#9869 Store at -20°C

Cyclin Antibody 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 A2 (BF683) Mouse mAb	4656	20 µl	55 kDa	Mouse IgE
Cyclin B1 Antibody	4138	20 µl	60 kDa	Rabbit IgG
Cyclin D1 (92G2) Rabbit mAb	2978	20 µl	36 kDa	Rabbit IgG
Cyclin D2 (D52F9) Rabbit mAb	3741	20 µl	31 kDa	Rabbit IgG
Cyclin D3 (DCS22) Mouse mAb	2936	20 µl	31 kDa	Mouse IgG1
Cyclin E1 (HE12) Mouse mAb	4129	20 µl	48-56 kDa	Mouse IgG1
Cyclin E2 Antibody	4132	20 µl	48 kDa	Rabbit IgG
Cyclin H Antibody	2927	20 µl	36 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.

Description: The Cyclin Antibody Sampler Kit provides an economical means of evaluating the presence of cyclin proteins in cells. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Control of the cell cycle is regulated by a multitude of cellular events and processes. The cyclin dependent kinases (CDK) regulate many of these pathways and constitute an active complex when associated with their cyclin partners. This activity is controlled primarily by phosphorylation, which determines subcellular localization of the CDK/cyclin complex (1,2). Some phosphorylation events control the function of cytoplasmic retention sequences while other events regulate nuclear localization and export sequence function (3,4). Cyclin and cyclin-dependent kinase inhibitor (CKI) levels are regulated by ubiquitination and degradation via the ubiquitin proteasome pathway (5). A variety of CKI proteins associate with these complexes and modulate access to regulatory domains on cyclins (6). Additional complexity is generated as the controlled protein levels of each cyclin oscillate with the stages of cell cycle. Increased expression of Cyclin D1 is associated with certain types of cancer (7,8) and may associate with TSC2 (tuberin) independent of its Cdk partner (9).

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

Source/Purification: Monoclonal antibody is produced by immunizing animals with synthetic peptides corresponding to residues within the carboxy terminus of cyclin D1, the amino terminus of cyclin D2, recombinant human cyclin E1, or cyclin A2. Polyclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues within the carboxy terminus of cyclin H, cyclin E2, or the amino terminus of cyclin B. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Krempler, A. et al. (2005) *Cell Mol Life Sci* 62, 1379–87.

(2) Toyoshima, F. et al. (1998) *EMBO J* 17, 2728–35.

(3) Porter, L.A. and Donoghue, D.J. (2003) *Prog Cell Cycle Res* 5, 335–47.

(4) Benzeno, S. et al. (2006) *Oncogene* 25, 6291–303.

(5) Diehl, J.A. et al. (1997) *Genes Dev* 11, 957–72.

(6) Hirai, H. et al. (1995) *Mol Cell Biol* 15, 2672–81.

(7) Sherr, C.J. (1996) *Science* 274, 1672–7.

(8) Kim, J.K. and Diehl, J.A. (2009) *J Cell Physiol* 220, 292–6.

(9) Zacharek, S.J. et al. (2005) *Cancer Res* 65, 11354–60.

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.

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 (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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

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 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.