western blot - Anti-Cdc34 antibody [EPR16808] (ab204515)

All lanes : Anti-Cdc34 antibody [EPR16808] (ab204515) at 1/5000 dilution

Lane 1 : C6 (Rat glial tumor cells) whole cell lysate
Lane 2 : RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysate
Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate
Lane 4 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

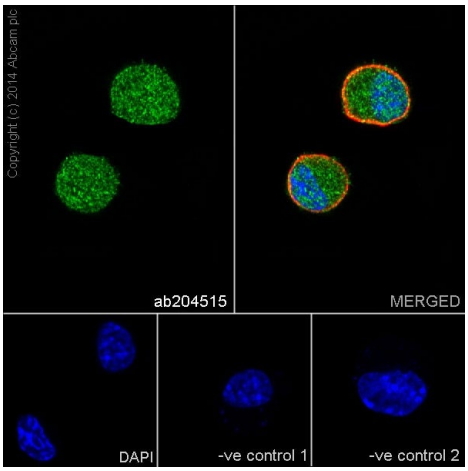
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 26 kDa
Observed band size: 35 kDa

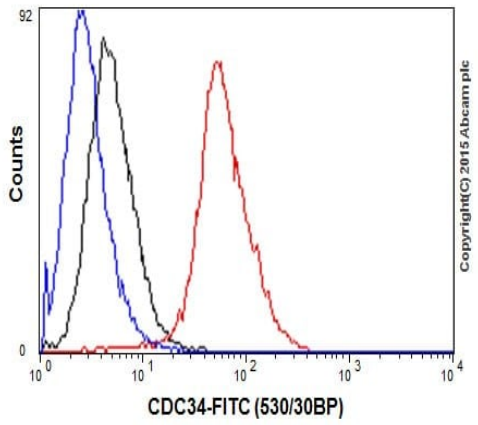
Exposure time: 2 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



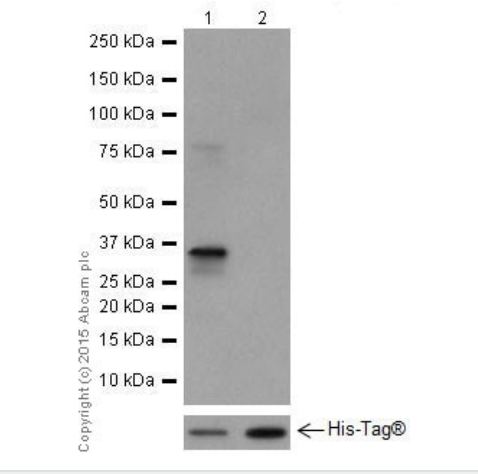
Immunocytochemistry/ Immunofluorescence - Anti-Cdc34 antibody [EPR16808] (ab204515)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Neuro-2a (Mouse neuroblastoma cells) cells labeling Cdc34 with ab204515 at 1/50 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and cytoplasmic staining on Neuro-2a cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).
The negative controls are as follows:
-ve control 1: ab204515 at 1/50 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Cdc34 antibody [EPR16808] (ab204515)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling Cdc34 with ab204515 at 1/100 dilution (red) compared with a rabbit monoclonal IgG isotype control (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.



Western blot - Anti-Cdc34 antibody [EPR16808] (ab204515)

All lanes : Anti-Cdc34 antibody [EPR16808] (ab204515) at 1/5000 dilution

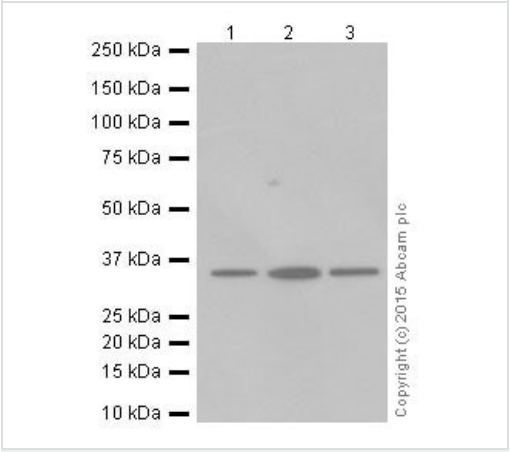
Lane 1 : Recombinant protein fragment human Cdc34
Lane 2 : Recombinant protein fragment human Cdc34B

Lysates/proteins at 0.01 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 26 kDa
Observed band size: 35 kDa

Exposure time: 1 second



Western blot - Anti-Cdc34 antibody [EPR16808] (ab204515)

All lanes : Anti-BTK (phospho Y223) antibody [EP420Y] (Alexa Fluor® 488) (**ab204516**) at 1/5000 dilution

Lane 1 : 293 (Human epithelial cells from embryonic kidney) whole cell lysate
Lane 2 : K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate
Lane 3 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate

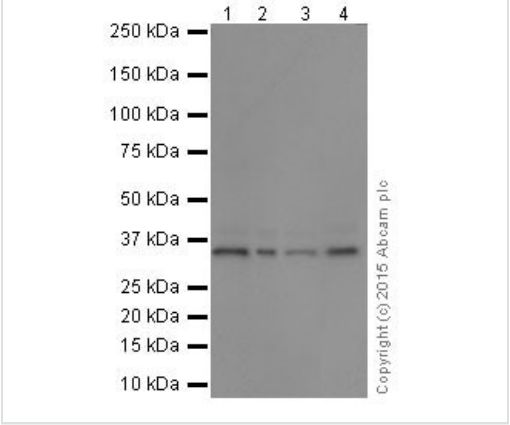
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/100000 dilution

Predicted band size: 26 kDa
Observed band size: 35 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Cdc34 antibody [EPR16808] (ab204515)

All lanes : Anti-Cdc34 antibody [EPR16808] (ab204515) at 1/1000 dilution

Lane 1 : Mouse brain tissue lysate
Lane 2 : Mouse heart tissue lysate
Lane 3 : Mouse kidney tissue lysate
Lane 4 : Rat heart tissue lysate

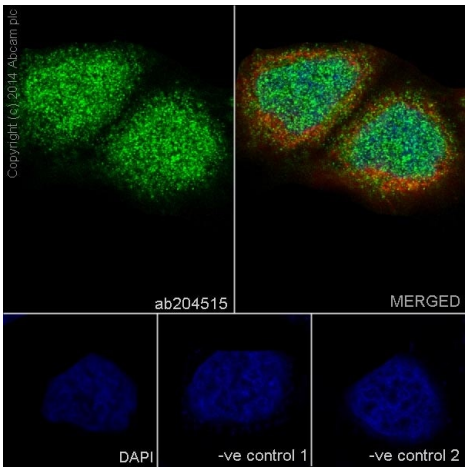
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 26 kDa
Observed band size: 35 kDa

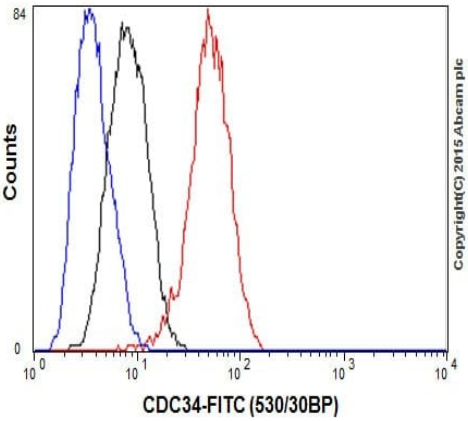
Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



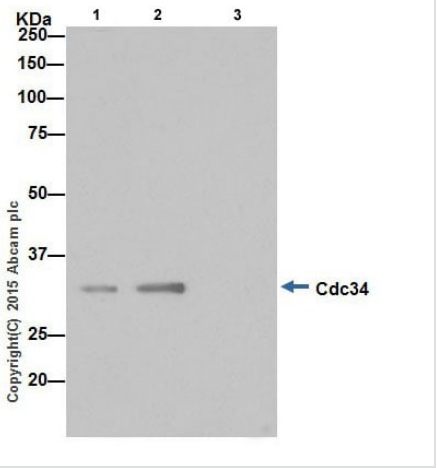
Immunocytochemistry/ Immunofluorescence - Anti-Cdc34 antibody [EPR16808] (ab204515)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Cdc34 with ab204515 at 1/50 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and cytoplasmic staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).
The negative controls are as follows:
-ve control 1: ab204515 at 1/50 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Cdc34 antibody [EPR16808] (ab204515)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Cdc34 with ab204515 at 1/100 dilution (red) compared with a rabbit monoclonal IgG isotype control (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Cdc34 antibody [EPR16808] (ab204515)

Cdc34 was immunoprecipitated from 1mg of K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate with ab204515 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab204515 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

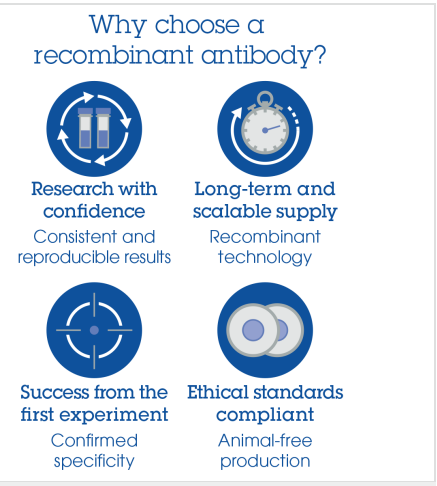
Lane 1: K562 whole cell lysate 10ug (Input).

Lane 2: ab204515 IP in K562 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab204515 in K562 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 5 seconds.



Anti-Cdc34 antibody [EPR16808] (ab204515)

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