PARP1 Enzyme human, lyophilized

Catalog #2090 Lot M1124

LIMITATIONS: THIS PRODUCT IS FOR RESEARCH USE ONLY AND IS NOT APPROVED FOR THERAPEUTIC OR DIAGNOSTIC USE.

Background:

Poly-ADP-ribose metabolism plays a mayor role in a wide range of biological processes, such as maintenance of genomic stability, transcriptional regulation, energy metabolism and apoptosis. At least six poly-ADP-ribose polymerase (PARP) mammalian enzymes have been identified: PARP1, PARP2, PARP3, PARP4/vPARP, PARP5/Tankyrases-1 and PARP6/Tankyrases-2. Poly(ADP-ribose) polymerase-1 (PARP1) is an abundant and ubiquitous nuclear enzyme that catalyzes the NAD(+)-dependent addition of ADP-ribose polymers on a variety of nuclear proteins.

Description:

PARP1 Enzyme, Cat. #2090, is highly purified and enzymatically active human PARP1, expressed in a baculovirus expression system. Upon addition of NAD^+ and cofactors the PARP1 is automodified by the addition of ADP-ribose monomers to form poly(ADP-ribose) polymer. The PARP1 is supplied lyophilized, and upon reconstitution with water, it retains its native poly ADP-ribosylation enzymatic activity. It is useful for high-throughput enzymatic assays, visualization of the automodification reaction by SDS-PAGE and Western blotting, ELISA, standard for SDS-PAGE and WB, and in other assays.

Supplied As:

50 µg PARP1 enzyme, human, lyophilized. When reconstituted with 100 µL H_2O, concentration will be 0.5 mg/mL (see below for reconstitution instructions).

Purity:

Purity >95% by SDS-PAGE.

Enzymatic Activity:

1000 U/vial, specific activity=20000 U/mg PARP1. 1U=10 fmol ADP-ribose incorporated into 5 µg immobilized histone in 30 min at room temperature. Note: Activity measurements are approximate values.

Storage and Stability:

Stable for 6 months from date of shipment when stored desiccated at 4˚C, or more stable at -70˚C.

Reconstitution/Storage:

Spin tube in a microfuge for 15 sec to sediment lyophilized material. Carefully open vial and add 100 µL dH_2O. Vortex gently for 20 sec (avoid air bubbles). Let stand for 5 min. Carefully triturate the sample 10-times using a pipetman (avoid air bubbles). Spin briefly in microfuge to consolidate. Upon reconstitution with 100 µL dH_20, the final concentrations are as follows: 0.5 mg/mL PARP1 enzyme, 20 mM Tris, pH8, 0.3 M NaCl, 0.1 mM EDTA, 1 mM DTT, plus lyophilization stabilizers. Store reconstituted PARP1 enzyme at -70˚C. *CAUTION: There is loss of PARP enzymatic activity upon each free/thaw cycle. It is suggested to aliquot the reconstituted enzyme into multiple tubes and freeze at -70˚C. Alternatively, add glycerol at 1:1 vol/vol to the reconstituted PARP1, mix gently by trituration, and store at -20˚C (do not store in a frost-free freezer!) for up to 6 months.*

SDS-PAGE/WB Standard Preparation:

To the reconstituted PARP1 enzyme, prepared as above, add an equal volume of SDS-PAGE 2x sample buffer containing reducing agent (βME or DTT). Vortex, heat at 90˚C for 2 minutes, then spin briefly. Store at -20 or -70˚C.

Applications and Suggested Dilutions:

Enzyme activity/inhibition assays
Western Blot, 100 ng/well using HRP/TMB; <20 ng/well for ECL.
SDS-PAGE standard, CB stained, 1 µg per well
ELISA, 10 ng/well

Please note: This information is intended as a guide. The optimal conditions must be determined by the user.

Tulip BioLabs Other Related Products:

PARP1, Automodified, human, **Cat. #2095**.
Anti-poly(ADP-ribose) polymer, clone 10H, mouse monoclonal antibody, **Cat. #1020**.
Anti-poly(ADP-ribose) polymer, IgY, chicken polyclonal antibody, **Cat. #1023**.
Anti-PARP1, whole protein, IgY, chicken polyclonal antibody, **Cat. #1051**.

Original Reference:

This product was developed at Tulip Biolabs, Inc.

Product References:

Y. Zhang *et al.* (2013) *Nature Methods* **10**, 981

Useful References:

D. Lamarre *et al.* (1988) *Biochim. Biophys. Acta* **950** 147
Y.A. Lazebnik *et al.* (1994) *Nature (Lond.)* **371** 346
P.J. Duriez *et al.* (1997) *Biochim. Biophys. Acta* **1334** 65
F.R. Sallmann *et al.* (1997) *Biochem. Cell Biol.* **75** 451
B.C. Woodhouse and G.L. Dianov (2008) *DNA Repair (Amst.)* **7** 1077
W.L. Kraus (2008) *Curr. Opin. Cell Biol.* **20** 294
P.O. Hassa and M.O. Hottiger (2008) *Frontiers Bioscience* **13** 3046

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SUGGESTED PROTOCOLS for PARP1 Enzyme, human, Catalog #2090

NOTE: These methods are meant to be used as a guideline. They have been used successfully in specific experiments, but the exact protocol may need to be altered depending on its intended use.

(Refs: Brochu *et al.* (1994) *Anal. Biochem.* **218**:265; Kameshita *et al.* (1984) *J. Biol. Chem.* **259**:4770; Affar *et al.* (1998) *Anal. Biochem.* **259**:280)

Final PARP1 Automodification Assay Conditions
(50mM Tris, pH8.0, 100mM NaCl, 10mM $MgCl_2$, 1mM DTT, 10µg/mL activated DNA, (0, 50µM, or 1mM) NAD^+, 100µg/mL PARP1)

Prepare:

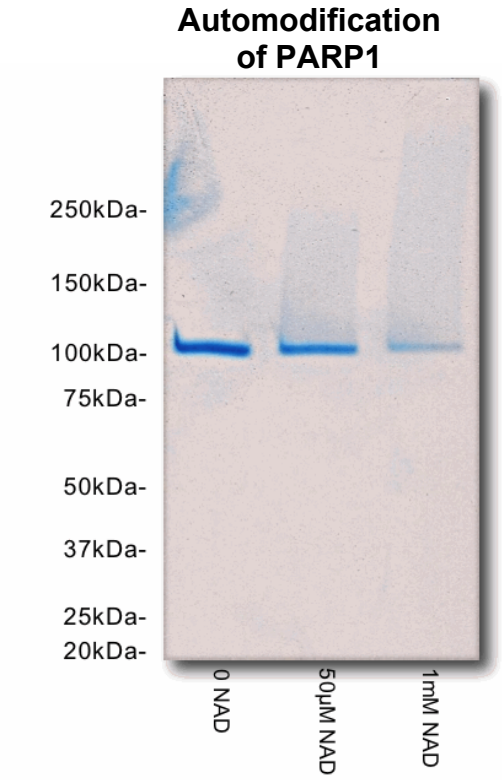
1.25x Reaction Buffer (0 NAD, 50µM NAD, 1mM NAD)

31µL Tris (1M, pH8)
21µL NaCl (3M)
6µL $MgCl_2$ (1M)
0.6µL DTT (1M)
6µL DNA activated (1mg/ml; Sigma D4522)
429µL H_2O

then add NAD:
for 0 add 6.25µL H_2O
for 50 µM add 6.25µL 20-fold diluted NAD (100mM in 100mM Tris, pH7.4)
for 1 mM add 6.25µL β-NAD (100mM in 100mM Tris, pH7.4)
-to make 500µL

REACTION:

1. Add 24µL of 1.25x Reaction Buffer into a microfuge tube.
2. Add 6µL PARP1 (Cat. #2090, reconstituted to 0.5mg/mL) and mix to start reaction.
3. Incubate at RT for 10 min. For highly extensive PARP1 automodification, incubate for 1 hr.
4. Add 30µL of 2x SDS-PAGE sample buffer (w/DTT or BME) and gently boil for 1 min to stop rxn.
5. Run the sample on a 4-15% SDS-PAGE, 25µL/well (1.25µg) for Coomassie blue staining, or use 2µL/well (100ng) for WB (see results).
6. Analyze the sample results directly by Coomassie blue staining, or for much greater sensitivity use Western blotting and visualize using anti-pADPr (Cat. #1023, see datasheet) or anti-PARP1 (Cat. #1051).



SDS-PAGE/Coomassie Blue staining of poly(ADP-ribose) automodified PARP1. PARP1 was incubated with 0, 50µM, or 1mM NAD for 10 min@RT in reaction buffer (see protocol). Samples (0.625µg/lane) were run on SDS-PAGE and stained with Coomassie Blue. With increasing NAD concentrations, the 113kDa PARP1 band decreases and there is a shift to a higher MW smear, indicating poly(ADP-ribose) PARP modified to various extents. At longer incubation times, there is a complete conversion from native to poly ADP-ribosylated PARP1 (not shown).

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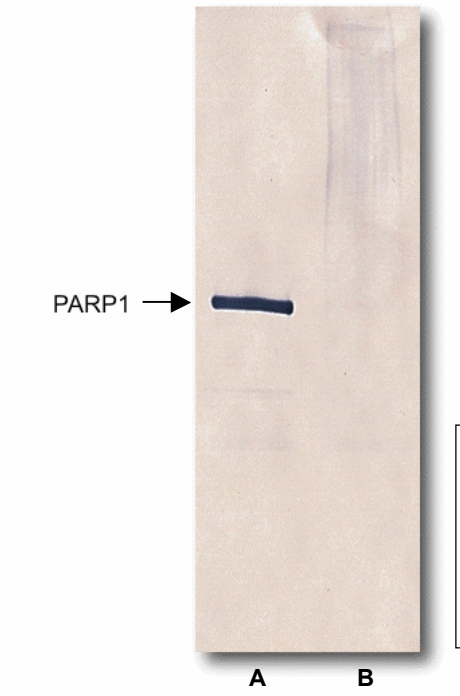
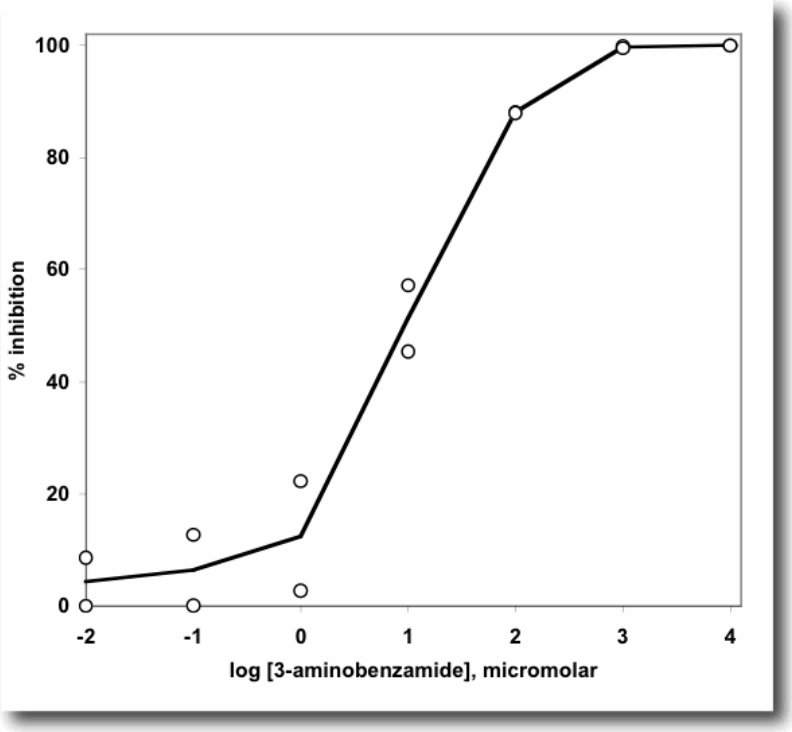
EXAMPLE RESULTS for PARP1 Enzyme, human, Catalog #2090

Automodification of PARP1



Western blot using anti-PARP1 (Cat. #1051). Lane A, PARP1; Lane B, PARP1 automodified by incubation with 1mM NAD for 1h@RT. Note the single PARP1 band in lane A, unmodified PARP. Lane B indicates a smear of staining above MW 113kDa representing polyADP-ribosylated PARP1 modified to various extents. All detectable PARP1 is automodified in B. Blot was developed with HRP/TMB. Each lane has 100ng PARP1.

Inhibition of PARP1 by 3-AB



PARP1 Enzyme Inhibition by 3-amino benzamide. PARP1 enzyme activity was assayed by ADP-ribose incorporation into immobilized histone for 1h@RT. Conditions were PARP1 (Cat. #2090), 15ng/well, preincubated with increasing concentrations of 3-AB, performed in duplicate.

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