

#9544 Store at -20°C

Cleaved PARP (Asp214) Antibody (Mouse Specific)

100 µl
 (10 Western mini-blot)



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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, IF-IC, IC	M	89 kDa	Rabbit

Background: PARP, a 116 kDa nuclear poly (ADP-ribose) polymerase, appears to be involved in DNA repair in response to environmental stress (1). This protein can be cleaved by many ICE-like caspases *in vitro* (2,3) and is one of the main cleavage targets of caspase-3 *in vivo* (4,5). In human PARP, the cleavage occurs between Asp214 and Gly215, which separates the PARP amino-terminal DNA binding domain (24 kDa) from the carboxy-terminal catalytic domain (89 kDa) (2,4). PARP helps cells to maintain their viability; cleavage of PARP facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis (6).

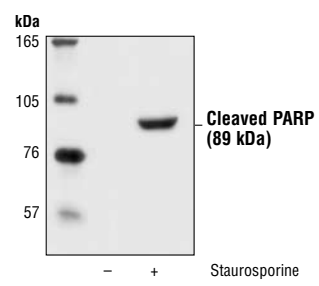
(This product is sold under license from Promega Corp., U.S. Patent No. 6,350,452.)

Specificity/Sensitivity: Cleaved PARP (Asp214) Antibody (Mouse Specific) detects endogenous levels of the large fragment (89 kDa) of mouse PARP1 resulting from caspase cleavage. The antibody does not recognize full length PARP1 or other PARP isoforms.

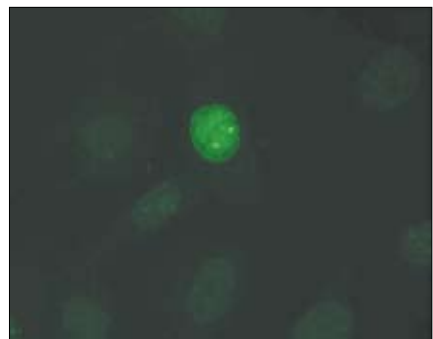
Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to carboxy-terminal residues surrounding Asp214 in mouse PARP. Antibodies are purified by protein A and peptide affinity chromatography.

Selected Application References:
 Garnier, P. et al. (2003) Ischemic preconditioning by caspase cleavage of poly(ADP-ribose) polymerase-1. *J. Neurosci.* 23 (22), 7967-73. Applications: IC-IF, W.

- Background References:**
 (1) Satoh, M.S. and Lindahl, T. (1992) *Nature* 356, 356-358.
 (2) Lazebnik, Y.A. et al. (1994) *Nature* 371, 346-347.
 (3) Cohen, G.M. (1997) *Biochem. J.* 326, 1-16.
 (4) Nicholson, D.W. et al. (1995) *Nature* 376, 37-43.
 (5) Tewari, M. et al. (1995) *Cell* 81, 801-809.
 (6) Oliver, F.J. et al. (1998) *J. Biol. Chem.* 273, 33533-33539.



Western blot analysis of NIH/3T3 cells, untreated or staurosporine-treated (1 µM), using Cleaved PARP (Asp214) Antibody (Mouse Specific).



Immunofluorescent analysis of NIH/3T3 cells, untreated (upper) or staurosporine-treated (lower), using Cleaved PARP (Asp214) Antibody (Mouse Specific).

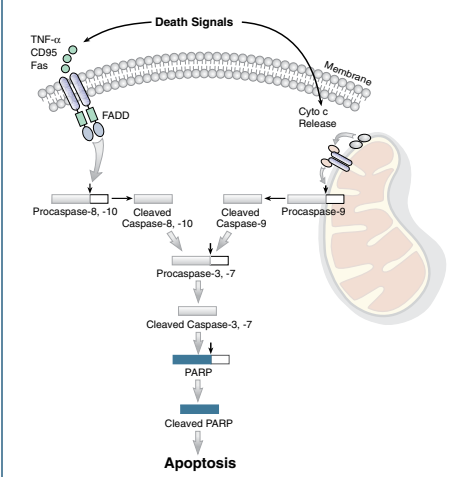
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunocytochemistry (ABC)	1:100
Immunofluorescence (IF-IC)	1:100

- Companion Products:**
 Cleaved PARP (Asp214) Antibody (Human Specific) #9541
 PARP Antibody #9542
 Cleaved PARP (Asp214) Antibody (Rat Specific) #9545
 Phospho-PTEN (Ser380) Antibody #9551
 PTEN Antibody #9552
 PTEN (26H9) Mouse mAb #9556
 Anti-rabbit IgG, HRP-linked Antibody #7074
 Prestained Protein Marker, Broad Range (Premixed Format) #7720
 Biotinylated Protein Ladder #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 All—all species expected

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 #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.

Immunocytochemistry Protocol for Immunofluorescence

This procedure works well when cells are grown in 6-well tissue culture plates containing sterile coverslips in the appropriate media and concentration of fetal bovine serum (FBS). If desired, provide extra coverslips for cell staining controls.

A Solutions and Reagents

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

1. Fetal Bovine Serum (FBS)
2. **10X Phosphate Buffered Saline (PBS):** To prepare 1L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O . Adjust pH to 7.4.
3. **4% Paraformaldehyde:** Prepare day of use either from commercial 16% stock solution or by dissolving paraformaldehyde in 1X PBS by heating at 60°C and stirring until it dissolves. Cool before use.
4. **Tris Buffered Saline (TBS):** 50 mM Tris-HCl (pH 7.4), 150 mM NaCl.
5. **0.1% Sodium Borohydride:** Dissolve in PBS on day of use.
6. **0.2% Triton X-100:** Prepare stock of 20% Triton in PBS; rotate tube overnight to dissolve. Dilute to 0.2%.
7. 1% Bovine Serum Albumin (BSA)
8. **Blocking Buffer:** 10% horse or goat serum, 1% BSA, 0.02% NaN₃, 1X PBS.
9. **ProLong™ Antifade Kit:** As directed by Molecular Probes (#P-7481), prepare just before use. Add approximately 1 ml of ProLong mounting medium to one vial of ProLong antifade reagent. Mix gently. Any unused mixture can be stored at -20°C (to slow reaction) for up to one month.

B Fixation

1. Prepare 4% paraformaldehyde and/or cool 100% methanol to -20°C in a tightly sealed container.
2. Treat cells as desired.
3. Wash cells on coverslips once with cool or room temperature TBS.
4. Aspirate off TBS completely and immediately fix as appropriate for your samples.

C Methanol (protein precipitation) Fixation

1. Immerse coverslips in -20°C 100% methanol for 10 minutes.
2. Rinse slips three times for 5 minutes each with room temperature TBS.
3. Quench cells in fresh 0.1% sodium borohydride in TBS for 5 minutes.
4. Wash slips three times for 5 minutes each with room temperature TBS. Aspirate off completely and return slips to TBS only. Continue protocol at "Blocking" step E1.

D Paraformaldehyde (cross-linking) Fixation

1. Immerse coverslips in 4% paraformaldehyde at room temperature for 10 minutes.
2. Wash coverslips once with TBS. Aspirate completely and then permeabilize cells on coverslips with 0.2% Triton X-100 for 5 minutes at room temperature or alternatively with -20°C methanol for 5 minutes.
3. Wash slips three times for 5 minutes each with TBS at room temperature.
4. Quench cells in fresh 0.1% sodium borohydride in TBS for 5 minutes. Aspirate off completely and return slips to TBS only. Continue protocol at blocking step.

E Blocking

1. Block all slips with blocking buffer at room temperature for 45–60 minutes. Wash once for 5 minutes with TBS.

F Staining

1. Dilute the primary antibody as appropriate in 1% BSA in TBS. Centrifuging the antibody for 20 minutes at 12,000 x g in a refrigerated microcentrifuge prior to use will remove any aggregated material, thereby reducing background. Apply the diluted antibody to the cells on coverslips and, most critically, incubate overnight at 4°C.

NOTE: When using any primary or fluorescence-labeled secondary antibody for the first time, titrate out the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.

NOTE: You may wish to leave one slip for a secondary antibody-only control.

2. Wash all slips three times for 5 minutes each with TBS.
3. Incubate all slips with a dilution of the fluorescence-labeled secondary antibody in 1% BSA in TBS for 30–45 minutes at room temperature in the dark.
4. Wash all slips three times for 5 minutes each with TBS in low lighting conditions.
5. Mount coverslips on slides using the ProLong™ Antifade Kit. Store slides at room temperature in the dark.

Immunocytochemistry Protocol for ABC Detection

This procedure works well with 50% confluent cells in a 6-well plate and can be performed without coverslips.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water. Be aware that azide will interfere with the HRP enzyme reaction and should not be added to solutions.

- 1. 10X Phosphate Buffered Saline (PBS):** To prepare 1L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O . Adjust pH to 7.4.
- 2. 4% Paraformaldehyde:** Prepare day of use either from commercial 16% stock solution or by dissolving paraformaldehyde in 1X PBS by heating at 60°C and stirring until it dissolves. Cool before use.
- 3. 100% Methanol**
- 4. Tris Buffered Saline (TBS):** 50 mM Tris-HCl (pH 7.4), 150 mM NaCl
- 5. 0.2% Triton X-100:** Prepare stock of 20% Triton in PBS. Rotate tube overnight to dissolve. Dilute to 0.2%.
- 6. TBS/Triton:** 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.1% Triton X-100
- 7. Blocking Buffer:** 5.5% normal goat serum in TBS/Triton
- 8. 3% Bovine Serum Albumin (BSA)**
- 9. 0.6% Hydrogen Peroxide:** 200 μl 30% H_2O_2 in 10 ml TBS
- 10. ABC Reagent:** (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) Prepare 30 minutes before using.
- 11. DAB Reagent:** Add 6.7 μl of 30% hydrogen peroxide to 10 ml dH_2O ; add this mixture to 10 ml of 1 mg/ml DAB (diaminobenzidine tetrahydrochloride) in PBS, filter.

B Fixation

1. Prepare 4% paraformaldehyde and/or cool 100% methanol to -20°C in a tightly sealed container.
2. Treat cells as desired.
3. Quickly wash cells once with cool or room temperature 1X PBS.
4. Aspirate off PBS completely and immediately fix as is appropriate. (Use either the Fixation or Paraformaldehyde methods in Steps C1 and D1–2.)
5. Aspirate fixative/permeabilizer and wash three times for 5 minutes each with 1 ml TBS/Triton. Rinse with TBS.

C Methanol (protein precipitation) Fixation

1. Immerse cells in -20°C 100% methanol for 10 minutes.

D Paraformaldehyde (cross-linking) Fixation

1. Immerse cells in 4% paraformaldehyde for 10 minutes at 4°C .
2. Rinse coverslips once with 1X TBS at room temperature. Aspirate completely, then permeabilize cells on coverslips with 0.2% Triton X-100 for 5 minutes at room temperature or alternatively with 100% methanol at -20°C for 5 minutes.

E Blocking

1. Aspirate TBS, then incubate with 1 ml blocking buffer for 45–60 minutes at room temperature.
2. Wash once for 5 minutes with 1 ml TBS.

F Staining

1. Aspirate, then incubate with primary antibody at suggested dilution in 3% BSA in TBS overnight at 4°C .

NOTE: When using any primary antibody for the first time, titrate out the primary to determine which dilution allows the strongest specific signal with the least background.

NOTE: You may wish to leave one slip for a secondary antibody-only control, and to provide another slip incubated in normal serum from the host animal (rabbit serum if using a rabbit primary antibody, and mouse serum if using a mouse primary antibody) instead of the primary antibody. Dilute normal serum to the lowest dilution shown to have no staining by your chosen fixation/permeabilization method.

2. Wash two times for 5 minutes each with 1 ml TBS/Triton. Wash once with TBS.
3. Incubate with biotinylated secondary antibody (diluted appropriately in TBS/3% BSA; 1:500 for secondary antibody from Vectastain ABC Kit) for 1 hour at room temperature.
4. Wash three times for 5 minutes each with 1 ml of TBS/Triton.
5. Wash once for 5 minutes with 1 ml TBS.
6. Incubate for exactly 30 minutes in 0.6% hydrogen peroxide at room temperature.
7. Wash three times for 5 minutes each with 1 ml of TBS/Triton. Wash once with TBS.
8. Incubate for 1 hour with 0.5–1.0 ml ABC reagent at room temperature. (Add 2 drops solution A into 5 ml PBS, mix, then add 2 drops solution B, mix.)
9. Wash two times for 5 minutes each with 1 ml TBS.
10. Add 1 ml DAB reagent. Monitor reaction progress under the microscope. Reaction may proceed for 10 minutes.
11. Terminate reaction by adding an equal volume of water.
12. Aspirate and wash once with 1 ml of water.
13. View cells in 6-well plate or mount coverslips with VectaMount (Vector Laboratories, Inc., Burlingame, CA) if necessary.