

#2600 Store at -20°C

Phospho-HP1 γ (Ser83) Antibody

100 μ l
 (10 western blots)



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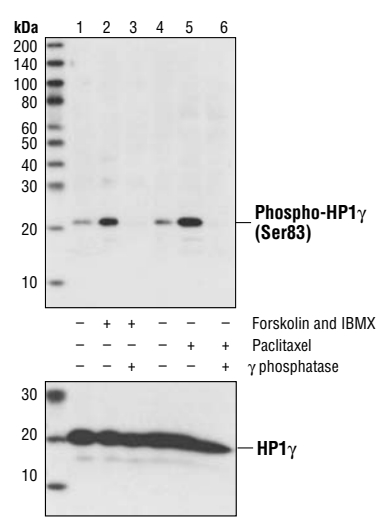
rev. 01/14/10

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IF-IC Endogenous	H, M, R, Mk, (B, Dm, Hr)	22 kDa	Rabbit**

Background: Heterochromatin protein 1 (HP1) is a family of heterochromatic adaptor molecules involved in both gene silencing and higher order chromatin structure (1). All three HP1 family members (α , β and γ) are primarily associated with centromeric heterochromatin; however, HP1 β and γ also localize to euchromatic sites in the genome (2,3). HP1 proteins are approximately 25 kDa in size and each contains a conserved amino-terminal chromodomain, followed by a variable hinge region and a conserved carboxy-terminal chromoshadow domain. The chromodomain facilitates binding to histone H3 tri-methylated on Lys9, a histone "mark" closely associated with centromeric heterochromatin (4,5). The variable hinge region binds both RNA and DNA in a sequence-independent manner (6). The chromoshadow domain mediates the dimerization of HP1 proteins, in addition to binding multiple proteins implicated in gene silencing and heterochromatin formation, including the SUV39H histone methyltransferase, the DNMT1 and DNMT3a DNA methyltransferases and the p150 subunit of chromatin-assembly factor-1 (CAF1) (7-9). In addition to contributing to heterochromatin formation and propagation, HP1 and SUV39H are also found complexed with retinoblastoma (Rb) and E2F6 proteins, both of which function to repress euchromatic gene transcription in quiescent cells (10,11). HP1 proteins are subject to multiple types of post-translational modifications, including phosphorylation, acetylation, methylation, ubiquitination and sumoylation, suggesting multiple means of regulation (12-14).

HP1 γ is phosphorylated on Ser83 by protein kinase A (PKA) *in vitro*, and activation of PKA by forskolin and IBMX treatment leads to increased phosphorylation *in vivo* (14). Phosphorylation of HP1 γ on Ser83 also increases in mitosis as demonstrated by our Phospho-HP1 γ (Ser83) Antibody, which shows increased immunofluorescent staining in untreated mitotic cells and increased Western blot signal in lysates from cells arrested in mitosis by treatment with paclitaxel. Phosphorylation of Ser83 only occurs on a subpopulation of HP1 γ found associated with euchromatin, specifically HP1 γ bound to coding regions of active genes (14). This phosphorylation impairs the ability of HP1 γ to silence transcription and may be a marker for transcription elongation (14).



Western blot analysis of whole cell lysates from HeLa cells, untreated (lanes 1 and 4), treated for 1 h with Forskolin (30 μ M) and IBMX (0.5 mM) (lanes 2 and 3), or treated 16 h with paclitaxel (500 nM) (lanes 5 and 6), using Phospho-HP1 γ (Ser83) Antibody (upper panel) or HP1 γ Antibody #2619 (lower panel).

Specificity/Sensitivity: Phospho-HP1 γ (Ser83) Antibody detects endogenous levels of HP1 γ protein only when phosphorylated on Ser83 (also referred to as Ser93 of the unprocessed form of HP1 γ). This antibody does not cross-react with HP1 α or HP1 β proteins.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide (KLH-coupled) corresponding to amino acids surrounding Ser83 of human HP1 γ . Antibodies are purified by affinity chromatography.

Entrez-Gene ID # 11335
Swiss-Prot Acc. # Q13185

Storage: Supplied in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 μ g/ml BSA and 50% glycerol. 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	1:25
Immunofluorescence (IF-IC)	1:200

For application 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.

Background References:

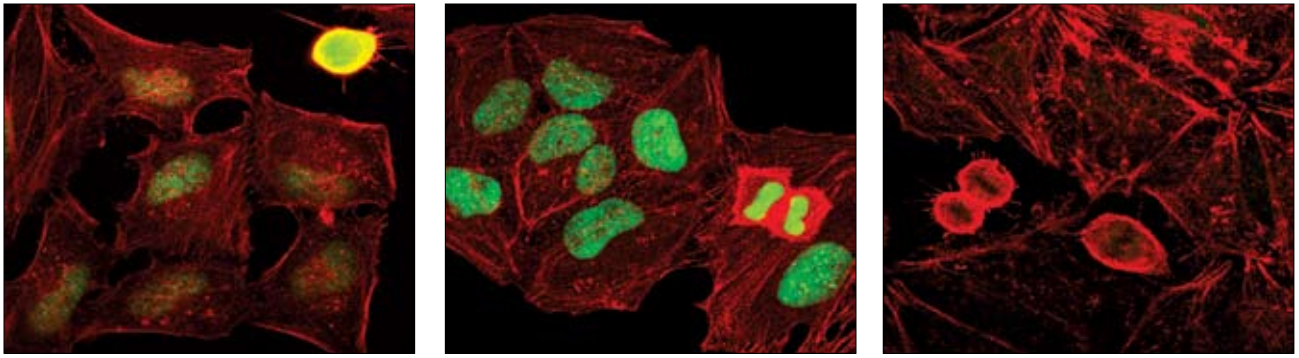
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IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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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.



Confocal immunofluorescent analysis of HeLa cells, untreated (left), Forskolin and IBMX-treated (center) or λ phosphatase-treated (right), using Phospho-HP1 γ (Ser83) Antibody (green). Actin filaments have been labeled with Alexa Fluor[®] 555 phalloidin (red).