abcam

Product datasheet

Anti-Glucocorticoid receptor antibody [EPR24889-231] ab305050

KO VALIDATED Recombinant RabMAb

9 Images

Overview

Product name	Anti-Glucocorticoid receptor antibody [EPR24889-231]
Description	Rabbit monoclonal [EPR24889-231] to Glucocorticoid Receptor
Host species	Rabbit
Tested applications	**Suitable for:** ChIP, IP, ICC/IF, WB, IHC-P **Unsuitable for:** Flow Cyt (Intra)
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse, Rat
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Whole cell lysates: HeLa (human cervix adenocarcinoma epithelial cell), Glucocorticoid receptor knockout HeLa, U-87 MG (human glioblastoma-astrocytoma epithelial cell), HEK-293T (human embryonic kidney epithelial cell), THP-1 (human monocytic leukemia monocyte). IHC-P: Human: liver, pancreas, and kidney. ICC/IF: NR3C1 knockout human cervical adenocarcinoma epithelial cell. IP: HeLa. ChIP: A549(Human lung carcinoma epithelial cell) treated with dexamethasone(100nM 1h) and A549 non treated.
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
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
Clonality	Monoclonal
Clone number	EPR24889-231
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab305050 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
ChIP		Use a concentration of 5 µg/ml.
IP		1/30.
ICC/IF		1/50.
WB		1/1000. Detects a band of approximately 94 kDa (predicted molecular weight: 85 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes Is unsuitable for Flow Cyt (Intra).

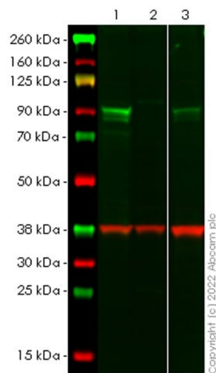
Target

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.

Cellular localization Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand, nuclear after ligand-binding and Nucleus. Localized largely in the nucleus.

Images



Western blot - Anti-Glucocorticoid receptor antibody [EPR24889-231] to (ab305050)

All lanes : Anti-Glucocorticoid receptor antibody [EPR24889-231] (ab305050) at 1/1000 dilution

Lane 1 : Wild-type HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 2 : Glucocorticoid receptor knockout HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 3 : U-87 MG (human glioblastoma-astrocytoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 85 kDa
Observed band size: 94 kDa

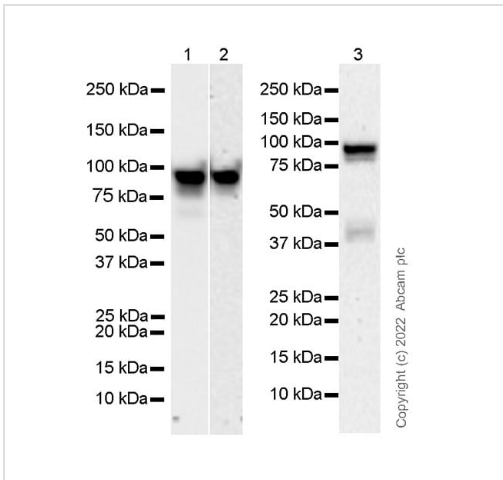
Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS.

The bands beneath the target band (94 kDa) are likely to be degraded target fragments.

False colour image of Western blot: Anti-Glucocorticoid receptor antibody [EPR24889-231] (ab305050) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red.

In Western blot, ab305050 was shown to bind specifically to Glucocorticoid receptor. A band was observed at 94 kDa in wild-type HeLa cell lysates with no signal observed at this size in Glucocorticoid receptor knockout cell line. To generate this image,

wild-type and Glucocorticoid receptor knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/10000 dilution.



Western blot - Anti-Glucocorticoid receptor antibody [EPR24889-231] to (ab305050)

All lanes : Anti-Glucocorticoid receptor antibody [EPR24889-231] (ab305050) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 2 : HEK-293T (human embryonic kidney epithelial cell), whole cell lysate
Lane 3 : THP-1 (human monocytic leukemia monocyte), whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Performed under non-reducing conditions.

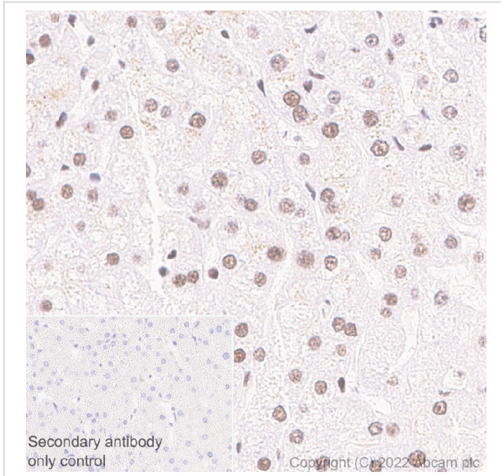
Predicted band size: 85 kDa
Observed band size: 94 kDa

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

The bands beneath the target band (94 kDa) in lane 3 are likely to be degraded target fragments.

Lysates were freshly made and used immediately to minimize protein degradation.

Exposure time: Lane 1: 37 seconds
Lanes 2-3: 3 minutes

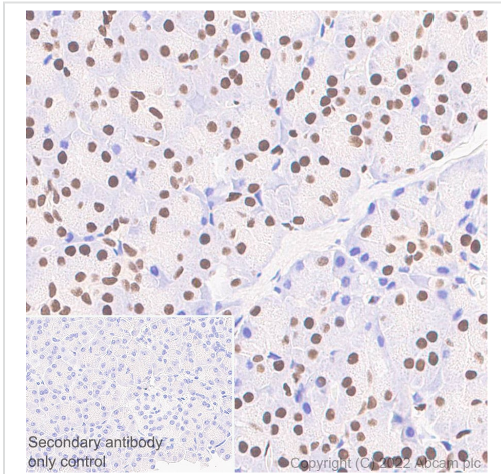


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Glucocorticoid receptor antibody [EPR24889-231] to (ab305050)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling Glucocorticoid receptor with ab305050 at 1/100 dilution (5.12 µg/ml), followed by a ready to use Leica DS9800 (Bond™ Polymer Refine Detection). Nuclear staining on human liver is observed. The section was incubated with ab305050 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (Bond™ Polymer Refine Detection) kit.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope retrieval solution 2) for 20 mins.

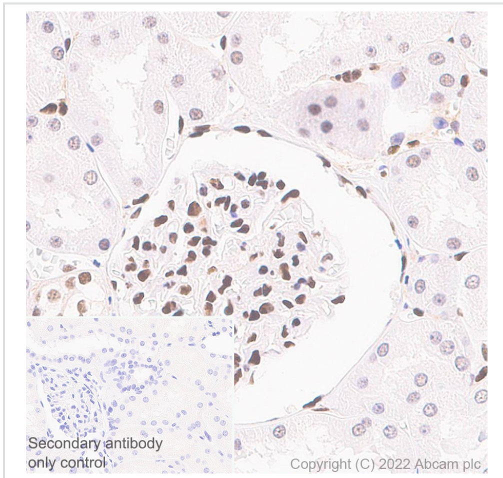


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Glucocorticoid receptor antibody [EPR24889-231] to (ab305050)

Immunohistochemical analysis of paraffin-embedded human pancreas tissue labeling Glucocorticoid receptor with ab305050 at 1/100 dilution (5.12 µg/ml), followed by a ready to use Leica DS9800 (Bond™ Polymer Refine Detection). Nuclear staining on human pancreas is observed (PMID: 32619553). The section was incubated with ab305050 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (Bond™ Polymer Refine Detection) kit.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope retrieval solution 2) for 20 mins.

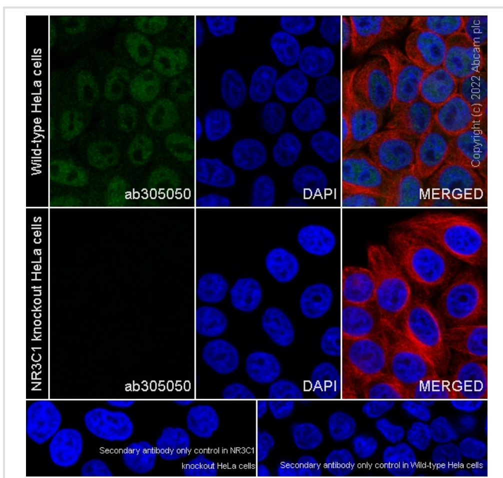


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Glucocorticoid receptor antibody [EPR24889-231] to (ab305050)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling Glucocorticoid receptor with ab305050 at 1/100 dilution (5.12 µg/ml), followed by a ready to use Leica DS9800 (Bond™ Polymer Refine Detection). Nuclear staining on human kidney is observed. The section was incubated with ab305050 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (Bond™ Polymer Refine Detection) kit.

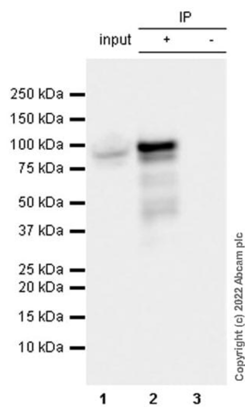
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope retrieval solution 2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-Glucocorticoid receptor antibody [EPR24889-231] to (ab305050)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NR3C1 KO HeLa (NR3C1 knockout human cervical adenocarcinoma epithelial cell) (**ab261766**) cells labeling Glucocorticoid receptor with AB305050 at 1/50 dilution (10.24 µg/mL), followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 µg/mL) (Green). Confocal image showing no staining in NR3C1 knockout HeLa cells and showing nuclear and weak cytoplasmic staining in wildtype HeLa cells. The image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 µg/mL) (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (2 µg/mL).



Immunoprecipitation - Anti-Glucocorticoid receptor antibody [EPR24889-231] to (ab305050)

Glucocorticoid receptor was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate with ab305050 at 1/30 dilution (2 µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab305050 at dilution. VeriBlot for IP secondary antibody (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg (Inset)

Lane 2: ab305050 IP in HeLa whole cell lysate

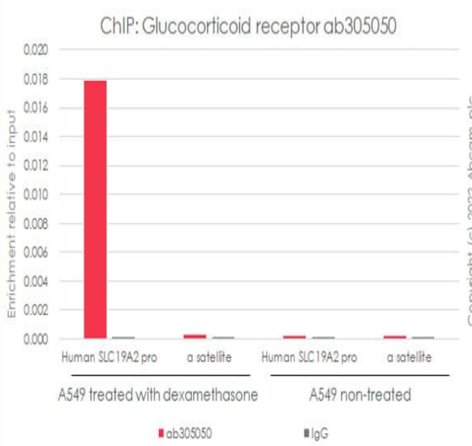
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab305050 in HeLa whole cell lysate

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

Exposure time: 6 seconds

Observed M_W (kDa): 94.

The bands beneath the target band (94 kDa) are likely to be degraded target fragments.



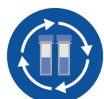
ChIP - Anti-Glucocorticoid receptor antibody [EPR24889-231] to (ab305050)

Chromatin was prepared from A549+dexamethasone(100 nM 1h) cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30 mins and then formaldehyde for 10 min.
The ChIP was performed with 25 µg of chromatin, 5 µg of 305050 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 25 µl of Protein A/G Dynabeads.

The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

http://www.abcam.com/resources?keywords=X%20ChIP%20protocol

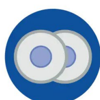
Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results

Long-term and scalable supply
Recombinant technology

Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-Glucocorticoid receptor antibody [EPR24889-231] to (ab305050)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors