

TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Anti-PAR Polyclonal, Affinity Purified

Catalog #: 4336-APC-050

Size: 50 µl

Description: A rabbit polyclonal antibody raised against poly(ADP-ribose) (PAR) polymer. The anti-PAR antibody can be used to detect ribosylated proteins by immunodetection. Trevigen's (PAR) (cat# 4336-100-01) and PAR-PARP (4500-10-P) may be used as positive controls.

Physical State: This antibody is provided as purified IgG fraction in 1X PBS, and 50% glycerol.

Immunogen: Poly(ADP-ribose) polymer (PAR (cat# 4336-100-01)).

Specificity: This polyclonal antibody detects free PAR and poly-ribosylated proteins.

Storage: -20°C (manual defrost freezer).

Applications: For western and dot blotting, an antibody dilution of 1:1000 is recommended. For ELISA a 1:4000 antibody dilution is recommended. Empirical determination of antibody dilutions will be required for optimum results.

Cell Lysates for Western Blotting:

To prepare total cell lysates, cells are solubilized in 1X SDS gel sample buffer (63 mM Tris-HCl, pH = 6.8; 10% glycerol; 2% SDS, 2.5% β-mercaptoethanol, and 0.0025% bromophenol blue) at 1 x 10⁷ cells per ml. The extracts are heated in a boiling water bath for 5 minutes, vortexed for 1 minute at the maximum speed and centrifuged at 10,000 x g for 10 minutes at room temperature. Electrophoresis on 4-20% Tris-Glycine SDS-PAGE gels.

Procedure for Immunoblotting using Peroxidase Detection:

Blotting buffer: 12 mM Tris base, 96 mM Glycine, and 15% MeOH.

Blocking solution: 5% (w/v) nonfat dry milk in TBS-0.1% Tween.

Antibody solution: 5% (w/v) nonfat dry milk, in TBS-0.1% Tween.

Transfer the electrophoresed proteins to a PVDF membrane and incubate the membrane for 1 hour at room temperature in blocking solution.

Incubate the membrane overnight at 4°C in antibody solution containing a 1:1000 dilution of anti-PAR rabbit polyclonal antibody. Empirical determination of primary antibody concentration will be required for optimal results.

Wash the membrane at room temperature for 5 minutes with 3 changes of TBS-0.1% Tween. Changing the membrane containers often reduces background.

Incubate the membrane at room temperature for 1 hour in antibody solution containing anti-rabbit conjugated to horseradish peroxidase.

Empirical determination of secondary antibody concentration will be required for optimal results.

Wash the membrane for 5 minutes with 4 changes of TBS-0.1% Tween.

Develop peroxidase reaction using e.g. chemiluminescence (Trevigen's PeroxyGlow™ A, cat# 4855-20-13, and PeroxyGlow™ B, cat# 4855-20-14).

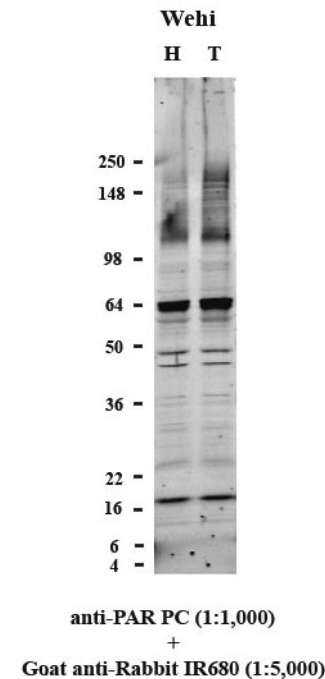


Figure 1. Western blot analysis of Wehi cells untreated (H) and treated (T) with 25 µM Etoposide for 4 hours at 37°C. Cells were lysed in Tris-Glycine SDS sample buffer at the concentration of 1 x 10⁷ cells/ml, and 10 µl of clarified lysate were loaded per well of 4-20% Tris-Glycine gel. Proteins were transferred onto an Immobilon FL membrane and ribosylated proteins were detected using Trevigen's polyclonal anti-PAR antibody (cat# 4336-APC-050) followed by an IR680-conjugated secondary antibody (Licor). The membrane was scanned using an Odyssey Infrared Imaging System (Licor).

References:

- Affar, E.B., et al. 1998. Immunodot blot method for the detection of poly(ADP-ribose) synthesized *in vitro* and *in vivo*. *Anal Biochem* **259**:280-3.
- Shah, G.M., et al. 1995. Methods for biochemical study of poly(ADP-ribose) metabolism *in vitro* and *in vivo*. *Anal Biochem* **227**:1-13.
- Gagné, J-P., et al. 2008. Proteome-wide identification of poly(ADP-ribose) binding proteins and poly(ADP-ribose)-associated protein complexes. *Nucleic Acids Res* **36**:6959-76.

Related Products:

Catalog#	Description	Size
4335-MC-100	Anti-PAR Monoclonal Antibody	100 µl
4510-096-K	PARP <i>in vivo</i> Pharmacodynamic Assay	96 samples

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(Manual Defrost Freezer)

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