

## **Product datasheet**

# Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] ab201008

Recombinant RabMAb

10 References 13 Images

Overview		
Product name	Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546]	
Description	Rabbit monoclonal [EPR19546] to CDK1 (phospho T161) + CDK2 (phospho T160) + CDK3 (phospho T160)	
Host species	Rabbit	
Specificity	ab201008 also recognizes CDK2 (phospho T160) and CDK3 (phospho T160).	
Tested applications	Suitable for: WB, Dot blot, IHC-P, IP, ICC/IF	
Species reactivity	Reacts with: Mouse, Rat, Human	
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.	
Positive control	WB: HeLa treated with UV for 90 minutes, HeLa treated with UV for 90 minutes then with alkaline phosphatase for 1 hour, C6, C6 treated with alkaline phosphatase for 1 hour and NIH/3T3 whole cell lysate. IHC-P: Human tonsil and cervix cancer tissues; Rat testis tissue. ICC/IF: HeLa cells. IP: HeLa treated with 25J/m2 UV for 1-hour 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 <u>see here</u> . Our RabMAb <sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u> .	

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

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19546
Isotype	lgG

### Applications

The Abpromise guarantee

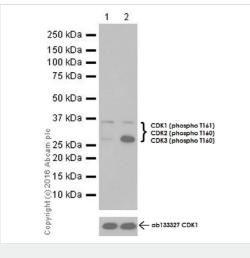
Our **Abpromise guarantee** covers the use of ab201008 in the following tested applications.

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

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 34, 28 kDa (predicted molecular weight: 34 kDa).
Dot blot		1/1000.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Recommended for human and rat only.
IP		1/30.
ICC/IF		1/100.

Target	
Cellular localization	CDK1: Nucleus.
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 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) All lanes : Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) at 1/1000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysateLane 2 : HeLa treated with UV for 90 minutes whole cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

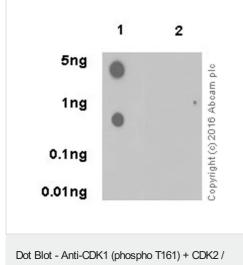
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 34 kDa Observed band size: 28,34 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



CDK3 (phospho T160) antibody [EPR19546] (ab201008)



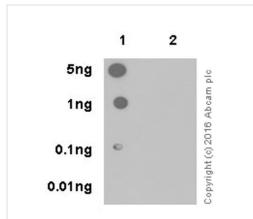
Lane 1: CDK1 (phospho T161) phospho peptide.

Lane 2: CDK1 non-phospho peptide.

Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Dot Blot - Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) Dot blot analysis of CDK2 (phospho T160) labeled with ab201008 at 1/1000 dilution.

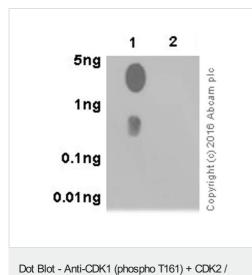
Lane 1: CDK2 (phospho T160) phospho peptide.

Lane 2: CDK2 non-phospho peptide.

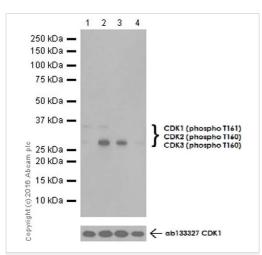
Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



CDK3 (phospho T160) antibody [EPR19546] (ab201008)



Western blot - Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) Dot blot analysis of CDK3 (phospho T160) labeled with ab201008 at 1/1000 dilution.

Lane 1: CDK3 (phospho T160) phospho peptide.

Lane 2: CDK3 non-phospho peptide.

Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

All lanes : Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) at 1/1000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : HeLa treated with UV for 90 minutes whole cell lysate Lane 3 : HeLa treated with UV for 90 minutes then with alkaline phosphatase for 1-hour whole cell lysate

Lane 4 : HeLa treated with UV for 90 minutes then with alkaline phosphatase overnight whole cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

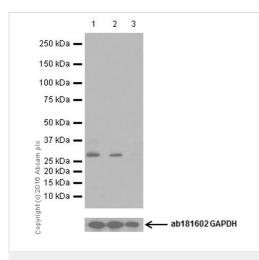
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 34 kDa Observed band size: 28,34 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) All lanes : Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) at 1/1000 dilution

Lane 1 : C6 (rat glial tumor cell line) whole cell lysateLane 2 : C6 treated with alkaline phosphatase for 1 hour whole cell lysate

Lane 3 : C6 treated with alkaline phosphatase overnight whole cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 34 kDa Observed band size: 28,34 kDa

Exposure time: 10 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

**All lanes :** Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) at 1/1000 dilution

Lane 1 : NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate

Lane 2: C6 (rat glial tumor cell line) whole cell lysate

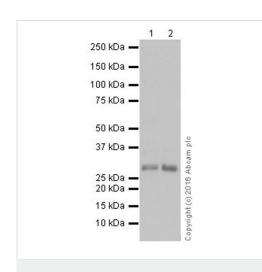
Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

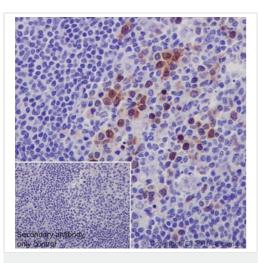
Developed using the ECL technique.

Predicted band size: 34 kDa



Western blot - Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) Exposure time: 3 seconds

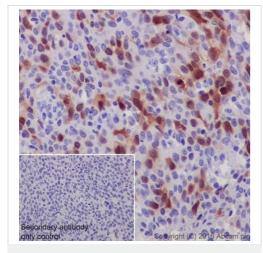
Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CDK1 (phospho T161) with ab201008 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nucleus and weak cytoplasm staining of germinal center from human tonsil is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

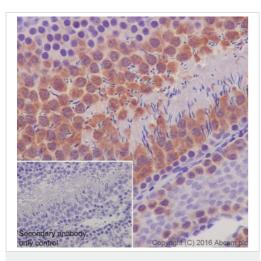
Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) Immunohistochemical analysis of paraffin-embedded human cervix cancer tissue labeling CDK1 (phospho T161) with ab201008 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nucleus and cytoplasm staining of cancer cells from human cervix cancer is observed [PMID: 15623629]. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

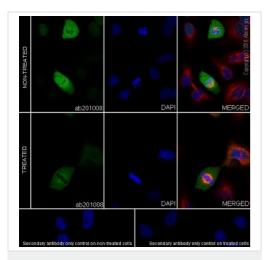


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008)

Immunohistochemical analysis of paraffin-embedded rat testis tissue labeling CDK1 (phospho T161) with ab201008 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nucleus and cytoplasm staining of rat testis is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

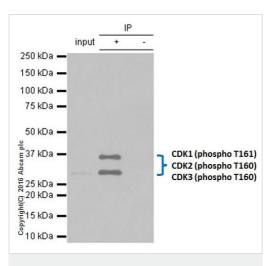
Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



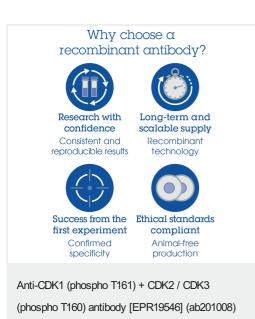
Immunocytochemistry/ Immunofluorescence - Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008) Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling CDK1 (phospho T161) with ab201008 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing specific signal in M phase cells. The signal decreased after treatment with lambda protein phosphatase 31°C for 5 hours.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-Alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (<u>ab195889</u>) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



Immunoprecipitation - Anti-CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160) antibody [EPR19546] (ab201008)



CDK1 (phospho T161) was immunoprecipitated from 0.35mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab201008 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab201008 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: HeLa treated with 25J/m2 UV for 1 hour whole cell lysate 10 µg (Input).

Lane 2: ab201008 IP in HeLa treated with 25J/m2 UV for 1 hour whole cell lysate.

Lane 3: Rabbit monoclonal lgG ( $\underline{ab172730}$ ) instead of ab201008 in HeLa treated with 25J/m2 UV for 1 hour whole cell lysate.

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

Exposure time: 3 minutes.

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