

PARP [Poly (ADP-ribose) Polymerase] Activity Test using Products No. ALX-201-063 or ALX-201-064 and ALX-840-040.

1. Additional Materials Required

- Glass Assay Tube, 25mm diameter Whatman Glass Microfibre Filters or equivalent, 25mm diameter vacuum filtration unit;
- Liquid scintillation counter, high flash-point LSC-cocktail aqueous and non-aqueous samples, Ultima Gold MV-Packard;
- 5% TCA, 1% inorganic phosphate solution, 95% ethanol.

2. PARP Activity Test

	Incubation Mixture
Tris-HCl pH 8.....	50 mM
MgCl ₂	4 mM
DTT.....	200 μM
DNA (purified) (DNase I activated) (Prod. No. ALX-840-040).....	200 ng
BSA.....	0.1 μg/μl
NAD.....	40 μM final
[³² P]-NAD.....	0.1 μCi/reaction
PARP-1 (Prod. No. ALX-201-063) or PARP-2 (Prod. No. ALX-201-064).....	200 ng
	Water for 100 μl final volume.

3. Procedure

1. The reaction should be done in triplicate. In a glass tube, incubate the reaction mixture for 10 minutes at room temperature; stop the reaction by adding 4ml of 5% TCA, 1% inorganic phosphate (this will precipitate the protein and the polymer).
2. Filter the reaction solution with the vacuum filtering system (the reaction specific material (pADP-ribose) is retained onto the filter); wash the glass tube 3 x with 4ml of 5% TCA, 1% inorganic phosphate, then with 4 ml of 95% ethanol. Dry the filter and count the radioactivity in a liquid scintillation counter.

4. References

1. *Identification of potential active-site residues in the human poly(ADP-ribose) polymerase:* F. Simonin, et al.; J. Biol. Chem. 268, 8529 (1993)
2. *A novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase:* J.C. Amé, et al.; J. Biol. Chem. 274, 17860 (1999)

The procedures listed above are intended only as a guide. Various assay conditions require that the investigator determine the optimal working concentrations. The results may vary depending on experimental conditions and technique. No warranty or guarantee of performance of above procedure is made or implied. Use good laboratory practices and handle all materials with care. These products and procedures are for in vitro experimental use only and are not intended for use in humans or clinical diagnosis.