

TREVIGEN® Product Data

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Anti-PARP Antibody C2-10

Catalog #: 4338-MC-50

Size: 50 µl

Description: Poly(ADP-ribose) polymerase 1 (PARP-1) is a 114 kDa eukaryotic enzyme, which uses NAD as a substrate to form poly(ADP-ribose) (PAR cat# 4336-100-01). Poly(ADP-ribose)ylation of proteins occurs by the addition of an ADP-ribose (generated from NAD⁺) to glutamic acid residues. PARP-1 ribosylates itself at an internal automodification domain. Self modification causes PARP-1 to lose enzymatic activity and dissociate from DNA. PARP-1 enzymatic activity is restored by poly(ADP-ribose) glycohydrolase, which degrades PAR into monomers. During apoptosis, PARP-1 activity initially increases, but later, falls due to auto-modification and cleavage by caspases. The specific rate of change in PARP-1 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 85 kDa and 26 kDa fragments. Trevigen's C2-10 antibody recognizes full length PARP and the 85 kDa cleavage product, which contains the catalytic, automodification and NAD-binding domains.

Physical State: C2-10 is an IgG₁ and is provided in 1X PBS/0.01% sodium azide.

Immunogen: Calf Thymus PARP-1.

Specificity: Mouse monoclonal antibody C2-10 detects the 114 kDa PARP-1 holoenzyme, 85 kDa apoptosis-related, and necrosis-related 50 kDa, 62 kDa and 74 kDa cleavage fragments. It cross-reacts with human, monkey, hamster, rat and mouse PARP-1. It does not recognize the chicken equivalent.

Storage: Store at 4°C for at least 1 month, or store aliquots at -20°C.

Sample Protocols:

I. Reagent Preparation:

For Method A: Phosphate Buffer Extraction

Buffer A: 50 mM Tris-Cl (pH = 7.4), 5 mM MgSO ₄ , 250 mM Sucrose, 1 mM PMSF, 0.5 µg/ml Leupeptin, 0.7 µg/ml Pepstatin, 5 µg/ml Antipain		Buffer B: 50 mM Tris-Cl (pH = 7.4), 5 mM MgSO ₄ , 250 mM Sucrose, 1 mM PMSF, 0.5 µg/ml Leupeptin, 0.7 µg/ml Pepstatin, 5 µg/ml Antipain, 1% (w/v) NP-40.	
For 20 ml of Buffer A , prepare just before use from stock solutions:		For 10 ml of Buffer B , prepare just before use from stock solutions:	
10.0 ml	Stock Buffer	5.0 ml	Stock Buffer
0.1 ml	200 mM PMSF in EtOH	50.0 µl	200 mM PMSF in EtOH
10.0 µl	1 mg/ml Leupeptin in H ₂ O	5.0 µl	1 mg/ml Leupeptin in H ₂ O
14.0 µl	1 mg/ml Pepstatin in MetOH	7.0 µl	1 mg/ml Pepstatin in MetOH
5.0 µl	20 mg/ml Antipain in H ₂ O	25.0 µl	20 mg/ml Antipain in H ₂ O
9.9 ml	H ₂ O	3.9 ml	H ₂ O
		1.0 ml	NP-40 (10% stock solution)

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Buffer C: 50 mM Tris-Cl (pH = 7.4), 300 mM K₂HPO₄, 5 mM MgSO₄, 250 mM Sucrose, 1 mM PMSF, 0.5 µg/ml Leupeptin, 0.7 µg/ml Pepstatin, 500 µg/ml Antipain
 For 5 ml of Buffer C, prepare just before use and chill on ice:

2.5 ml	Stock Buffer	2.5 µl	1 mg/ml Leupeptin in H ₂ O
1.5 ml	1M K ₂ HPO ₄	3.5 µl	1 mg/ml Pepstatin in MetOH
25 µl	200 mM PMSF in EtOH	12.5 µl	20 mg/ml Antipain in H ₂ O
		960 µl	H ₂ O

For methods A and B:

Sample Buffer: 62.5 mM Tris-Cl, pH = 6.8, 4M Urea, 10% Glycerol, 2% SDS, 0.00125% bromophenol blue, 5% β-mercaptoethanol (freshly added)
Note: For storage, omit the β-mercaptoethanol and store at -20 °C. Add fresh β-mercaptoethanol just before use.

Cell Lysates for Western Blotting:

II. Preparation of Cell Extracts for SDS-PAGE

Choose one of the following methods for the preparation of cell extracts for Western blotting application. Two methods are provided. The purpose of urea in the sample buffer and sonication step is to effectively dissociate PARP:DNA interactions. Alternatively, you may want to use a non-denaturing procedure:

A. Phosphate buffer extraction method (non-denaturing):

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 1-2 x 10⁷ 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 3,000 x g for 10 min at 4°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 x g for 10 min at 4°C. Recover supernatant and combine with sample recovered in step 7.
9. Store the cell extract at -20°C until analysis.
10. Add 2 volumes of urea sample buffer and subject sample to SDS-PAGE and Western blot analysis.

B. Sonication/Urea 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 x g for 5 minutes at 4 °C.
3. Wash cell pellet in ice cold 1X PBS.
4. Resuspend cells at 10⁷ cells/ml in Sample Buffer. Use a 1000 µl pipette to resuspend the cells. The sample will be viscous due to release of DNA during lysis.
5. Sonicate sample on ice for 15 seconds, and incubate at 65 °C for 15 minutes.
6. Store sample at -20°C until analysis.
7. Add 2 volumes of sample buffer and use in SDS-PAGE and Western analysis.

III. SDS-PAGE and Western Analysis

Methods for SDS-PAGE and Western transfer are available in a wide variety of techniques manuals. For Western blotting a 1:2000 dilution using enhanced chemiluminescence (ECL) or 1:1000 using alkaline phosphatase should be used but titration may be needed for optimal 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; 150 mM NaCl, for 1 hr at room temperature, with gentle agitation.
5. Incubate membrane with a 1:2000 dilution of Anti-PARP (C2-10) in 1X PBS, 5% non-fat dried milk, 0.1% Tween® 20; 150 mM NaCl, 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.

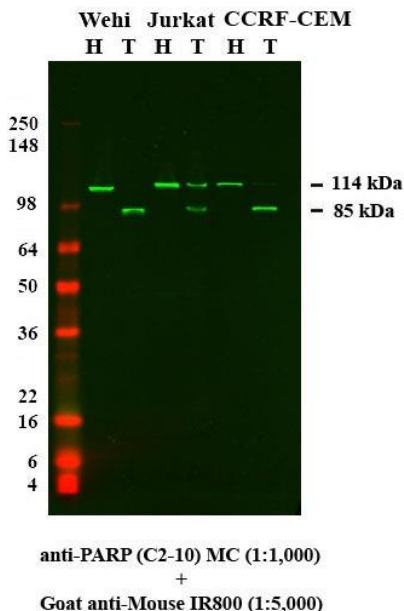


Figure 1. Western blot analysis of Wehi, Jurkat and CCRF-CEM cell lines untreated (H) and treated (T) with 25 µM Etoposide for 16 hours at 37°C. Cells were lysed in Tris-Glycine SDS sample buffer at the concentration 1 x 10⁷ cells/ml and 10 µl of each lysate were loaded per well of a 4-20% Tris-Glycine gel. Proteins were transferred onto an Immobilon FL membrane and PARP was detected with Trevigen's anti-PARP (C2-10) antibody followed by an IR800-conjugated secondary antibody. The membrane was scanned using an Odyssey Infrared Imaging System (Licor).

IV. Immunocytochemistry

C2-10 can also be used in immunocytochemistry applications. The recommended antibody dilution is 1:1000 but for optimal results the antibody should be titrated. Note that the antibody will recognize the full length and 85 KDa cleaved fragment generated during apoptosis.

References:

- 1) Lamarre D, B. Talbot, G de Murcia, C Laplante, Y Leduc, A Mazen, GG Poirier. (1988). Structural and functional analysis of poly(ADP-ribose) polymerase: an immunological study. *Biochim. Biophys. Acta* 950:147-60.
- 2) Simonin F, JP Briand, S Muller, G de Murcia (1991) Detection of Poly(ADP-ribose) Polymerase in Crude Extracts by Activity-Blot. *Anal Biochem.* 195:226-31.
- 3) Shah GM, D Poirier, C Duchaine, G Brochu, S Desnoyers, J Lagueux, A Verreault, JC Hoflack, JB Kirkland, GG Poirier. (1995) Methods for biochemical study of poly(ADP-ribose) metabolism *in vitro* and *in vivo*. *Anal Biochem.* 227:1-13.

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(Manual Defrost Freezer)
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