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#17498

Cell Cycle Phase Determination Antibody Sampler Kit



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

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
Geminin (E5Q9S) XP® Rabbit mAb	52508	20 µl	25 kDa	Rabbit IgG
CDT1 (D10F11) Rabbit mAb	8064	20 µl	65 kDa	Rabbit IgG
Thymidine Kinase 1 (E2H7Z) Rabbit mAb	28755	20 µl	26 kDa	Rabbit IgG
P-Histone H3 (Ser10) (D7N8E) XP® Rabbit mAb	53348	20 µl	17 kDa	Rabbit IgG
Cyclin A2 (E1D9T) Rabbit mAb	91500	20 µl	55 kDa	Rabbit IgG
Cyclin B1 (D5C10) XP® Rabbit mAb	12231	20 µl	55 kDa	Rabbit IgG
Cyclin E1 (D7T3U) Rabbit mAb	20808	20 µl	48 kDa	Rabbit IgG
P-cdc2 (Tyr15) (10A11) Rabbit mAb	4539	20 µl	34 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Cell Cycle Phase Determination Antibody Sampler Kit provides an economical means of detecting total proteins or post-translational modifications present in cells at various phases of the cell cycle. Geminin is degraded in G1 phase, while CDT1 is degraded in S, G2, and M phases. Thymidine Kinase 1 accumulates in G1 phase, peaks in S phase, and is degraded before cell division. Phospho-Histone H3 (Ser10) is present only in M phase, while Phospho-cdc2 (Tyr15) is absent in M phase. Cyclins A2, B1, and E1 peak at G2 phase, late G2/M phase, and late G1/early S phase, respectively. The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

Background: The entry of eukaryotic cells into mitosis is regulated by cdc2/CDK1 kinase activation, a process controlled at several steps including cyclin B1 nuclear accumulation and binding, and phosphorylation of cdc2/CDK1 at Thr161 (1). At the end of mitosis, cyclin B1 is targeted for degradation by the anaphase-promoting complex (APC), allowing for cell cycle progression (2). A critical regulatory step in activating cdc2 during progression into mitosis is dephosphorylation of cdc2/CDK1 at Thr14 and Tyr15 (3).

Phosphorylation of Histone H3 at Ser10 is tightly correlated with chromosome condensation during both mitosis and meiosis (4). Overcoming the G1/S checkpoint to commence DNA replication requires cyclin E, traversing the G2/M checkpoint to initiate mitosis requires cyclin B, and cyclin A is required for both S-phase and M-phase (5). Cyclin A availability is apparently the rate-limiting step for entry into mitosis, and cyclin A is required for completion of prophase (6).

Thymidine kinases play a critical role in generating the DNA synthetic precursor deoxythymidine triphosphate (dTTP). Cytoplasmic thymidine kinase 1 (TK1) expression and activity are regulated in a cell cycle-dependent manner, accumulating

during G1-phase to peak levels in S-phase before being degraded prior to cell division (7). The initiation of S phase begins with the formation of the pre-replication complex (pre-RC) in late mitosis/early G1 phase. CDT1 and cdc6 bind to the origin of DNA replication, which allows binding of the MCM2-7 complex. In order to ensure that replication occurs only once per cell cycle, geminin inhibits and destabilizes CDT1 during the S, G2 and M phases. At the metaphase/anaphase transition, geminin is degraded by the anaphase-promoting complex (APC) allowing for the formation of new pre-RC (8).

Specificity/Sensitivity: Phospho-Histone H3 (Ser10) (D7N8E) XP® Rabbit mAb recognizes endogenous levels of histone H3 protein only when phosphorylated at Ser10. This antibody detects phosphorylation at Ser10 in the presence of acetylated or methylated Lys9, but not in the presence of phosphorylated Thr11. This antibody does not cross-react with histone H3 phosphorylated at Ser28. Cyclin A2 (E1D9T) Rabbit mAb recognizes endogenous levels of total cyclin A2 protein. Cyclin B1 (D5C10) XP® Rabbit mAb recognizes endogenous levels of total cyclin B1 protein. This antibody also detects a 100 kDa protein of unknown origin in some cell lines. Cyclin E1 (D7T3U) Rabbit mAb recognizes endogenous levels of total cyclin E1 protein. Based on the sequence of the peptide antigen, this antibody is expected to detect all isoforms of cyclin E1. This antibody does not cross-react with cyclin E2. Phospho-cdc2 (Tyr15) (10A11) Rabbit mAb detects endogenous levels of cdc2 protein only when phosphorylated at tyrosine 15. Geminin (E5Q9S) XP® Rabbit mAb recognizes endogenous levels of total geminin protein. CDT1 (D10F11) Rabbit mAb recognizes endogenous levels of total CDT1 protein. Thymidine Kinase 1 (E2H7Z) Rabbit mAb recognizes endogenous levels of total thymidine kinase 1 protein.

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.

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

Background References:

- (1) Atherton-Fessler, S. et al. (1994) *Mol Biol Cell* 5, 989-1001.
- (2) Gong, D. and Ferrell, J.E. (2010) *Mol Biol Cell* 21, 3149-61.
- (3) Norbury, C. et al. (1991) *EMBO J* 10, 3321-9.
- (4) Hendzel, M.J. et al. (1997) *Chromosoma* 106, 348-60.
- (5) Pagano, M. et al. (1992) *EMBO J* 11, 961-71.
- (6) Furuno, N. et al. (1999) *J Cell Biol* 147, 295-306.
- (7) Munch-Petersen, B. (2010) *Nucleosides Nucleotides Nucleic Acids* 29, 363-9.
- (8) Caillat, C. and Perrakis, A. (2012) *Subcell Biochem* 62, 71-87.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding phosphorylated Ser10 of human histone H3 protein, phosphorylated Tyr15 of human cdc2 protein, Val421 of human cyclin A2 protein, Pro209 of human thymidine kinase 1 protein, Gly351 of human cyclin E1 protein, Pro70 of human geminin protein, residues near the amino terminus of human CDT1 protein, and residues near the amino terminus of human cyclin B1 protein.

U.S. Patent No. 5,675,063.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide **Species Cross-Reactivity:** 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.**