# abcam

## Product datasheet

## Anti-CDK1 (phospho Y15) + CDK2 (phospho Y15) + CDK3 (phospho Y15) + CDK5 (phospho Y15) antibody [EPR ab133463

Recombinant RabMAb

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Overview

**Product name** Anti-CDK1 (phospho Y15) + CDK2 (phospho Y15) + CDK3 (phospho Y15) + CDK5 (phospho

Y15) antibody [EPR

**Description** Rabbit monoclonal [EPR7875] to CDK1 (phospho Y15) + CDK2 (phospho Y15) + CDK3

(phospho Y15) + CDK5 (phospho Y15)

**Host species** Rabbit

Specificity The antibody will cross-react with phosphorylated CDK1 (pY15), CDK2 (pY15), CDK3 (pY15) and

CDK5 (pY15) but not with non-phosphorylated CDK1, CDK2, CDK3 and CDK5. Please see our

ELISA results on the images section.

**Tested applications** Suitable for: WB, IHC-P, ELISA, Dot blot

Species reactivity Reacts with: Mouse, Rat, Human, Recombinant fragment

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control HeLa cell lysate treated with UV, human colon, C6 cell lysate, NIH/3T3 cell lysate, Saso2 cells,

human breast carcinoma tissue

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.

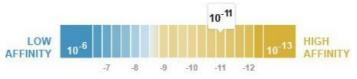
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

**Properties** 

**Form** 

Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C. Storage instructions

 $K_D = 4.10 \times 10^{-11} M$ Dissociation constant (K<sub>D</sub>)



#### Learn more about K<sub>D</sub>

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

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

Purity Protein A purified

ClonalityMonoclonalClone numberEPR7875

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab133463 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
WB	****(1)	1/1000 - 1/2000. Detects a band of approximately 34 kDa (predicted molecular weight: 34 kDa).
IHC-P	****(1)	1/50 - 1/75. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ELISA		Use at an assay dependent concentration.
Dot blot		1/1000.

## **Target**

Cellular localization CDK1: Nucleus. CDK5: Cytoplasm. Cell projection > lamellipodium. Cell projection > growth

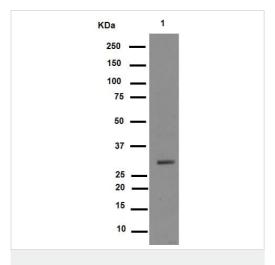
cone. In axonal growth cone with extension to the peripheral lamellipodia.

Form CDK1: CDK1 can be located to the Nucleus, cytoplasm and Mithocondria. It's cytoplasmic during

interphase and reversibly translocated from cytoplasm to the nucleus when phosphorilated before G2-M transition when associated with cyclin-B1. Accumulates in mitochondria in G2-arrested cells

upon DNA-damage.

## Images



Western blot - Anti-CDK1+CDK2+CDK3+CDK5 (phospho Y15) antibody [EPR7875] (ab133463)

Anti-CDK1 (phospho Y15) + CDK2 (phospho Y15) + CDK3 (phospho Y15) + CDK5 (phospho Y15) antibody [EPR (ab133463) at 1/1000 dilution (purified) + HeLa cell lysate treated with UV at 10  $\mu g$ 

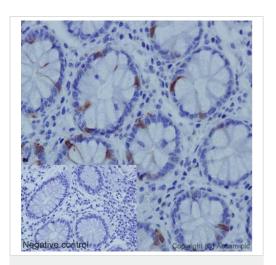
#### Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Predicted band size:** 34 kDa **Observed band size:** 34 kDa

Blocking buffer: 5% NFDM/TBST

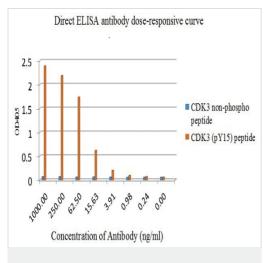
Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-

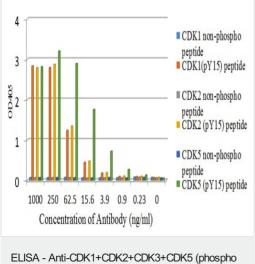
CDK1+CDK2+CDK3+CDK5 (phospho Y15) antibody [EPR7875] (ab133463)

Immunohistochemical staining of paraffin embedded human colon with purified ab133463 at a working dilution of 1 in 75. The secondary antibody used is a HRP polymer for rabbit lgG. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



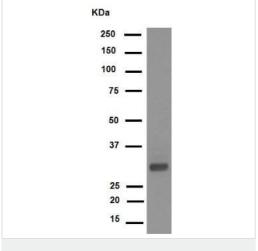
Direct ELISA antibody dose-response curve using purified ab133463 at 0-1000 ng/ml. Antigen concentration of 1000 ng/mL. An alkaline phosphatase-conjugated goat anti-rabbit lgG (H+L) (1/2500) was used as the secondary antibody.





Y15) antibody [EPR7875] (ab133463)

Direct ELISA antibody dose-response curve using purified ab133463 at 0-1000 ng/ml. Antigen concentration of 1000 ng/mL. An alkaline phosphatase-conjugated goat anti-rabbit lgG (H+L) (1/2500) was used as the secondary antibody.



Anti-CDK1 (phospho Y15) + CDK2 (phospho Y15) + CDK3 (phospho Y15) + CDK5 (phospho Y15) antibody [EPR (ab133463) at 1/2000 dilution (purified) + C6 cell lysate at  $10 \mu g$ 

#### Secondary

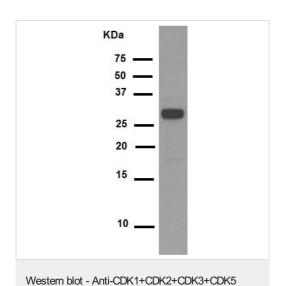
HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Predicted band size:** 34 kDa **Observed band size:** 34 kDa

Western blot - Anti-CDK1+CDK2+CDK3+CDK5 (phospho Y15) antibody [EPR7875] (ab133463)

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



(phospho Y15) antibody [EPR7875] (ab133463)

Anti-CDK1 (phospho Y15) + CDK2 (phospho Y15) + CDK3 (phospho Y15) + CDK5 (phospho Y15) antibody [EPR (ab133463) at 1/2000 dilution (purified) + NIH/3T3 cell lysate at  $10 \mu g$ 

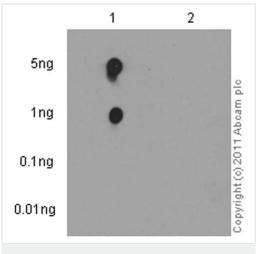
#### Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Predicted band size:** 34 kDa **Observed band size:** 34 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

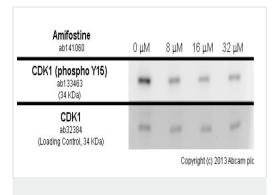


Dot Blot - Anti-CDK1+CDK2+CDK3+CDK5 (phospho Y15) antibody [EPR7875] (ab133463)

Dot blot analysis of CDK1+CDK2+CDK3+CDK5 (pY15) phospho peptide (lane 1) and CDK1+CDK2+CDK3+CDK5 non-phospho peptide (lane 2) labelling CDK1+CDK2+CDK3+CDK5 (phospho Y15) with unpurified ab133463 at a dilution of 1/1000. A peroxidase-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/2500).

Blocking and dilution buffer: 5% NFDM/TBST.

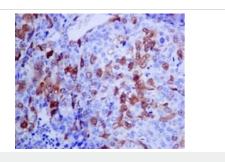
Exposure time: 10 seconds.



Western blot - Anti-CDK1+CDK2+CDK3+CDK5 (phospho Y15) antibody [EPR7875] (ab133463)

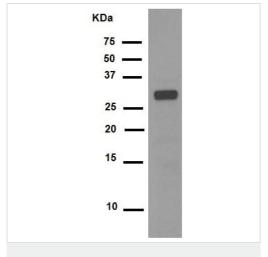
Saso2 cells were incubated at  $37^{\circ}$ C for 24 hours with vehicle control (0  $\mu$ M) and different concentrations of Amifostine (<u>ab141060</u>). Decreased expression of CDK1 (phospho Y15) (unpurified ab133463) in Saso2 cells correlates with an increase in Amifostine concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 10 $\mu$ g of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with unpurified ab133463 at 1  $\mu$ g/ml and ab32384 at 1  $\mu$ g/ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (ab97051) at 1/10000 and visualised using ECL development solution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CDK1+CDK2+CDK3+CDK5 (phospho Y15) antibody [EPR7875] (ab133463)

Immunohistochemical analysis of paraffin embedded human breast carcinoma tissue labelling CDK1+CDK2+CDK3+CDK5 with unpurified ab133463 at 1/50 dilution. Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-CDK1+CDK2+CDK3+CDK5 (phospho Y15) antibody [EPR7875] (ab133463)

Anti-CDK1 (phospho Y15) + CDK2 (phospho Y15) + CDK3 (phospho Y15) + CDK5 (phospho Y15) antibody [EPR (ab133463) at 1/1500 dilution (unpurified) + NIH/3T3 cell lysate at 1/1000 dilution

#### **Secondary**

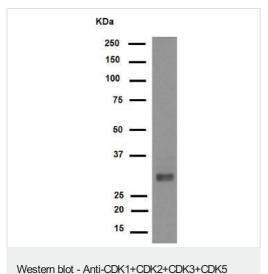
HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Predicted band size:** 34 kDa **Observed band size:** 34 kDa



Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



(phospho Y15) antibody [EPR7875] (ab133463)

Anti-CDK1 (phospho Y15) + CDK2 (phospho Y15) + CDK3 (phospho Y15) + CDK5 (phospho Y15) antibody [EPR (ab133463) at 1/1500 dilution (unpurified) + C6 cell lysate at 10 µg

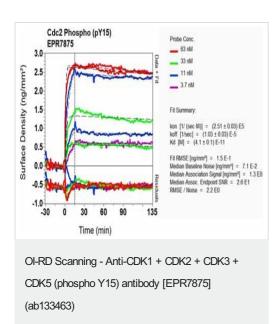
#### Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Predicted band size:** 34 kDa **Observed band size:** 34 kDa

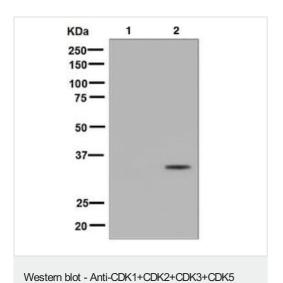
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Equilibrium disassociation constant ( $K_D$ ) Learn more about  $K_D$ 

#### Click here to learn more about K<sub>D</sub>



(phospho Y15) antibody [EPR7875] (ab133463)

**All lanes :** Anti-CDK1 (phospho Y15) + CDK2 (phospho Y15) + CDK3 (phospho Y15) + CDK5 (phospho Y15) antibody [EPR (ab133463) at 1/1000 dilution (unpurified)

Lane 1: HeLa cell lysate

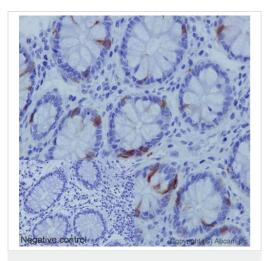
Lane 2: HeLa cell lysate treated with UV

Lysates/proteins at 10 µg per lane.

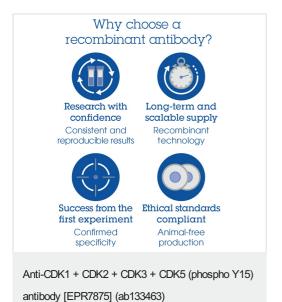
#### **Secondary**

All lanes: HRP labelled goat anti-rabbit lgG at 1/2000 dilution

**Predicted band size:** 34 kDa **Observed band size:** 34 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CDK1+CDK2+CDK3+CDK5 (phospho Y15) antibody [EPR7875] (ab133463) Immunohistochemical staining of paraffin embedded human colon with unpurified ab133463 at a working dilution of 1 in 50. The secondary antibody used is a HRP polymer for rabbit lgG. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



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