#6886 Store at -20°C

PhosphoPlus® Histone H3 (Ser10) In-Cell Duet (ICW Compatible)

☐ Small Kit
(1 x 96 well plate)

☐ Large Kit
(5 x 96 well plates)

New 05/11

Cell Signaling TECHNOLOGY®

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Products Included	Product #	Volume	Applicaton	Dilution	Species Cross-Reactivity
Primary Cocktail	6900	500 µl	ICW	1:10	H, M, R, Mk
Detection Cocktail	5531	500 µl	ICW	1:10	N/A

Kit Analytes	Detection Dye	$Ex_{(max)}$ (nm)	$Em_{(max)}$ (nm)
Phospho-Histone H3 (Ser10)	DyLight® 800	777	794
β-Actin	DyLight® 680	692	712

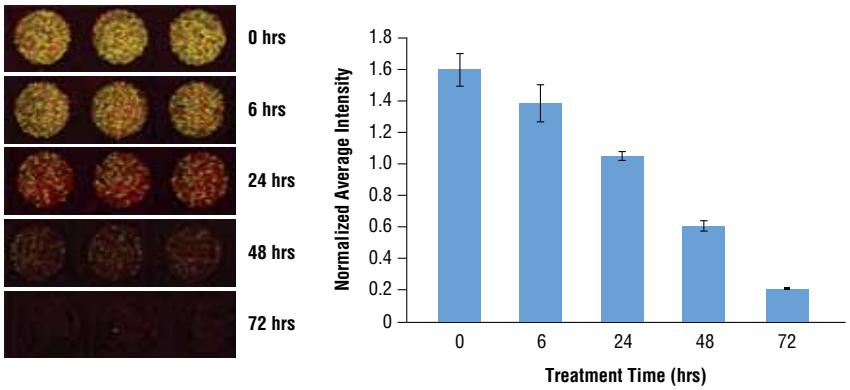
Description: PhosphoPlus® Histone H3 (Ser10) In-Cell Duet from Cell Signaling Technology (CST) provides an easy method to assess protein activation status using a multi-well plate scanner with near infrared detection capabilities, such as the LI-COR® Biosciences Odyssey® Infrared Imaging System. This kit contains a pre-optimized activation state and total protein antibody cocktail, selected based on superior performance. Phosphorylated and total protein are detected simultaneously in the same well, allowing levels of phosphorylated protein to be normalized to total protein. A near infrared detection cocktail is also included.

Specificity/Sensitivity: Phospho-Histone H3 (Ser10) antibody recognizes endogenous levels of histone H3 only when phosphorylated at Ser10. This antibody does not cross-react with other phosphorylated histones or with acetylated histones. β-Actin antibody recognizes endogenous levels of total β-actin protein.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser10 of human histone H3 protein or by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human β-actin protein.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, and 50% glycerol. Store at –20°C. *Do not aliquot either cocktail.*

Species cross-reactivity is determined by western blot with parent antibodies.



Analysis of HCC827 cells exposed to 1 µM gefitinib for the indicated times. The phosphorylation status of histone H3 and expression level of β-actin were measured simultaneously using the PhosphoPlus® Histone H3 (Ser10) In-Cell Duet (ICW Compatible). With increased exposure to gefitinib, a significant decrease (~3-8-fold) in phospho-histone H3 signal (green) was observed. Levels of β-actin (red) decreased after 24 hrs of exposure to gefitinib due to cell death. Data and images were generated on the LI-COR® Biosciences Odyssey® Infrared Imaging System.

Applications Key: **ICW**—In-Cell Western

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.

Background: Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of DNA wound around eight core histone proteins (two each of H2A, H2B, H3, and H4), is the primary building block of chromatin (1). The amino-terminal tails of core histones undergo various post-translational modifications, including acetylation, phosphorylation, methylation, and ubiquitination (2-5). These modifications occur in response to various stimuli and have a direct effect on the accessibility of chromatin to transcription factors and, therefore, on gene expression (6). In most species, histone H2B is primarily acetylated at Lys5, 12, 15, and 20 (4,7). Histone H3 is primarily acetylated at Lys9, 14, 18, 23, 27, and 56. Acetylation of H3 at Lys9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms (2,3). Phosphorylation at Ser10, Ser28, and Thr11 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis (8-10). Phosphorylation of Thr3 of histone H3 is highly conserved among many species and is catalyzed by the kinase haspin. Immunostaining with phospho-specific antibodies in mammalian cells reveals mitotic phosphorylation of H3 Thr3 in prophase and its dephosphorylation during anaphase (11).

Background References:

(1) Workman, J.L. and Kingston, R.E. (1998) *Annu Rev Biochem* 67, 545-79.

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(3) Strahl, B.D. and Allis, C.D. (2000) *Nature* 403, 41-5.

(4) Cheung, P. et al. (2000) *Cell* 103, 263-71.

(5) Bernstein, B.E. and Schreiber, S.L. (2002) *Chem Biol* 9, 1167-73.

(6) Jaskelioff, M. and Peterson, C.L. (2003) *Nat Cell Biol* 5, 395-9.

(7) Thorne, A.W. et al. (1990) *Eur J Biochem* 193, 701-13.

(8) Hendzel, M.J. et al. (1997) *Chromosoma* 106, 348-60.

(9) Goto, H. et al. (1999) *J Biol Chem* 274, 25543-9.

(10) Preuss, U. et al. (2003) *Nucleic Acids Res* 31, 878-85.

(11) Dai, J. et al. (2005) *Genes Dev* 19, 472-88.

PhosphoPlus® In-Cell Duet (ICW Compatible) Protocol

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. **10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O. Adjust pH to 7.4.
2. **Formaldehyde**, use fresh, dilute in PBS for use.
3. **Blocking Buffer (1X PBS/5% normal goat serum/0.3% Triton X-100):** To prepare 25 ml, add 2.5 ml 10X PBS, 1.25 ml normal goat serum and 21.25 ml dH_2O and mix well. While stirring, add 75 µl Triton X-100.
4. **Antibody Dilution Buffer (1X PBS/1% BSA/0.3% Triton X-100):** To prepare 25 ml, add 2.5 ml 10X PBS to 22.5 ml dH_2O, mix. Add 0.25 g BSA and mix well. While stirring, add 75 µl Triton X-100.

B Specimen Preparation

NOTE: Cells should be grown, treated, fixed, and stained directly in multi-well plates.

1. Aspirate culture medium, and then cover cells to a depth of 2–3 mm with 4% formaldehyde diluted in 1X PBS.
   NOTE: Formaldehyde is toxic, use only in fume hood.
2. Allow cells to fix for 15 minutes at room temperature.
3. Aspirate fixative, rinse three times in PBS for 5 minutes each.
4. Proceed with immunostaining.

C Immunostaining

NOTE: Include control well(s) for detection cocktail staining alone (no primary cocktail) for nonspecific background correction.

1. Block specimen in Blocking Buffer for 60 minutes.
2. While blocking, prepare primary cocktail by diluting as indicated on datasheet in Antibody Dilution Buffer.
3. Aspirate blocking solution, apply diluted primary cocktail.
4. Incubate overnight at 4°C.
5. Rinse three times in PBS for 5 minutes each.
6. Prepare detection cocktail by diluting as indicated on datasheet in Antibody Dilution Buffer.
7. Incubate 1-2 hours at room temperature in the dark.
8. Rinse three times in PBS for 5 minutes each.
9. For best results examine specimens immediately using appropriate excitation wavelengths.