Cell Cycle Regulation Antibody Sampler Kit I

HAK21010

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Cdk2 [ET1602-6]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M	34 kDa
Cdk4 [ET1612-23]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,FC	H,M,R	34 kDa
Cdk6 [ET1612-3]	20μl	IF-Cell,IF-Tissue,IHC-P,FC,WB	H	37 kDa
Cyclin A2 [ET1612-26]	20μl	WB,IF-Cell,IF-Tissue,IHC-P	H,M,R	49 kDa
Cyclin D1 [ET1601-31]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC	H,M,R	34 kDa
Cyclin E1 [ET1612-16]	20μl	WB,IF-Cell,IF-Tissue,IHC-P	H,M	47 kDa
Cyclin E2 [ET1612-17]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,FC	H,M,R	47 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: Cell Cycle Regulation Antibody Sampler kit offers an economical way of detecting eight integral cell cycle regulation proteins. The kit contains enough primary and secondary 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

Eukaryotic cell cycle progression is dependent, in part, on the tightly regulated activity of cyclin dependent kinases (CDKs). Cyclin D/CDK4/6 activity occurs in mid-late G1 phase, upstream of CDK2/cyclin E activity. Both of these activities are required for hyperphosphorylation of the retinoblastoma gene product (pRb). This point in the cell cycle is known as the restriction point.

Cyclin protein levels oscillate throughout the cell cycle, and their availability is a means of controlling CDK activity and cell proliferation. Cyclin D is degraded through the ubiquitin proteasome pathway in the absence of mitogenic signaling. Ubiquitination of cyclin D1 is enhanced by phosphorylation at Thr286 by glycogen synthase kinase 3b (GSK-3b). They form heterotrimeric complexes with cyclins and CDKs, inhibiting kinase activity and blocking progression through G1/S phase. However, p21 may enhance assembly and activity of cyclin D/CDK4/6 complexes. All INK4 proteins selectively inhibit CDK4/6 activity, either in a binary complex, or in a ternary complex including cyclin D, resulting in inhibition of cell division.

Database links: UniProt ID: P24941, P97377, Q63699, P11802, P30285, P35426, Q00534, P20248, P51943, 13094, P24385, P25322, P39948, P24864, Q61457, O96020, Q9Z238, 362485

Images

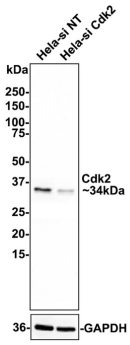

Fig1: Western blot analysis of Cdk2 on different lysates with Rabbit anti-Cdk2 antibody (ET1602-6) at 1/1,000 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si Cdk2 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 34 kDa
Observed band size: 34 kDa

Exposure time: 1 minute;
ECL: merk

4-20% SDS-PAGE gel.

ET1602-6 was shown to specifically react with Cdk2 in Hela-si NT cells. Weakened band was observed when Hela-si Cdk2 sample was tested. Hela-si NT and Hela-si Cdk2 samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1602-6, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

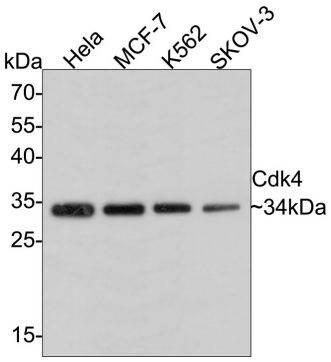

Fig2: Western blot analysis of Cdk4 on different lysates with Rabbit anti-Cdk4 antibody (ET1612-23) at 1/1,000 dilution.

Lane 1: Hela cell lysate
Lane 2: MCF-7 cell lysate
Lane 3: K562 cell lysate
Lane 4: SKOV-3 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 34 kDa
Observed band size: 34 kDa

Exposure time: 2 minutes;

12% 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 (ET1612-23) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

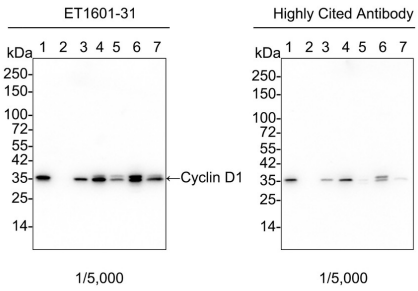

Fig3: Western blot analysis of Cyclin D1 on different lysates with Rabbit anti-Cyclin D1 antibody (ET1601-31) at 1/5,000 dilution and competitor's antibody at 1/5,000 dilution.

Lane 1: MCF7 cell lysate
Lane 2: K-562 cell lysate (negative)
Lane 3: A431 cell lysate
Lane 4: Neuro-2a cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: C6 cell lysate
Lane 7: SH-SY5Y cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 34 kDa
Observed band size: 35 kDa

Exposure time: 20 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 (ET1601-31) at 1/5,000 dilution and competitor's antibody at 1/5,000 dilution were 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.

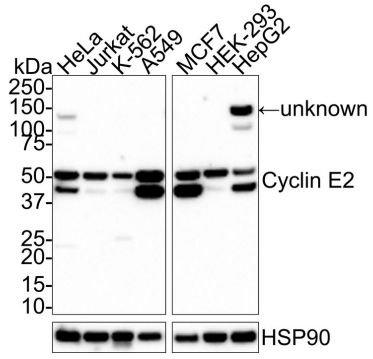

Fig4: Western blot analysis of Cyclin E2 on different lysates with Mouse anti-Cyclin E2 antibody (M0407-15) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: K-562 cell lysate
Lane 4: A549 cell lysate
Lane 5: MCF7 cell lysate
Lane 6: HEK-293 cell lysate
Lane 7: HepG2 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 47 kDa
Observed band size: 51/47 kDa

Exposure time: 2 minutes;

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 (M0407-15) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:150,000 dilution was used for 1 hour at room temperature.

Background References

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2. Pestell, R.G. et al. (1999) Endocr Rev 20, 501-34.
3. Cheng, M. et al. (1999) EMBO J 18, 1571-83.
4. Sheaff, R.J. et al. (2000) Mol Cell 5, 403-10.
5. Shin, I. et al. (2002) Nat Med 8, 1145-52.
6. Guan, K.L. et al. (1994) Genes Dev 8, 2939-52.
7. Hirai, H. et al. (1995) Mol Cell Biol 15, 2672-81.