#12007 Store at 4°C

Glucocorticoid Receptor (D8H2) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate)

☑ 100 µl
(50 tests)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Entrez-Gene ID #2908
UniProt ID #P04150

Applications	Species Cross-Reactivity*	Isotype
IF-IC, F	H, M, R, Mk	Rabbit IgG
Endogenous		

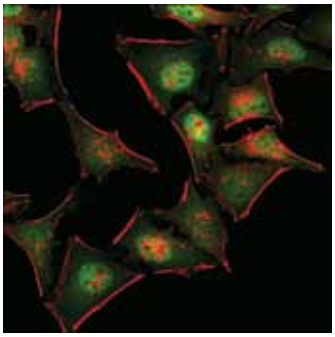
Description: This Cell Signaling Technology antibody is conjugated to Alexa Fluor® 488 fluorescent dye and tested in-house for direct immunofluorescent analysis in human cells. This antibody is expected to exhibit the same species cross-reactivity as the unconjugated Glucocorticoid Receptor (D8H2) XP® Rabbit mAb #3660.

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 mutiple 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).

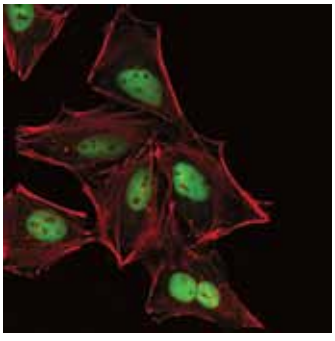
Specificity/Sensitivity: Glucocorticoid Receptor (D8H2) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) recognizes endogenous levels of total glucocorticoid receptor protein. Based upon sequence alignment, this antibody is predicted to cross-react with all known alternative translation start site generated isoforms of glucocorticoid receptor-α and glucocorticoid receptor-β. This antibody does not cross-react with mineralocorticoid receptor.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu378 of human glucocorticoid receptor protein.

Untreated



Dexamethasone-treated



Confocal immunofluorescent analysis of HeLa cells, grown in phenol red-free media containing 5% charcoal-stripped FBS for 2 days and either untreated (upper) or dexamethasone-treated (100 nM, 2 hr; lower), using Glucocorticoid Receptor (D8H2) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red).

Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide and 2 mg/ml BSA. Store at 4°C. *Do not aliquot the antibody. Protect from light. Do not freeze.*

***Species cross-reactivity other than human is determined by western blot using the unconjugated antibody.**

Recommended Antibody Dilutions:

Immunofluorescence (IF-IC)	1:50
Flow Cytometry	1:100

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

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.

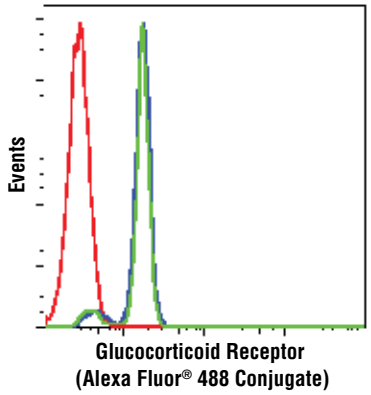
(5) Krstic, M. D. et al. (1997) *Mol. Cell. Biol.* 17, 3947-3954.

(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.

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.



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 (D8H2) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate). CD3 (blue) and CD19 (green) population gates were applied to a histogram depicting the mean fluorescence intensity of glucocorticoid and compared to Rabbit (DA1E) mAb IgG XP®Isotype Control (Alexa Fluor® 488 Conjugate) #2975 (red).