Revision 1

#17498 Store at -20C

Cell Cycle Phase Determination Antibody Sampler Kit

1 Kit (8 x 20 microliters)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	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
Phospho-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
Phospho-cdc2 (Tyr15) (10A11) Rabbit mAb	4539	20 µl	34 kDa	Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

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.

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.*

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).

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.

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Limited Uses