abcam

Product datasheet

Anti-Glucocorticoid Receptor antibody [EPR19621] ab183127





重组 RabMAb

★★★★★ 2 Abreviews 6 References 12 图像

概述

产品名称 Anti-Glucocorticoid Receptor抗体[EPR19621]

描述 兔单克隆抗体[EPR19621] to Glucocorticoid Receptor

宿主 Rabbit

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

种属反应性 与反应: Mouse, Rat, Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

阳性对照 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.

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

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: 7.2

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 EPR19621 克隆编号

同种型 ΙgG

应用

Abpromise™承诺保证使用ab183127于以下的经测试应用 The Abpromise guarantee

"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。

应用	Ab评论	说明
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. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

46000	1-
2111	*
35	A7A

功能 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.

组织特异性 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.

疾病相关 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.

序列相似性 Belongs to the nuclear hormone receptor family. NR3 subfamily.

Contains 1 nuclear receptor DNA-binding domain.

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

terminal ligand-binding domain.

翻译后修饰 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-211phosphorylated form is nuclear. Transcriptional activity correlates with the amount of

phosphorylation at Ser-211.

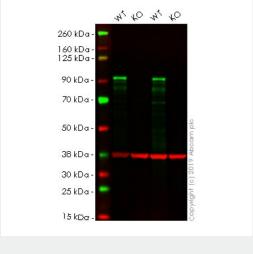
Sumoylated; this reduces transcription transactivation.

Ubiquitinated; restricts glucocorticoid-mediated transcriptional signaling.

细胞定位 Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand, nuclear after ligand-binding and

Nucleus. Localized largely in the nucleus.

2



Western blot - Anti-Glucocorticoid Receptor 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 (**ab216773**) at 1/20000 dilution

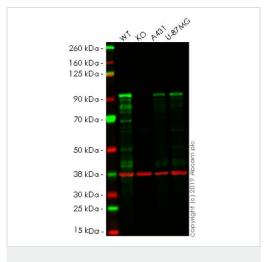
Performed under reducing conditions.

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

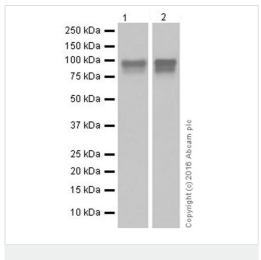
Lysates/proteins at 20 µg per lane.

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 ab8245 (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 ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 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.

1 2 3
250 kDa —
150 kDa —
100 kDa —
75 kDa —
37 kDa —
25 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —

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) (ab97051) 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.

Secondary

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

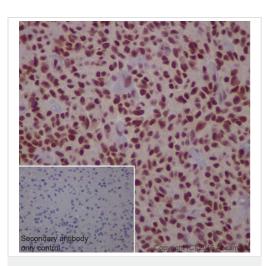
Predicted band size: 86 kDa **Observed band size:** 112 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 0.5 second.

250 kDa — 150 kDa — 150 kDa — 25 kDa — 20 kDa —

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

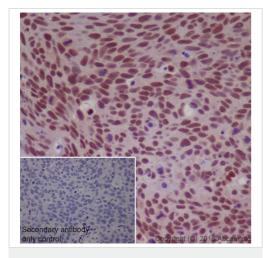


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

Immunohistochemical analysis of paraffin-embedded Human glioma tissue labeling Glucocorticoid Receptor with ab183127 at 1/2000 dilution, followed by Goat Anti-Rabbit lgG 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.

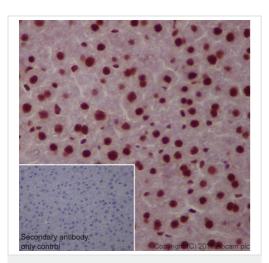


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

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 **ab97051** at 1/500 dilution.

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

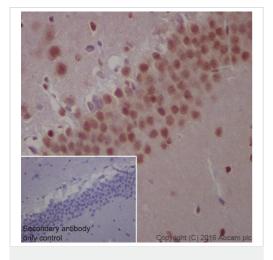


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

Immunohistochemical analysis of paraffin-embedded Mouse liver 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 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.

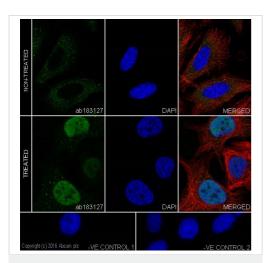


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Glucocorticoid Receptor antibody [EPR19621] (ab183127)

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 IgG (Alexa Fluor[®] 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.

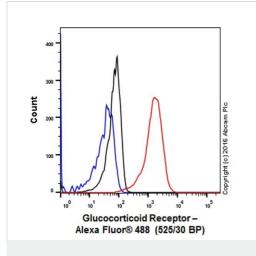
The nuclear counter stain is DAPI (blue).

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

The negative controls are as follows:

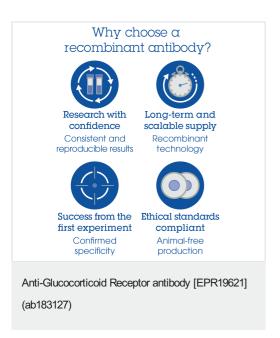
-ve control 1: ab183127 at 1/500 dilution followed by **ab150120** at 1/1000 dilution.

-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 lgG,monoclonal [EPR25A]-lsotype control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit lgG (Alexa Fluor® 488) at 1/500 dilution was used as the secondary antibody.



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