Cell Cycle Phase Determination Antibody Kit

K2004

Contains Product	Specification	Applications	Species reactivity	MW(kDa)
Geminin[HA722239]	20μl	WB,IF-Cell,IHC-P	H,M,R	24 kDa
CDT1[ET1704-67]	20μl	WB,IHC-P,IP	H	60 kDa
Thymidine Kinase 1[ET1702-31]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M	25 kDa
Phospho-Histone H3 (S10)[ET1601-30]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M,R	15 kDa
Cyclin A2[ET1612-26]	20μl	WB,IF-Cell,IF-Tissue,IHC-P	H,M,R	49 kDa
Cyclin B1[ET1608-27]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP	H	48 kDa
Cyclin E1[ET1612-16]	20μl	WB,IF-Cell,IF-Tissue,IHC-P	H	47 kDa
HRP-Alpaca anti-Rabbit IgG Fc, Recombinant VHH[HA1031]	100μl	IP,ELISA,IHC-P,WB	Rab	

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. 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 Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

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. At the end of mitosis, cyclin B1 is targeted for degradation by the anaphase-promoting complex (APC), allowing for cell cycle progression.

A critical regulatory step in activating cdc2 during progression into mitosis is dephosphorylation of cdc2/CDK1 at Thr14 and Tyr15.Phosphorylation of Histone H3 at Ser10 is tightly correlated with chromosome condensation during both mitosis and meiosis.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. Cyclin A availability is apparently the rate-limiting step for entry into mitosis, and cyclin A is required for completion of prophase.

Database links: UniProt ID: O75496, O88513, 291137, Q9H211, P04183, P04184, P68431, P84243, Q16695, Q6NXT2, Q71DI3, P84244, P84245, P20248, P51943, 13094, P14635, P24864, Q61457

Images

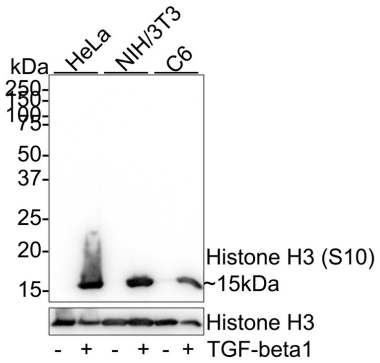

Fig1: Western blot analysis of Phospho-Histone H3 (S10) on different lysates with Rabbit anti-Phospho-Histone H3 (S10) antibody (ET1601-30) at 1/5,000 dilution.

Lane 1: HeLa whole cell lysate (20 µg/Lane)
Lane 2: HeLa treated with 50nM Calyculin A for 30 minutes whole cell lysate (20 µg/Lane)
Lane 3: NIH/3T3 whole cell lysate (20 µg/Lane)
Lane 4: NIH/3T3 treated with 100nM Calyculin A for 30 minutes whole cell lysate (20 µg/Lane)
Lane 5: C6 whole cell lysate (20 µg/Lane)
Lane 6: C6 treated with 100nM Calyculin A for 30 minutes whole cell lysate (20 µg/Lane)

Predicted band size: 15 kDa
Observed band size: 15 kDa

Exposure time: 10 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1601-30) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

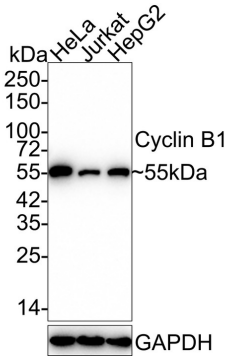

Fig2: Western blot analysis of Cyclin B1 on different lysates with Rabbit anti-Cyclin B1 antibody (ET1608-27) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: HepG2 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 48 kDa
Observed band size: 55 kDa

Exposure time: 3 minutes 10 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1608-27) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

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.