Revision 1

#97285 Store at -20C

Phospho-Glucocorticoid Receptor (Ser226) (D9D3V) Rabbit mAb

Cell Signaling TECHNOLOGY®

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP, IF-IC, ChIP	H M	Endogenous	94, 91	Rabbit IgG	#P04150	2908

Product Usage Information

For optimal ChIP results, use 5 µl of antibody and 10 µg of chromatin (approximately 4 x 10^6 cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (Immunocytochemistry)	1:400
Chromatin IP	1:100

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.

Specificity / Sensitivity

Phospho-Glucocorticoid Receptor (Ser226) (D9D3V) Rabbit mAb recognizes endogenous levels of glucocorticoid receptor protein only when phosphorylated at Ser226.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser226 of human glucocorticoid receptor protein.

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).
Phosphorylation of GR at serine 226 by JNK enhances nuclear export after ligand depletion (6,7). Phosphorylation of various serine residues, including serine 226 also affect GR binding to different target genes, contributing to an additional layer of transcriptional regulation (8). Serine 226 phosphorylation has also been linked to depression disorders as well as inflammation (9-11).

Background References

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8. Blind, R.D. and Garabedian, M.J. (2008) *J Steroid Biochem Mol Biol* 109, 150-7.
9. Mitic, M. et al. (2013) *Neuropharmacology* 70, 100-11.
10. Lukic, I. et al. (2015) *J Mol Neurosci* 55, 951-8.
11. Verhoog, N.J. et al. (2011) *J Biol Chem* 286, 19297-310.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation **IF-IC:** Immunofluorescence (Immunocytochemistry)
ChIP: Chromatin IP

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **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
GP: Guinea Pig **Rab:** rabbit **All:** all species expected

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