#9868 Store at -20°C

CDK Antibody Sampler Kit

☑ 1 Kit
(6 x 20 µl)

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
CDK6 (D4S8S) Rabbit mAb	13331	20 µl	36 kDa	Rabbit IgG
CDK2 (78B2) Rabbit mAb	2546	20 µl	33 kDa	Rabbit IgG
CDK4 (D9G3E) Rabbit mAb	12790	20 µl	30 kDa	Rabbit IgG
cdc2 (POH1) Mouse mAb	9116	20 µl	34 kDa	Mouse IgG2a
CDK7 (MO1) Mouse mAb	2916	20 µl	40 kDa	Mouse IgG1
CDK9 (C12F7) Rabbit mAb	2316	20 µl	42, 55 kDa	Rabbit IgG
Anti-Rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-Mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

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

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:

Western blotting	1:1000

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

Description: The CDK Antbody Sampler Kit provides an economical means of evaluating Cdk proteins. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Cyclin-dependent kinases (CDKs) are the core effectors of cell cycle progression. CDK activity is regulated through association with their cyclin partners and cyclin-dependent kinase inhibitors (CKIs) as well as by activating and inhibitory phosphorylation events. Inhibition is mediated by Wee1 and Myt1 kinases that target residues at the amino terminus of CDK1 (1,2). Dephosphorylation of these residues by cdc25 phosphatase leads to activation of CDK kinase activity (3). The CDK7/cyclinH complex is the ubiquitous mammalian CDK-activating kinase (CAK) that phosphorylates a conserved threonine residue in the T-loop domain of CDKs. The carboxy-terminal domain of RNA polymerase II is also a target of CAK as well as CDK9/cyclinT (4,5). CDK4/6 associate with cyclinD and phosphorylate retinoblastoma protein and initiate progression through the restriction point in G1 (6). CDK2 associates with cyclinE in early S phase and cyclinA later in G2. CDK1/cyclinB regulates the initiation of mitotic events (7).

Specificity/Sensitivity: Each antibody in the CDK Antibody Sampler Kit detects endogenous levels of its respective target protein and does not cross-react with other family members.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a recombinant human cdc2 fusion protein, synthetic peptides corresponding to residues of human CDK2 and recombinant human CDK7, and residues near the carboxy terminus of human CDK4, human CDK6, and human CDK9 proteins.

Background References:

(1) Fattaey, A. and Booher, R.N. (1997) *Prog Cell Cycle Res* 3, 233-40.

(2) Booher, R.N. et al. (1997) *J Biol Chem* 272, 22300-6.

(3) Jessus, C. and Ozon, R. (1995) *Prog Cell Cycle Res* 1, 215-28.

(4) Kaldis, P. (1999) *Cell Mol Life Sci* 55, 284-96.

(5) De Falco, G. and Giordano, A. *Cancer Biol Ther* 1, 342-7.

(6) Sherr, C.J. (1995) *Trends Biochem Sci* 20, 187-90.

(7) Morgan, D.O. (1997) *Annu Rev Cell Dev Biol* 13, 261-91.

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

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.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.