

Cell Cycle Regulation Sampler Kit

1 Kit
 (8 x 40 µl)



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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Cyclin D1 (DCS6) Mouse mAb	2926	40 µl	36 kDa	Mouse IgG2a
p27 Kip1 Antibody	2552	40 µl	27 kDa	Rabbit IgG
p15 INK4B Antibody	4822	40 µl	15 kDa	Rabbit IgG
p18 INK4C (DCS118) Mouse mAb	2896	40 µl	18 kDa	Mouse IgG2a
CDK6 (DCS83) Mouse mAb	3136	40 µl	36 kDa	Mouse IgG1
Cyclin D3 (DCS22) Mouse mAb	2936	40 µl	31 kDa	Mouse IgG1
p21 Waf1/Cip1 (DCS60) Mouse mAb	2946	40 µl	21 kDa	Mouse IgG2a
CDK4 (DCS156) Mouse mAb	2906	40 µl	30 kDa	Mouse IgG1
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.

Description: Cell Cycle Regulation Sampler kit offers an economical way of detecting eight integral cell cycle regulation proteins. The kit contains 40 µL of each primary antibody and 100 µL of HRP-conjugated anti-mouse and 50 µL of HRP-conjugated anti-rabbit.

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 antibody is produced by immunizing animals with recombinant human proteins.

Background References:

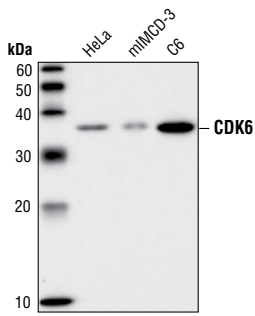
- (1) Diehl, J.A. et al. (1997) *Genes Dev.* 11, 957–972.
- (2) Pestell, R.G. et al. (1999) *Endocrine Rev.* 20, 501–534.
- (3) Cheng, J. et al. (1999) *EMBO J.* 18, 1571–1573.
- (4) Sheaff, R.J. et al. (2000) *Cell* 5, 403–410.
- (5) Shin, I. et al. (2002) *Nat. Med.* 8, 1145–1152.
- (6) Guan, K.L.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

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.*

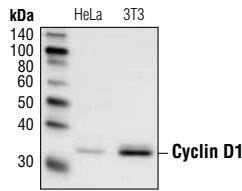
Recommended Antibody Dilutions:
Western blotting 1:1000

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

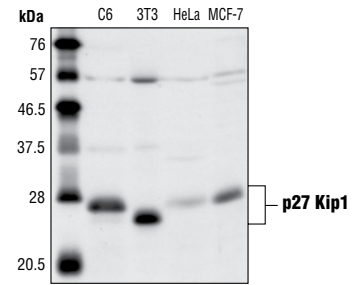
Please visit www.cellsignal.com for a complete listing of recommended companion products.



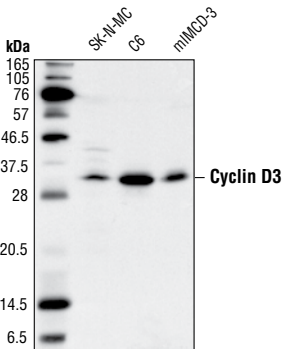
Western blot analysis of extracts from HeLa, IM-CD-3 and C6 cell lysates using **CDK6 (DCS83) mAb #3136**.



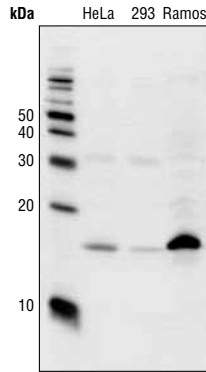
Western blot analysis of extracts from HeLa and NIH/3T3 cells using **Cyclin D1 (DCS) mAb #2926**.



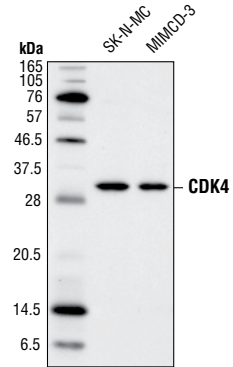
Western blot analysis of whole cell extracts from C6, NIH/3T3, HeLa and MCF-7 cells using **p27 Kip1 Antibody #2552**.



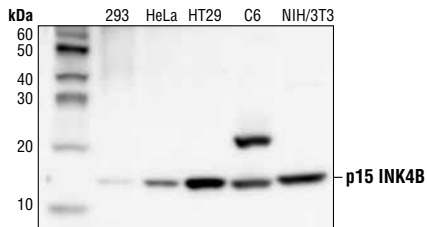
Western blot analysis of extracts from SK-N-MC, C6 and IMCD3 cells using **Cyclin D3 (DCS22) mAb #2936**.



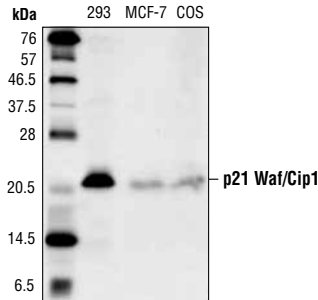
Western blot analysis of extracts from HeLa, 293 and Ramos cells, using **p18 INK4C (DCS118) Mouse mAb #2896**.



Western blot analysis of SK-N-MC and IMCD3 cell lysates using **CDK4 (DCS156) mAb #2906**.



Western blot analysis of whole cell extracts from 293, HeLa, HT29, C6 and NIH/3T3 cells using **p15 INK4B Antibody #4822**.



Western blot analysis of whole cell lysates from 293, MCF-7 and COS cells using **p21 Waf1/Cip1 (DCS60) mAb #2946**.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.

Western Immunoblotting Protocol (Primary Antibody Incubation in Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight marker (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.