

Anti-PARP Antibody C2-10

Cat #: 4338-MC-50

Monoclonal antibody recognizing
poly(ADP-ribose) polymerase (50 μ l)

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I Introduction

Background

Poly(ADP-ribose) polymerase (PARP) is a 116 kDa eukaryotic post-translational protein modification enzyme, which uses NAD as a substrate to form polymers of ADP. Poly(ADP-ribosyl)ation of proteins occurs by the addition of an ADP-ribose (generated from NAD⁺) to glutamic acid residues. The PARP ribosylates itself at an internal automodification domain, and ribosylates a number of additional nuclear proteins. Self modification causes the PARP to dissociate from the DNA. The activity of the PARP can be restored by the activity of the enzyme poly(ADP-ribose) glycohydrolase.

During apoptosis, the activity of PARP is modulated. PARP activity is initially increased but due to automodification and cleavage by the caspase family of proteins, activity is ultimately abrogated. The specific rate of change in PARP activity is dependent upon a variety of factors including cell type, method of induction of DNA damage or apoptosis, and culture conditions. Specific proteolytic cleavage of PARP has been demonstrated to be a reliable marker for apoptosis in a wide variety of cell types generating a 85 KDa and a 26 KDa fragment.

The Anti-PARP Antibody C2-10 recognizes the full length PARP and the 85 KDa cleavage product.

Application

The C2-10 antibody can be used for analysis of PARP cleavage by Western transfer and blotting and for immunocytochemistry. Since the antibody's recognition site overlaps with the DNA binding site of the protein, it is important to follow the instructions for preparation of cell extracts for optimal results in Western analysis. The antibody is provided in phosphate buffered saline containing 0.1% BSA and 0.1% sodium azide and should be stored at -20°C. Repeat freezing and thawing should be avoided therefore it is advisable to prepare small aliquots when the sample is first thawed.

II Reagent Preparation

For Method A: Phosphate Buffer extraction

- Buffer A:** 50 mM Tris-Cl (pH 7.4), 5 mM MgSO₄, 250 mM Sucrose, 1 mM PMSF, 0.5 µg/ml Leupeptin, 0.7 µg/ml Pepstatin, 5 µg/ml Antipain.

For 20 ml of Buffer A, prepare just before use from stock solutions:

10.0 ml	Stock Buffer
0.1 ml	200 mM PMSF in ethanol
10.0 µl	1 mg/ml Leupeptin in H ₂ O
14.0 µl	1 mg/ml Pepstatin in methanol
5.0 µl	20 mg/ml Antipain in H ₂ O
9.9 ml	H ₂ O

- Buffer B:** 50 mM Tris-Cl (pH 7.4), 5 mM MgSO₄, 250 mM Sucrose, 1 mM PMSF, 0.5 µg/ml Leupeptin, 0.7 µg/ml Pepstatin, 50 µg/ml Antipain, 1% (w/v) NP-40.

For 10 ml of Buffer B, prepare just before use and chill on ice:

5.0 ml	Stock Buffer
1.0 ml	NP-40 (10% stock solution)
0.05 ml	200 mM PMSF in ethanol
5.0 µl	1 mg/ml Leupeptin in H ₂ O
7.0 µl	1mg/ml Pepstatin in methanol
25.0 µl	20 mg/ml Antipain in H ₂ O
3.9 ml	H ₂ O

- 4. Buffer C** 50 mM Tris-Cl (pH 7.4), 300 mM K_2HPO_4 , 5 mM $MgSO_4$, 250 mM Sucrose, 1 mM PMSF, 0.5 μ g/ml Leupeptin, 0.7 μ g/ml Pepstatin, 500 μ g/ml Antipain.

For 5 ml of Buffer C, prepare just before use and chill on ice:

2.5 ml	Stock Buffer
1.5 ml	1 M K_2HPO_4
25 μ l	200 mM PMSF in ethanol
2.5 μ l	1 mg/ml Leupeptin in H_2O
3.5 μ l	1 mg/ml Pepstatin in methanol
12.5 μ l	20 mg/ml Antipain in H_2O
960 μ l	H_2O

For methods A and B

5. Gel Loading Buffer

62.5 mM Tris-Cl, pH 6.8, 6 M urea, 10% glycerol, 2% SDS, 0.00125 % bromophenol blue, 5% β -mercaptoethanol.

For storage omit the β -mercaptoethanol and store at $-20^\circ C$.
Add fresh β -mercaptoethanol just before use.

III Preparation of Cell Extracts for SDS-PAGE

Choose one of the following methods for the preparation of cell extracts for Western blotting application.

A. Phosphate buffer extraction method

The protocol given below is for adherent cells. For suspension cells, harvest cells by centrifugation, and resuspend the cell pellet in each buffer by tapping the tube gently.

1. For best results use around 2×10^7 cells.
2. Chill plates or dishes on ice.
3. Aspirate medium.
4. Rinse in ice cold Buffer A.
5. Cover cells with 5 ml of cold Buffer B, let stand on ice for 20 min. Drain buffer.
6. Cover cells with 2 ml of cold Buffer C, let stand on ice for 20 min.
7. Recover Buffer C-cell mixture and transfer to a microcentrifuge tube prechilled on ice. Centrifuge at $3000 \times g$ for 10 min at $4^\circ C$. Recover supernatant to prechilled tube and place on ice.
8. Optional: to increase protein recovery, add 1 ml ice cold Buffer C to the cell pellet and vortex to resuspend. Leave on ice for 10 minutes. Centrifuge at $3000 \times g$ for 10 min at $4^\circ C$. Recover supernatant and combine with sample recovered in step 7.
9. Store the cell extract at $-20^\circ C$ until analysis.
10. Add 2 volumes of urea loading buffer and use in SDS-PAGE and Western analysis.

B. Urea/sonication extraction method

1. If necessary, scrape cells from dish or plate in a small volume ice cold 1X PBS.
2. Harvest cells by centrifugation at $500 \times g$ for 5 minutes at $4^\circ C$.
3. Wash cell pellet in ice cold 1X PBS.
4. Resuspend cells at 1×10^7 cells/ml in Gel Loading Buffer. Use a 1000 μ l pipette to resuspend the

cells. The sample will be viscous due to release of DNA during lysis.

5. Sonicate sample on ice for 20 to 40 seconds.
6. Store sample at -20°C until analysis.

IV SDS-PAGE and Western Analysis

Methods for SDS-PAGE and Western transfer are available in a wide variety of techniques manuals. A method that is routinely used in our laboratories is given below as a guide. Typically a 1 in 2 000 dilution of the Anti-PARP Antibody is required but titrations should be performed to optimize results.

1. Heat prepared cell extract at 95°C for 10 minutes.
2. Load sample (ideally, 20 - 40 µg total protein) onto 10% or 12% SDS polyacrylamide gel. Include prestained markers for reference. Run SDS-PAGE.
3. Transfer proteins to nitrocellulose by Western blot.
4. Block membrane with 1X PBS, 5% non-fat dried milk, 0.1% Tween®20 for 1 hr. at room temperature, with gentle agitation.
5. Incubate membrane with 1 in 2000 dilution of Anti-PARP antibody 4338-MC-50 in 1X PBS, 5% non-fat dried milk, 0.1% Tween®20 for 1 hour at room temperature or overnight at 4°C.
6. Wash membrane three times with 1X PBS, 0.1% Tween®20, for 10 minutes per wash.
7. Incubate membrane with secondary antibody for 1 hr at room temperature (use dilution recommended by antibody manufacturer).
8. Wash membrane three times with 1X PBS, 0.1% Tween®20, for 10 minutes per wash.
9. Incubate with appropriate color development or chemiluminescent reagents according to manufacturer's instructions.

Note: If a phosphatase conjugated secondary antibody is used, the 1X PBS should be replaced with 50 mM Tris-Cl, pH 7.4, 150 mM NaCl.

V Immunocytochemistry

The Anti-PARP Antibody 4338-MC-50 can be used in immunocytochemistry applications. The recommended antibody dilution is 1 in 1000 but for optimal results the antibody should be titrated. Note that the antibody will recognize both the full length and the cleaved 85 KDa fragment generated during apoptosis.

VI Appendix

Related products

<u>Name</u>	<u>Catalog Number</u>	<u>Amount provided</u>
Anti-poly(ADP-ribose), monoclonal	4335-MC-100	100 µl
Anti-poly(ADP-ribose), polyclonal	4336-BPC-100	100 µl
Poly-ADP(riboseyl)ated Protein	4500-050-P	50 µl
Poly(ADP-ribose) Polymerase	4667-50-01, 4667-250-01	100 units, 500 units
Poly(ADP-ribose) Polymerase Activity Assay	4667-50-K	50 reactions
PARP HSA	4868-100-01	100 units
PARP HSA	4868-500-01	500 units
PARP HSA	4868-2K-01	2000 units
PARP catalytic domain	4869-100-01	100 µg
PARP inhibition assay	4669-96-K	96 samples
Biotinylated NAD	4670-500-01	500 µl

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