#12041 Store at -20°C

Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb

☐ Small 100 µl
(10 western blots)

☐ Petite 40 µl
(4 western blots)

rev. 01/05/15

Cell Signaling TECHNOLOGY®

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Entrez-Gene ID #2908
UniProt ID #P04150

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, ChIP, F	H, M, R, Mk	94, 91 kDa	Rabbit IgG**
Endogenous			

Background: Glucocorticoid hormones control cellular proliferation, inflammation, and metabolism through their association with the glucocorticoid receptor (GR)/NR3C1, a member of the nuclear hormone receptor superfamily of transcription factors (1). GR is composed of several conserved structural elements, including a carboxy-terminal ligand-binding domain (which also contains residues critical for receptor dimerization and hormone-dependent gene transactivation), a neighboring hinge region containing nuclear localization signals, a central zinc-finger-containing DNA-binding domain, and an amino-terminal variable region that participates in ligand-independent gene transcription. In the absence of hormone, a significant population of GR is localized to the cytoplasm in an inactive form via its association with regulatory chaperone proteins, such as HSP90, HSP70, and FKBP52. On hormone binding, GR is released from the chaperone complex and translocates to the nucleus as a dimer to associate with specific DNA sequences termed glucocorticoid response elements (GREs), thereby enhancing or repressing transcription of specific target genes (2). It was demonstrated that GR-mediated transcriptional activation is modulated by phosphorylation (3-5). Although GR can be basally phosphorylated in the absence of hormone, it becomes hyperphosphorylated upon binding receptor agonists. It has been suggested that hormone-dependent phosphorylation of GR may determine target promoter specificity, cofactor interaction, strength and duration of receptor signaling, receptor stability, and receptor subcellular localization (3). Indeed Ser211 of human GR is phosphorylated to a greater extent in the presence of hormone, and biochemical fractionation studies following hormone treatment indicate that Ser211-phosphorylated GR is found in the nucleus (3). Thus, Ser211 phosphorylation is a biomarker for activated GR *in vivo*. An added layer of complexity to GR signaling lies in the ability of multiple isoforms to be generated through both alternative splicing and the use of alternative translation intiation start sites, thus increasing the repertoire of functional signaling homo- and heterodimers (6,7).

Specificity/Sensitivity: Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb recognizes endogenous levels of total GR protein. This antibody reacts with GR-α and GR-β but does not cross-react with mineralocorticoid receptor.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a recombinant protein specific to the amino terminus of human GR protein.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

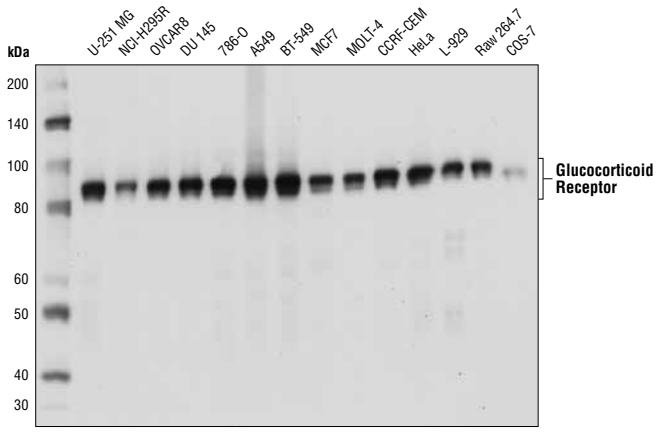
Western blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:400†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:50
Chromatin IP	1:50
Flow Cytometry	1:200

For product specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

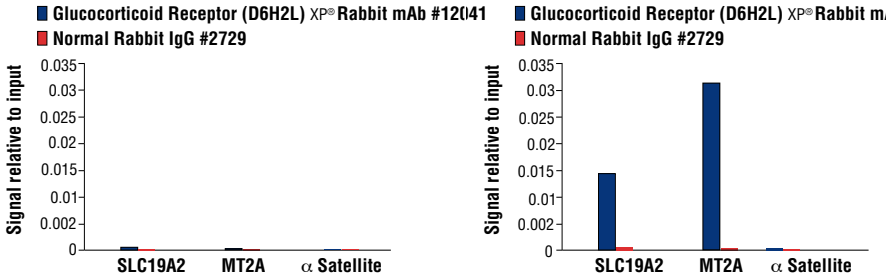


Western blot analysis of extracts from various cell lines using Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb.

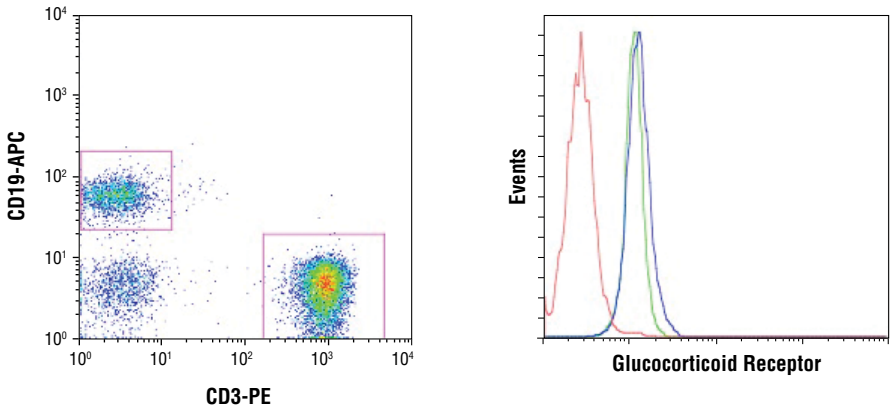
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

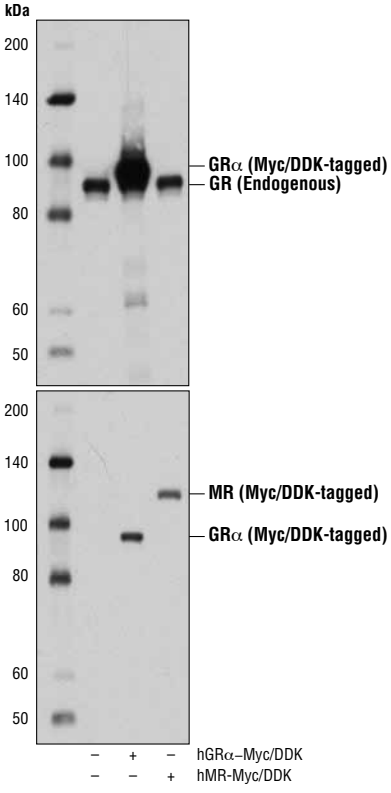
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



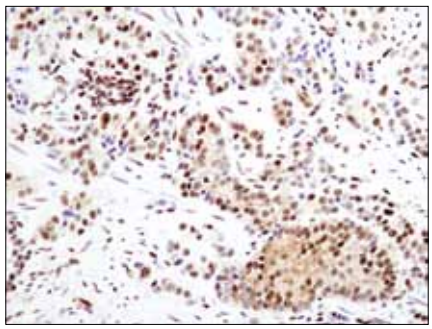
A549 cells were cultured in media with 5% charcoal-stripped FBS for 3 d and then either untreated (left panel) or dexamethasone-treated (100 nM, 1 hr; right panel). Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 cells and 10 µl of Glucocorticoid Receptor (D6H2L) Rabbit mAb or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human SLC19A2 Promoter Primers #7681, human MT2A promoter primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.



Human whole blood was fixed, lysed, and permeabilized as per the Cell Signaling Technology Flow Cytometry (Alternate) Protocol, and stained with CD3-PE, CD19-APC, and Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb. B cell (green) and T cell (blue) population gates (left) were applied to a histogram depicting the mean fluorescence intensity of glucocorticoid receptor, compared to a nonspecific negative control antibody (red; right). Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.



Western blot analysis of extracts from 293T cells, either mock transfected (-) or transfected with constructs expressing Myc/DDK-tagged full-length human glucocorticoid receptor-α (hGRα-Myc/DDK; +) or Myc/DDK-tagged full-length human mineralocorticoid receptor (hMR-Myc/DDK; +), using Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb (upper) or DYKDDDDK Tag Antibody #2368 (lower).



Immunohistochemical analysis of paraffin-embedded human prostate carcinoma using Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb.

Background References:

(1) Yamamoto, K.R. (1985) *Annu. Rev. Genet* 19, 209-252.

(2) Necela, B.M. and Cidlowski, J.A. (2003) *Trends Pharmacol. Sci.* 24, 58-61.

(3) Wang, Z. et al. (2002) *J. Biol. Chem.* 277, 26573-26580.

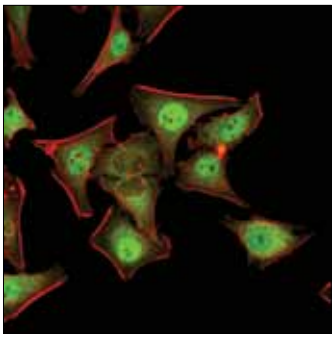
(4) Rogatsky, I. et al. (1998) *J. Biol. Chem.* 273, 14315-14321.

(5) Krstic, M. D. et al. (1997) *Mol. Cell. Biol.* 17, 3947-3954.

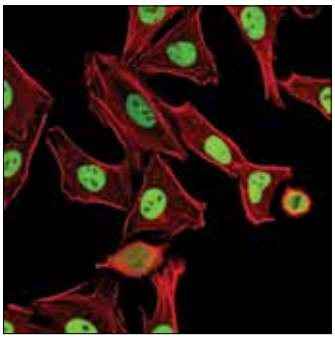
(6) Yudt, M.R. and Cidlowski, J.A. (2001) *Mol Endocrinol* 15, 1093-103.

(7) Lu, N.Z. and Cidlowski, J.A. (2005) *Mol Cell* 18, 331-42.

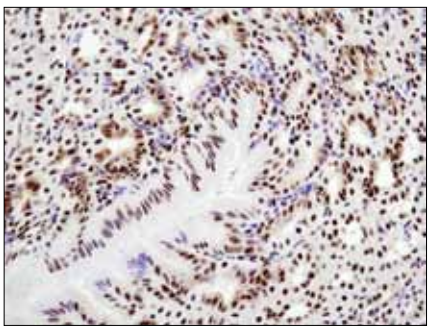
Untreated



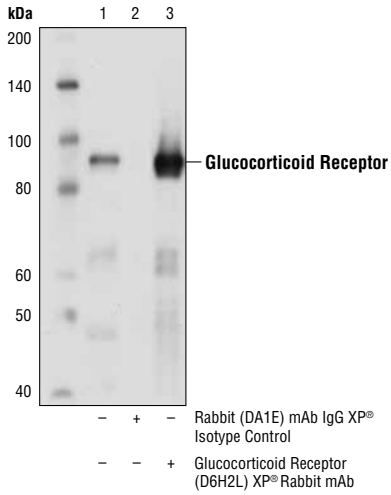
Dexamethasone-treated



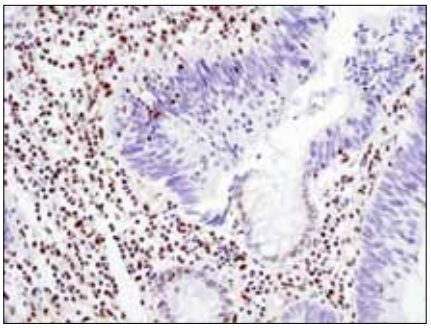
Confocal immunofluorescent analysis of HeLa cells, grown in phenol red-free media containing 5% charcoal-stripped FBS for 2 d and either untreated (upper) or treated with dexamethasone (100 nM, 2 hr; lower), using Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red).



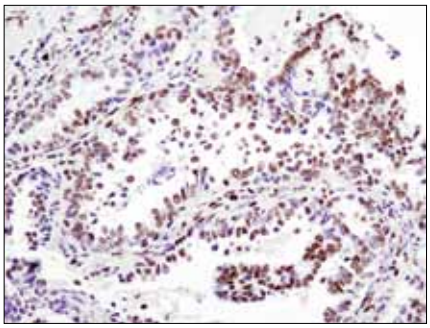
Immunohistochemical analysis of paraffin-embedded mouse stomach using Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb.



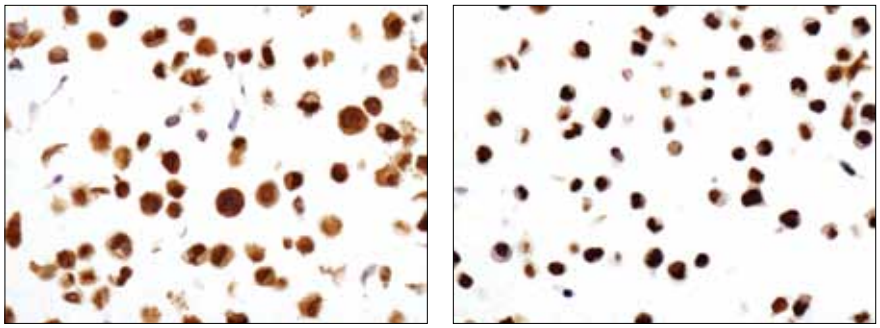
Immunoprecipitation of glucocorticoid receptor from HeLa cell extracts using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or Glucocorticoid Receptor (D6H2L) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using Glucocorticoid Receptor (D6H2L) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded HeLa cell pellets, untreated (left) or dexamethasone-treated (right), using Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb.