Store at -20°C

#9932

Cell Cycle Regulation Sampler Kit

☑ 1 Kit
(8 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Cyclin D1 (92G2) Rabbit mAb	2978	20 µl	36 kDa	Rabbit IgG
p27 Kip1 (D69C12) XP® Rabbit mAb	3686	20 µl	27 kDa	Rabbit IgG
CDK2 (78B2) Rabbit mAb	2546	20 µl	33 kDa	Rabbit IgG
p18 INK4C (DCS118) Mouse mAb	2896	20 µl	18 kDa	Mouse IgG2a
CDK6 (DCS83) Mouse mAb	3136	20 µl	36 kDa	Mouse IgG1
Cyclin D3 (DCS22) Mouse mAb	2936	20 µl	31 kDa	Mouse IgG1
p21 Waf1/Cip1 (12D1) Rabbit mAb	2947	20 µl	21 kDa	Rabbit IgG
CDK4 (D9G3E) Rabbit mAb	12790	20 µl	30 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. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Note: Please use the nonfat dry milk protocol for the monoclonal antibodies and the BSA protocol for the polyclonal antibodies.

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

Description: Cell Cycle Regulation Antibody Sampler kit offers an economical way of detecting eight integral cell cycle regulation proteins. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

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). pRb phosphorylation allows the release of S phase-promoting transcription factors and is indicative of the cell's commitment to proliferate. 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) (1). p27/Kip1, p57 Kip2 and p21 Waf1/Cip1 are members of the Cip/Kip family of cyclin-dependent kinase inhibitors. They form heterotrimeric complexes with cyclins and CDKs, inhibiting kinase activity and blocking progression through G1/S phase (2). However, p21 may enhance assembly and activity of cyclin D/CDK4/6 complexes (3). Levels of p21 and p27 protein are controlled through ubiquitination and proteasomal degradation (4). Levels of p27 are upregulated in quiescent cells and in cells treated with negative cell cycle regulators. p27 nuclear localization is controlled by Akt-dependent phosphorylation at Thr157 (5). The inhibitors of CDK4 (INK4) family include p15 INK4B, p16 INK4A, p18 INK4C, and p19 INK4D. 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 (6,7).

Specificity/Sensitivity: Antibodies detect endogenous levels of their respective proteins.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic peptides and are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with recombinant human proteins or synthetic peptides.

Background References:

(1) Diehl, J.A. et al. (1997) *Genes Dev.* 11, 957-972.
(2) Pestell, R.G. et al. (1999) *Endocrine Rev.* 20, 501-534.
(3) Cheng, J. et al. (1999) *EMBO J.* 18, 1571-1573.
(4) Sheaff, R.J. et al. (2000) *Cell* 5, 403-410.
(5) Shin, I. et al. (2002) *Nat. Med.* 8, 1145-1152.
(6) Guan, K.L. et al. (1994) *Genes Dev.* 8, 2939-2952.
(7) Hirai, H. et al. (1995) *Mol. Cell. Biol.* 15, 2672-2681.

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 **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.