# abcam

# Product datasheet

# Anti-Chk1 antibody [E250] ab32531



重组 RabMAb

★★★★★ 4 Abreviews 11 References 7 图像

概述

产品名称 Anti-Chk1抗体[E250]

描述 兔单克隆抗体[E250] to Chk1

宿主 Rabbit

经测试应用 适用于: Flow Cyt (Intra), IP, ICC/IF, WB

不适用干: IHC

种属反应性 与反应: Mouse. Rat. Human

免疫原 Synthetic peptide within Human Chk1 aa 1-100 (N terminal). The exact sequence is proprietary.

阳性对照 IP: K-562 whole cell lysate. ICC/IF: HeLa cells. Flow Cyt (intra): K-562 cells. WB: K-562, Hela,

NIH/3T3, MEF and PC-12 whole cell lysates

常规说明 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**.

性能

形式 Liquid

存放说明 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.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯度 Protein A purified

克隆 单克隆 克隆编号 E250

### 应用

The Abpromise guarantee Abpromise™承诺保证使用ab32531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。

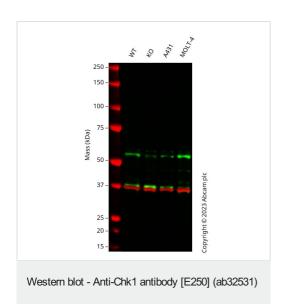
应 <b>用</b>	Ab评论	说明
Flow Cyt (Intra)		1/30.
IP		1/20.
ICC/IF	<b>★★★☆☆</b> (2)	1/50.
WB	**** <u>(2)</u>	1/1000. Detects a band of approximately 54 kDa (predicted molecular weight: 54 kDa).

应用说明

Is unsuitable for IHC.

#### 靶标

#### 图片



All lanes: Anti-Chk1 antibody [E250] (ab32531) at 1/1000 dilution

Lane 1: Wild-type A549 cell lysate

Lane 2: CHEK1 knockout A549 cell lysate

Lane 3 : A431 cell lysate
Lane 4 : MOLT-4 cell lysate

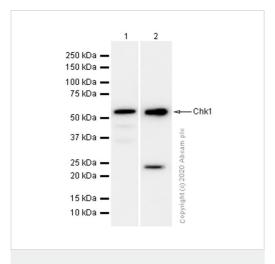
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 54 kDa **Observed band size:** 57 kDa

Anti-CHEK1 antibody [E250] (ab32531) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32531 was shown to bind specifically to CHEK1. A

band was observed at 57 kDa in wild-type A549 cell lysates with a reduction in signal observed at this size in CHEK1 heterozygous knockout cell line. To generate this image, wild-type and CHEK1 heterozygous knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween<sup>®</sup> 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit lgG H&L 800CW and Goat anti-Mouse lgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-Chk1 antibody [E250] (ab32531)

**All lanes :** Anti-Chk1 antibody [E250] (ab32531) at 1/10000 dilution (Purified)

**Lane 1 :** MEF (Mouse embryonic fibroblast (immortalized)) whole cell lysate

Lane 2: PC-12 (Rat adrenal gland pheochromocytoma ) whole cell lysate

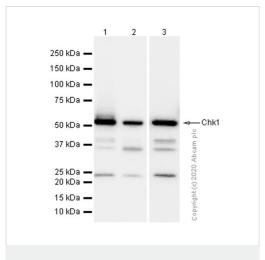
Lysates/proteins at 15 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

**Predicted band size:** 54 kDa **Observed band size:** 54 kDa

We are unsure how to define the extra bands.



Western blot - Anti-Chk1 antibody [E250] (ab32531)

All lanes: Purfiied at 1/1000 dilution

**Lane 1**: K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate

**Lane 2 :** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 3: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

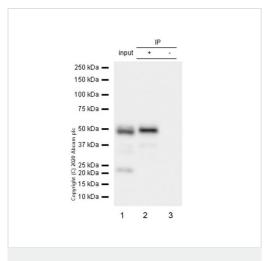
Lysates/proteins at 20 µg per lane.

# Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/20000 dilution

**Predicted band size:** 54 kDa **Observed band size:** 54 kDa

We are unsure how to define the extra bands.



Immunoprecipitation - Anti-Chk1 antibody [E250] (ab32531)

Purified ab32531 at 1/20 dilution (1 $\mu$ g) immunoprecipitating Chk1 in K-562 whole cell lysate.

Lane 1 (input): K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate 10µg

Lane 2 (+): ab32531 + K-562 whole cell lysate.

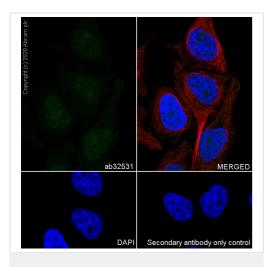
Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab32531 in K-562 whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

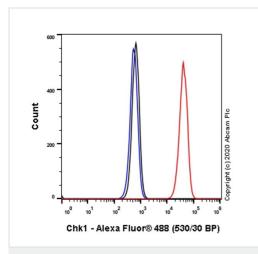
Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 54 kDa



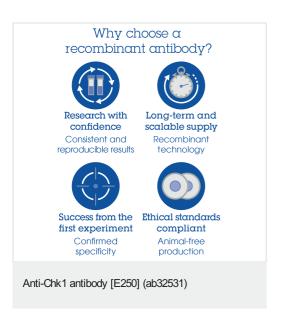
Immunocytochemistry/ Immunofluorescence - Anti-Chk1 antibody [E250] (ab32531)

Immunocytochemistry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Chk1 with purified ab32531 at 1/50 dilution (5.06 μg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 μg/mL). Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 μg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-Chk1 antibody [E250] (ab32531)

Intracellular Flow Cytometry analysis of K-562 (Human chronic myelogenous leukemia lymphoblast) cells labeling Chk1 with purified ab32531 at 1/30 dilution (10  $\mu$ g/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



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