**SignalSlide® Phospho-Stat1/3/5** 

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

**Description:** Each control slide contains formalin fixed, paraffin-embedded HeLa cells, untreated, treated with Human Interferon- $\alpha$ 1 (hIFN- $\alpha$ 1) #8927 or treated with Human Epidermal Growth Factor (hEGF) #8916 that serve as a control for Phospho-Stat1 (Tyr701), Phospho-Stat3 (Tyr705) and Phospho-Stat5 (Tyr694) immunostaining. Western blot analysis was performed on extracts derived from the same cells to verify the efficacy of the hIFN- $\alpha$ 1 and hEGF treatments. SignalSlide<sup>®</sup> Phospho-Stat1/3/5 IHC Control slides have not been tested as controls for Phospho-Stat1 (Ser727) or Phospho-Stat3 (Ser727) immunostaining.

Background: Stat proteins serve as transcription factors in growth and survival pathways stimulated by growth factor and cytokine activation of receptor proteins. Receptor activation promotes tyrosine phosphorylation of Stat proteins, resulting in Stat dimerization and translocation to the nucleus where they regulate expression of numerous proteins that control cell growth, survival, differentiation and pathogen resistance (1). Stat1 is essential in IFN- $\alpha$ and IFN-y stimulated pathways and is abnormally activated in many tumors (2,3). Both Stat1 $\alpha$  (91 kDa) and Stat1 $\beta$ (84 kDa) isoforms are activated by IFN- $\alpha$  but only Stat1 $\alpha$ responds to IFN-y. Phosphorylation of Stat1 at Tyr701 induces Stat1 dimerization, nuclear translocation and DNA binding (4). Transcription factor Stat3 possesses oncogenic potential and anti-apoptotic activities; a number of human tumors display constitutively activated Stat3 (5,6). Activation of Stat3 follows phosphorylation at Tyr705, resulting in dimerization, nuclear translocation and DNA binding (7). Expression of Stat3 $\alpha$  (86 kDa) and Stat3 $\beta$  (79 kDa) isoforms correlates with cell type, ligand and cell maturation stage (8). Phosphorylation of Stat5a at Tyr694 is essential for transcription factor activation (9). Stat5 is activated by interleukins and other cytokines (i.e. CSF1); presence of phosphorylated Stat5 is in some IL-3-treated cells suggests a role in angiogenesis and cell motility (10). Altered Stat5 expression and activity is associated with abnormal cell proliferation and apoptosis in some forms of leukemia (11).

Applications: These slides are intended for use in immunohistochemical assays. Please see our Companion Products list for products that can be used with these slides

### Background References:

(1) Ihle, J.N. (2001) Curr Opin Cell Biol 13, 211-7.

- (2) Bromberg, J. (2002) *J Clin Invest* 109, 1139-42.
- (3) Frank, D.A. (1999) *Mol Med* 5, 432-56.
- (4) Ihle, J.N. et al. (1994) Trends Biochem Sci 19, 222-7.

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(6) Catlett-Falcone, R. et al. (1999) *Immunity* 10, 105-15.

- (7) Darnell, J.E. (1997) *Science* 277, 1630-5.
- (8) Biethahn, S. et al. (1999) *Exp Hematol* 27, 885-94.
- (9) Gouilleux, F. et al. (1994) EMBO J 13, 4361-9.
- (10) Buitenhuis, M. et al. (2004) *Int J Biochem Cell Biol* 36, 2120-4.
- (11) Baśkiewicz-Masiuk, M. et al. (2003) *Cell Prolif* 36, 265-78.

#### Entrez-Gene ID # 6774 Swiss-Prot Acc. # P40763

#### Storage: Store at 4º C.

Optimal staining is achieved if slides are stained following CST's standard IHC protocols and are used within 8 weeks of assay date; however, signals may persist beyond two months.

#### 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.



IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF-Immunofluorescence E-P-ELISA-Peptide Applications Key: W-Western IP—Immunoprecipitation F—Flow cytometry Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mi—mink C—chicken Mk-monkey Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Da-dog Pa-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.



Immunohistochemical analysis of paraffin-embedded HeLa cell pellets, untreated (left), Human Interferon- $\alpha$ 1 (hIFN- $\alpha$ 1)-treated #8927 (middle) or Human Epidermal Growth Factor (hEGF)-treated #8916 (right), using Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb #9167 (top), Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145 (middle) or Phospho-Stat5 (Tyr694) (C11C5) Rabbit mAb #9359 (bottom). Immunohistochemical analysis of paraffin-embedded HeLa cell pellets, untreated (left), treated with Human Interferon- $\alpha$ 1 (hIFN- $\alpha$ 1) #8927 (middle), or treated with Human Epidermal Growth Factor (hEGF) #8916 (right), using Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb #9167 (top), Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145 (middle), or treated with Human Epidermal Growth Factor (hEGF) #8916 (right), using Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb #9167 (top), Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145 (middle), or Phospho-Stat5 (Tyr694) (C11C5) Rabbit mAb #9359 (bottom).

# **Certificate of Non-Hazardous Material**

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