## abcam

### Product datasheet

# Anti-Glucocorticoid Receptor antibody [EPR19621] ab183127





RabMAb

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### Overview

Product name Anti-Glucocorticoid Receptor antibody [EPR19621]

**Description**Rabbit monoclonal [EPR19621] to Glucocorticoid Receptor

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human fetal heart and fetal kidney lysates; HeLa, A549, U-87 MG, HEK-293 and A431 whole

cell lysates; HEK-293 whole cell lysate transfected with human Glucocorticoid Receptor with GFP-

Myc tag. IHC-P: Human glioma and cervix carcinoma tissues; Mouse liver tissue; Rat

hippocampus tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells.

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

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ClonalityMonoclonalClone numberEPR19621

**Isotype** IgG

### **Applications**

### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab183127 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	**** <u>(2)</u>	1/2000. Detects a band of approximately 86, 83 kDa (predicted molecular weight: 86 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.
Flow Cyt (Intra)		1/500.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

T	a	r	α	et

**Function** 

Receptor for glucocorticoids (GC). Has a dual mode of action: as a transcription factor that binds to glucocorticoid response elements (GRE) and as a modulator of other transcription factors. Affects inflammatory responses, cellular proliferation and differentiation in target tissues. Could act as a coactivator for STAT5-dependent transcription upon growth hormone (GH) stimulation and could reveal an essential role of hepatic GR in the control of body growth. Involved in chromatin remodeling. Plays a significant role in transactivation. Involved in nuclear translocation.

Tissue specificity

Widely expressed. In the heart, detected in left and right atria, left and right ventricles, aorta, apex, intraventricular septum, and atrioventricular node as well as whole adult and fetal heart.

Involvement in disease

Defects in NR3C1 are a cause of glucocorticoid resistance (GCRES) [MIM:138040]; also known as cortisol resistance. It is a hypertensive, hyperandrogenic disorder characterized by increased serum cortisol concentrations. Inheritance is autosomal dominant.

Sequence similarities

Belongs to the nuclear hormone receptor family. NR3 subfamily.

Contains 1 nuclear receptor DNA-binding domain.

**Domain** 

Composed of three domains: a modulating N-terminal domain, a DNA-binding domain and a C-terminal ligand-binding domain.

Post-translational modifications

Increased proteasome-mediated degradation in response to glucocorticoids.

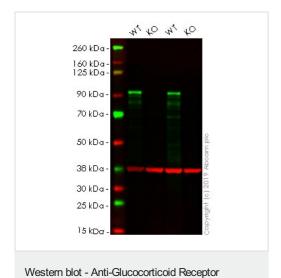
Phosphorylated in the absence of hormone; becomes hyperphosphorylated in the presence of glucocorticoid. The Ser-203-phosphorylated form is mainly cytoplasmic, and the Ser-211-phosphorylated form is nuclear. Transcriptional activity correlates with the amount of

phosphorylation at Ser-211.

Sumoylated; this reduces transcription transactivation.

Ubiquitinated; restricts glucocorticoid-mediated transcriptional signaling.

### **Images**



antibody [EPR19621] (ab183127)

**All lanes :** Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: NR3C1 knockout HeLa cell lysate

Lane 3: Wild-type A549 cell lysate

Lane 4: NR3C1 knockout A549 cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

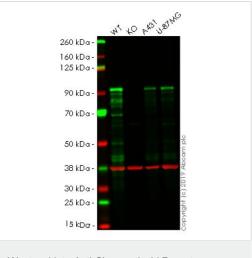
**All lanes :** Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/20000 dilution

Performed under reducing conditions.

**Predicted band size:** 86 kDa **Observed band size:** 90-100 kDa

**Lanes 1-4:** Merged signal (red and green). Green - ab183127 observed at 90-100 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

ab183127 Anti-Glucocorticoid Receptor antibody [EPR19621] was shown to specifically react with Glucocorticoid Receptor in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261766 (knockout cell lysate ab257009) was used. Wild-type and Glucocorticoid Receptor knockout samples were subjected to SDS-PAGE. ab183127 and Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

**All lanes :** Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127) at 1/2000 dilution

**Lane 1 :** Wild-type A549 (Human lung carcinoma cell line) whole cell lysate

Lane 2: NR3C1 knockout A549 (Human lung carcinoma cell line) whole cell lysate

**Lane 3**: A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 4: U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) whole cell lysate

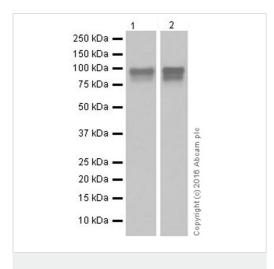
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 86 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab183127 observed at 90 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab183127 was shown to recognize NR3C1 in wild-type A549 cells as signal was lost at the expected MW in NR3C1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and NR3C1 knockout samples were subjected to SDS-PAGE. Ab183127 and <a href="mailto:ab8245">ab8245</a> (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/2000 dilution and 1/1000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed <a href="mailto:ab216773">ab216773</a> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <a href="mailto:ab216776">ab216776</a> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

**All lanes :** Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127) at 1/2000 dilution

Lane 1 : Human fetal heart lysate

Lane 2 : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

**Predicted band size:** 86 kDa **Observed band size:** 83,86 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1: 30 seconds; Lane 2: 15 seconds.

This antibody may recognize eight isoforms. The predicted MW are from 61KDa to 86KDa in human, respectively.



Western blot - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

**All lanes :** Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127) at 1/2000 dilution

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

**Lane 2**: HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 3: A431 (Human epidermoid 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:** 86 kDa **Observed band size:** 83,86 kDa

Exposure time: 10 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

This antibody may recognize eight isoforms. The predicted MW are from 61KDa to 86KDa in human, respectively.

**All lanes :** Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127) at 1/20000 dilution

**Lane 1 :** Empty vector with GFP-Myc tag (vector control) transfected HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

**Lane 2**: HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate transfected with human Glucocorticoid Receptor with GFP-Myc tag

Lysates/proteins at 10 µg per lane.

# 250 kDa — 150 kDa — 100 kDa — 75 kDa — 25 kDa — 20 kDa —

Western blot - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 86 kDa

Observed band size: 112 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

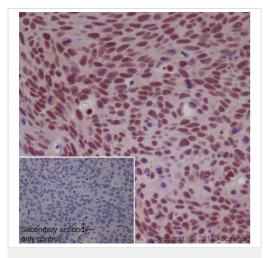
Exposure time: 0.5 second.

Secondary entibody
only control
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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

Immunohistochemical analysis of paraffin-embedded Human glioma tissue labeling Glucocorticoid Receptor with ab183127 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on tumor cells of the Human glioma is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

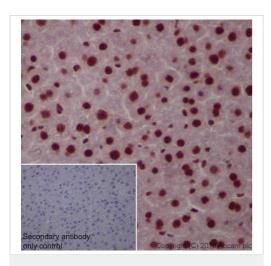


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma tissue labeling Glucocorticoid Receptor with ab183127 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on tumor cells of the cervix carcinoma is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab97051</u> at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

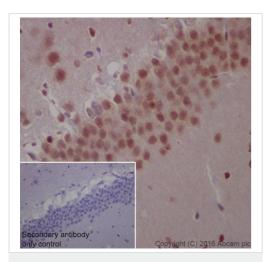


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling Glucocorticoid Receptor with ab183127 at 1/2000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on hepatocytes of the mouse liver is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

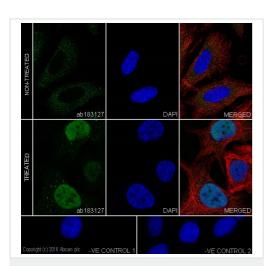


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

Immunohistochemical analysis of paraffin-embedded Rat hippocampus tissue labeling Glucocorticoid Receptor with ab183127 at 1/2000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on rat hippocampus is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Glucocorticoid Receptor with ab183127 at 1/500 dilution, followed by Goat Anti-Rabbit lgG (Alexa Fluor<sup>®</sup> 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

The results show signal translocation after dexamethasone (100 nM for 2 hours) treatment on Hela cells. PMID: 24291004.

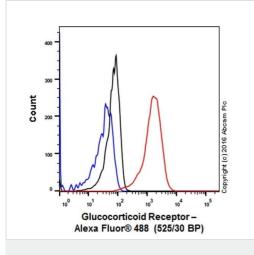
The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (<u>ab7291</u>) at 1/1000 dilution and Goat Anti-Mouse lgG H&L (Alexa Fluor® 594) preadsorbed (<u>ab150120</u>) at 1/1000 dilution (red).

The negative controls are as follows:

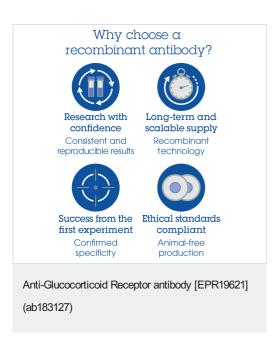
-ve control 1: ab183127 at 1/500 dilution followed by  $\underline{ab150120}$  at 1/1000 dilution.

-ve control 2: <u>ab7291</u> at 1/1000 dilution followed by <u>ab150077</u> at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Glucocorticoid Receptor with ab183127 at 1/500 dilution (red) compared with a Rabbit IgG,monoclonal [EPR25A]-Isotype control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluor® 488) at 1/500 dilution was used as the secondary antibody.



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