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 into the nucleus (2). GR-mediated transcriptional activation is modulated by phosphorylation of several residues surrounding Leu378 of human 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. 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.

Specificity/Sensitivity: Glucocorticoid Receptor (D8H2) XP® Rabbit mAb 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.

Recommended Antibody Dilutions:
- Western blotting: 1:1000
- Immunoprecipitation: 1:100
- Immunofluorescence (F-IC): 1:100
- Chromatin IP: 1:100
- Flow Cytometry: 1:200

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.

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IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight. Do not aliquot the antibody.
A549 cells were cultured in media with 5% charcoal-stripped FBS for 3 days 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 10⁶ cells and 5 µl of Glucocorticoid Receptor (D8H2) XP® 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 Satellites 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.