

#2946 Store at **-20°C**

p21 Waf1/Cip1 (DCS60) Mouse mAb

- Small 100 µl (20 Western mini-blot)
- Large 300 µl (60 Western mini-blot)



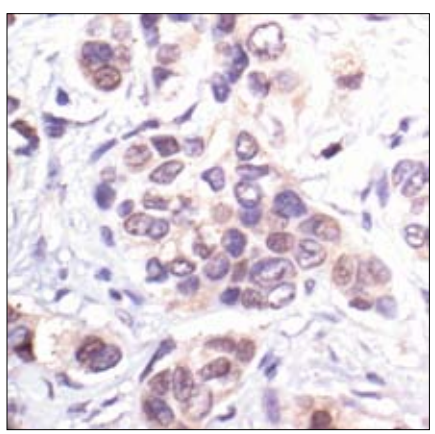
Orders ■ 877-616-CELL (2355)
 orders@cellsignal.com
Support ■ 877-678-TECH (8324)
 info@cellsignal.com
Web ■ www.cellsignal.com

rev. 01/29/08

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	Isotype
W, IP, IHC-P	H, Mk	21 kDa	Mouse	IgG2a

Background: The tumor suppressor protein p21 Waf1/Cip1 acts as an inhibitor of cell cycle progression. It functions in stoichiometric relationships forming heterotrimeric complexes with cyclins and cyclin-dependent kinases. In association with CDK2 complexes, it serves to inhibit kinase activity and block progression through G1/S (1). However, it may also enhance assembly and activity in complexes of CDK4 or CDK6 and cyclin D (2). The carboxy-terminal region of p21 is sufficient to bind and inhibit PCNA, a subunit of DNA polymerase, and may coordinate DNA replication with cell cycle progression (3). Upon UV damage, or during stages of the cell cycle when cdc2/cyclin B or CDK2/cyclin A are active, p53 is phosphorylated and upregulates p21 transcription via a p53 responsive element (4). Protein levels of p21 are downregulated through ubiquitination and proteasomal degradation (5).



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing nuclear and cytoplasmic localization, using p21 Waf1/Cip1 (DCS60) Mouse mAb.

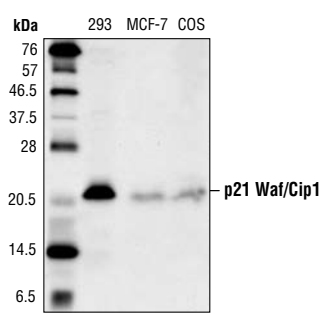
Specificity/Sensitivity: p21 Waf1/Cip1 (DCS60) Mouse mAb detects endogenous levels of total p21 protein. The antibody does not cross-react with other CDK inhibitors.

Source/Purification: Monoclonal antibody is produced by immunizing mice with recombinant human p21 corresponding to the amino-terminal portion of p21.

Selected Application References:
 Thullberg, M. et al. (2000) Monoclonal antibody probes for p21 Waf1/Cip1 and the INK4 family of cyclin-dependent kinase inhibitors. *Hybridoma* 19 (1), 63–72. Applications: IC-IF, Western Blotting.

Zhu, H. et al. (2004) Induction of S-phase arrest and p21 overexpression by a small molecule 2[[3-(2,3-dichlorophenoxy)propyl] amino]ethanol in correlation with activation of ERK. *Oncogene* 23, 4984–4992. Application: Western Blotting.

- Background References:**
- Pestell, R.G. et al. (1999) *Endocrine Rev.* 20, 501–534.
 - Cheng, J. et al. (1999) *EMBO J.* 18, 1571–1583.
 - Flores-Rozas, H. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91, 8655–8659.
 - Wang, Y. and Prives, C. (1995) *Nature* 376, 88–91.
 - Sheaff, R.J. et al. (2000) *Cell* 101, 403–410.



Western blot analysis of extracts from 293, MCF-7 and COS cells, using p21 Waf1/Cip1 (DCS60) Mouse mAb.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at **-20°C**. Do not aliquot the antibody.
***Species cross-reactivity is determined by Western blot.**

Recommended Antibody Dilutions:
 Western blotting 1:2000
 Immunoprecipitation 1:100
 Immunohistochemistry (Paraffin) 1:100

Companion Products:
 Anti-mouse IgG, HRP-linked Antibody #7076
 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% 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
 F—Flow cytometry E—ELISA D—DELFIATM
 Z—zebra fish B—bovine All—all species expected
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

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)
- 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[®]-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.

Immunoprecipitation Protocol / (For Analysis By Western Immunoblotting)

A Solutions and Reagents

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

- 1X Phosphate Buffered Saline (PBS)
- 1X Cell Lysis Buffer:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM Sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na₃VO₄, 1 μg/ml Leupeptin

NOTE: CST recommends adding 1 mM PMSF before use*.

- Transfer Buffer:** 25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)
- Protein A or G Agarose Beads:** (Can be stored for 2 weeks at 4°C.) Please prepare according to manufacturer's instructions. Use Protein A for rabbit IgG pull down and Protein G for mouse IgG pull down.
- 3X SDS Sample Buffer:** 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

B Preparing Cell Lysates

- Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.

- Remove PBS and add 0.5 ml 1X ice-cold cell lysis buffer plus 1 mM PMSF* to each plate (10 cm) and incubate the plates on ice for 5 minutes.
- Scrape cells off the plates and transfer to microcentrifuge tubes. Keep on ice.
- Sonicate samples on ice four times for 5 seconds each.
- Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. If necessary, lysate can be stored at -80°C.

C Immunoprecipitation

- Take 200 μl cell lysate and add primary antibody. Incubate with gentle rocking overnight at 4°C.
- Add either protein A or G agarose beads (20 μl of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
- Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500 μl of 1X cell lysis buffer. Keep on ice during washes.
- Resuspend the pellet with 20 μl 3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
- Heat the sample to 95–100°C for 2–5 minutes.
- Load the sample (15–30 μl) on SDS-PAGE gel (12–15%).
- Analyze sample by Western blotting (see Western Immunoblotting Protocol).

Immunohistochemistry Protocol for Paraffin Sections

A Solutions and Reagents

- 10X Phosphate Buffered Saline (PBS):** 0.58 M sodium phosphate dibasic (Na₂HPO₄), 0.17 M sodium phosphate monobasic (NaH₂PO₄), 0.68 M NaCl. To prepare 1 liter of 10X PBS, combine 82.33 g Na₂HPO₄, 23.45 g NaH₂PO₄•H₂O and 40 g NaCl. Adjust pH to 7.4.
- 10 mM Sodium Citrate Buffer:** To prepare 1 liter, add 2.94 g sodium citrate to 1 liter distilled H₂O (dH₂O). Adjust pH to 6.0.
- EDTA (optional)*
- 1% Hydrogen Peroxide:** To prepare, add 10 ml 30% H₂O₂ to 290 ml dH₂O.
- Blocking Solution:** 5% horse serum or goat serum in PBS
- ABC Reagent:** (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) Prepare according to manufacturer's instructions 30 minutes before use.
- DAB Reagent:** Add 6.7 μl of 30% hydrogen peroxide to 10 ml dH₂O; add this mixture to 10 ml of 1 mg/ml DAB (diaminobenzidine tetrahydrochloride) in PBS, filter.

B Protocol

- Deparaffinize/hydrate sections:**
 - Incubate sections in three washes of xylene for 5 minutes each.
 - Incubate sections in two washes of 100% ethanol for 10 minutes each.
 - Incubate sections in two washes of 95% ethanol for 10 minutes each.
- Wash sections twice in dH₂O for 5 minutes each.
- Wash sections in PBS for 5 minutes.
- For antigen unmasking, heat sections in 10 mM sodium citrate buffer (pH 6.0) for 1 minute at full power followed by 9 minutes at medium power.* (Keep slides fully immersed in buffer and maintain temperature at or just below boiling.) Cool slides for 20 minutes after antigen unmasking.

* Alternatively in Step B4, use 1 mM EDTA (pH 8.0), which gives superior results for EGF receptor and HER2/ErbB2 antibodies. See the Tyrosine Kinases/Docking Proteins section of the catalog for these products.

- Wash sections in dH₂O three times for 5 minutes each.
- Incubate sections in 1% Hydrogen Peroxide for 10 minutes.
- Wash sections in dH₂O three times for 5 minutes each.
- Wash section in PBS for 5 minutes.
- Block each section with 100–400 μl blocking solution for 1 hour at room temperature.
- Remove blocking solution and add 100–400 μl diluted primary antibody to each section. (Dilute antibody in blocking solution.) Incubate overnight at 4°C.
- Remove antibody solution and wash sections in PBS three times for 5 minutes each.
- Add 100–400 μl secondary antibody, diluted in blocking solution, to each section. Incubate 30 minutes at room temperature.
- If using ABC avidin/biotin method, make ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
- Remove secondary antibody solution and wash sections three times with PBS for 5 minutes each.
- Add 100–400 μl ABC reagent to each section and incubate for 30 minutes at room temperature.
- Remove ABC reagent and wash sections three times in PBS for 5 minutes each.
- Add 100–400 μl DAB reagent to each section and monitor staining closely.
- As soon as the section turns brown, immerse slides in dH₂O.
- If desired, counterstain sections in hematoxylin for 10 seconds.
- Wash sections in dH₂O two times for 5 minutes each.
- Dehydrate sections:**
 - Incubate sections in 95% ethanol two times for 10 seconds each.
 - Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
 - Repeat in xylene, incubating sections two times for 10 seconds each.
- Mount coverslips.