# abcam

# Product datasheet

# Anti-GRK2 antibody [EPR22465] ab227825





# 6 Images

#### Overview

**Product name** Anti-GRK2 antibody [EPR22465]

**Description** Rabbit monoclonal [EPR22465] to GRK2

**Host species** Rabbit

Suitable for: WB, IP **Tested applications** 

Unsuitable for: ICC/IF or IHC-P

Reacts with: Human Species reactivity

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

Positive control WB: HeLa, HEK-293 and HepG2 whole cell lysates; Wild-type HAP1 whole cell lysate; Human

skeletal muscle lysate. IP: HeLa and HEK-293T whole cell lysates.

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

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long Storage instructions

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 EPR22465

### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab227825 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. Predicted molecular weight: 80 kDa.
IP		1/30.

#### **Application notes**

Is unsuitable for ICC/IF or IHC-P.

#### **Target**

<b>Function</b> Sp	pecifically phosphorylates the agonist-occupied for	rm of the beta-adrenergic and closely related
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receptors, probably inducing a desensitization of them. Key regulator of LPAR1 signaling.

Competes with RALA for binding to LPAR1 thus affecting the signaling properties of the receptor.

Desensitizes LPAR1 and LPAR2 in a phosphorylation-independent manner.

Tissue specificity Expressed in peripheral blood leukocytes.

Sequence similarities Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. GPRK subfamily.

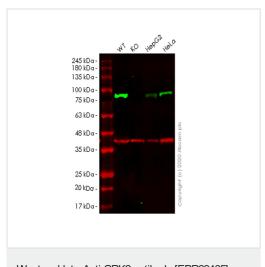
Contains 1 AGC-kinase C-terminal domain.

Contains 1 PH domain.

Contains 1 protein kinase domain.

Contains 1 RGS domain.

#### **Images**



Western blot - Anti-GRK2 antibody [EPR22465]

(ab227825)

All lanes: Anti-GRK2 antibody [EPR22465] (ab227825) at 1/1000

dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: ADRBK1 knockout HEK293T cell lysate

Lane 3: HepG2 cell lysate Lane 4: HeLa cell lysate

Lysates/proteins at 20 µg per lane.

## Secondary

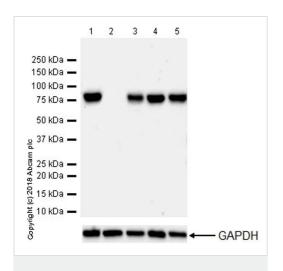
All lanes: Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 80 kDa

Observed band size: 80 kDa

**Lanes 1-4:** Merged signal (red and green). Green - ab227825 observed at 80 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab227825 Anti-GRK2 antibody [EPR22465] was shown to specifically react with GRK2 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line <a href="mailto:ab266352">ab266352</a> (knockout cell lysate <a href="mailto:ab257345">ab257345</a>) was used. Wild-type and GRK2 knockout samples were subjected to SDS-PAGE. ab227825 and Anti-GAPDH antibody [6C5] - Loading Control (<a href="mailto:ab8245">ab8227825</a> and Anti-GAPDH antibody [6C5] - Loading Control (<a href="mailto:ab8245">ab82478</a>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-GRK2 antibody [EPR22465] (ab227825)

**All lanes :** Anti-GRK2 antibody [EPR22465] (ab227825) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: GRK2 knockout HAP1 whole cell lysate

**Lane 3**: HEK-293 (human epithelial cell line from embryonic kidney) whole cell lysate

**Lane 4**: HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 5**: HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

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

Predicted band size: 80 kDa

Blocking/Dilution buffer: NFDM/TBST.

ab227825 was shown to specifically react with GRK2 in wild-type

HAP1 cells as signal was lost in GRK2 knockout cells. Wild-type and GRK2 knockout samples were subjected to SDS-PAGE. ab227825 and <u>ab181602</u> (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RAD<sup>®</sup> ChemiDoc™ MP instrument using the ECL technique.

250 kDa —
150 kDa —
150 kDa —
75 kDa —
37 kDa —
37 kDa —
25 kDa —
25 kDa —
15 kDa —
15 kDa —
16 kDa —

Western blot - Anti-GRK2 antibody [EPR22465]

(ab227825)

Anti-GRK2 antibody [EPR22465] (ab227825) at 1/1000 dilution + Human skeletal muscle lysate at 20 µg

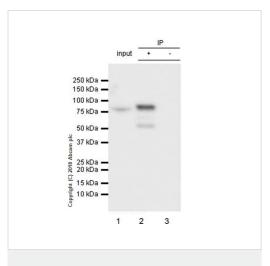
#### Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

Predicted band size: 80 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunoprecipitation - Anti-GRK2 antibody [EPR22465] (ab227825)

GRK2 was immunoprecipitated from 0.35 mg HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab227825 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab227825 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.

Lane 1: HeLa whole cell lysate 10 µg (Input).

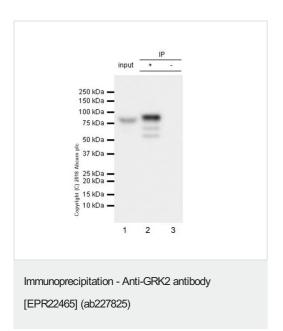
Lane 2: ab227825 IP in HeLa whole cell lysate.

**Lane 3:** Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab227825 in HeLa whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 seconds.

GRK2 was found readily degradable by proteolytic process (PMID:9857063; PMID:12738776). The bands smaller than 80-kDa detected in the immune-precipitate may represent degraded GRK2.



GRK2 was immunoprecipitated from 0.35 mg HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate with ab227825 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab227825 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)

(ab131366), was used for detection at 1/1000 dilution.

Lane 1: HEK-293T whole cell lysate 10 µg (Input).

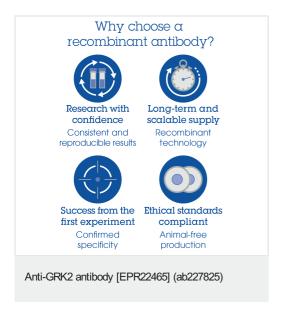
Lane 2: ab227825 IP in HEK-293T whole cell lysate.

**Lane 3:** Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab227825 in HEK-293T whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 seconds.

GRK2 was found readily degradable by proteolytic process (PMID:9857063; PMID:12738776). The bands smaller than 80-kDa detected in the immune-precipitate may represent degraded GRK2.



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