

SignalSilence® Glucocorticoid Receptor siRNA I



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

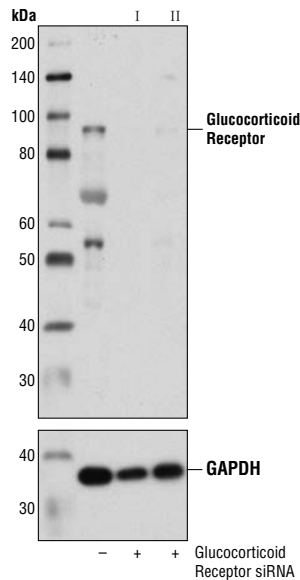
Species Cross-Reactivity: H, (Mk)

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).

Description: SignalSilence® Glucocorticoid Receptor siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit glucocorticoid receptor expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

Specificity/Sensitivity: SignalSilence® Glucocorticoid Receptor siRNA I inhibits human and monkey glucocorticoid receptor expression.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® Glucocorticoid Receptor siRNA I 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.



Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® Glucocorticoid Receptor siRNA I (+), or SignalSilence® Glucocorticoid Receptor siRNA II #7699 (+), using Glucocorticoid Receptor (D8H2) XP® Rabbit mAb #3660 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The Glucocorticoid Receptor (D8H2) XP® Rabbit mAb confirms silencing of glucocorticoid receptor expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

Each vial contains the equivalent of 100 transfections, which corresponds to a final siRNA concentration of 100 nM per transfection in a 24-well plate with a total volume of 300 µl per well.

Quality Control: Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.

Entrez-Gene ID #2908
Swiss-Prot Acc. #P04150

Storage: Glucocorticoid Receptor siRNA I is supplied in RNase-free water. Aliquot and store at -20°C.

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

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.