

# p15 INK4B Antibody

✓ 100 µl  
(10 Western mini-blot)

Orders ■ 877-616-CELL (2355)  
orders@cellsignaling.com

Support ■ 877-678-TECH (8324)  
info@cellsignaling.com

Web ■ www.cellsignaling.com

rev. 05/08/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity	Molecular Wt.	Source
W, F	H, M, R	15 kDa	Rabbit

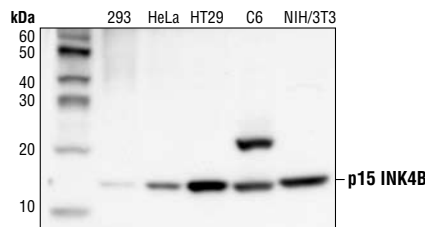
**Background:** Cyclin-dependent kinases (CDKs) are activated in part by forming complexes with cyclins. For example, CDK4 and CDK6 associate with the D-type cyclins and phosphorylate the retinoblastoma protein. This phosphorylation is a necessary event for cells to enter S-phase (1). The inhibitors of CDK4 (INK4) family include p15 INK4B, p16 INK4A, p18 INK4C and p19 INK4D. p18 has been shown to function as a haploinsufficient tumor suppressor *in vivo* (2). All INK4 proteins are composed of 32 amino acid ankyrin motifs and selectively inhibit CDK4/6 activity. Mutational analyses of p18 implicate the third and the amino-terminal portion of the fourth ankyrin repeat in mediating binding to CDK4/6 (3). The interaction of INK4 family members can be a binary complex with CDK4/6 or ternary complex with cyclin D-bound CDK4/6 and ultimately results in the inhibition of cell cycle progression (4,5). The gene encoding p15 INK4B is often silenced by hypermethylation in acute myeloid leukemia and deleted in acute lymphocytic leukemia (6).

**Specificity/Sensitivity:** p15 INK4B Antibody detects endogenous levels of p15 INK4B.

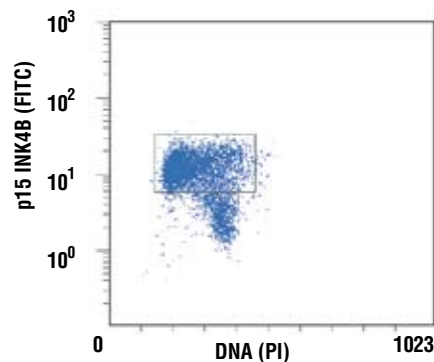
**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to the amino terminus of human p15 INK4B. Antibodies are purified by protein A and peptide affinity chromatography.

#### Background References:

- (1) Lukas, J. et al. (1996) *Mol. Cell. Biol.* 16, 6917–6925.
- (2) Bai, F. et al. (2003) *Mol. Cell. Biol.* 23, 1269–1277.
- (3) Noh, S.J. et al. (1999) *Cancer Res.* 59, 558–564.
- (4) Guan, K.L. et al. (1994) *Genes Dev.* 8, 2939–2952.
- (5) Hirai, H. et al. (1995) *Mol. Cell. Biol.* 15, 2672–2681.
- (6) Drexler, H.G. (1998) *Leukemia* 12, 845–859.



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



Flow cytometric analysis of untreated C6 cells, using p15 INK4B Antibody versus propidium iodide (DNA content). Boxed population indicates p15 INK4B-positive cells.

**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 antibody.

#### Recommended Antibody Dilutions:

Western Blotting 1:1000  
Flow Cytometry 1:25

#### Companion Products:

p18 INK4C (DCS118) Mouse mAb #2896

CDK4 (DCS156) Mouse mAb #2906

CDK6 (DCS83) Mouse mAb #3136

Cyclin D1 (DCS6) Mouse mAb #2926

Cyclin D3 (DCS22) Mouse mAb #2936

Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071

Anti-rabbit IgG, HRP-linked Antibody #7074

Prestained Protein Marker, Broad Range (Premixed Format) #7720

Biotinylated Protein Ladder Detection Pack #7727

20X LumiGLO® Reagent and 20X Peroxide #7003

**IMPORTANT: 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.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence

**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFIATM

Z—zebra fish B—bovine AII—all species expected

## Western Immunoblotting Protocol (Primary Ab Incubation In Nonfat Dry 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)
- Bovine Serum Albumin (BSA)
- 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<sup>®</sup>-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<sup>®</sup> 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 cm<sup>2</sup>) 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<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> 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<sup>®</sup> incubation and declines over the following 2 hours.

# Flow Cytometry Protocol for Intracellular Staining Using Conjugated Secondary Antibodies

## A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g  $\text{Na}_2\text{HPO}_4$  and 0.24 g  $\text{KH}_2\text{PO}_4$  in 800 mL distilled water ( $\text{dH}_2\text{O}$ ). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100mL 1X PBS. Store at 4°C

## B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

## C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

## D Staining Using Unlabeled Primary and Conjugated Secondary Antibodies

**NOTE:** Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot 0.5-1x10<sup>6</sup> cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
3. Resuspend cells in 100  $\mu\text{l}$  Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add the primary antibody at the appropriate dilution to the assay tubes (see individual antibody data sheet for the appropriate dilution).
6. Incubate for 30-60 minutes at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in fluorochrome-conjugated secondary antibody\*, diluted in Incubation Buffer according to the manufacturer's recommendations.
9. Incubate for 30 minutes at room temperature.
10. Rinse as before in Incubation Buffer by centrifugation.
11. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.

\*Recommended Secondary Antibodies from Invitrogen.

A-11070 Alexa Fluor® 488 F(ab')<sub>2</sub> fragment of goat anti-rabbit IgG (H+L) (1:1000 dilution)

A-11017 Alexa Fluor® 488 F(ab')<sub>2</sub> fragment of goat anti-mouse IgG (H+L) (1:1000 dilution)