TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Anti-Poly (ADP-ribose) Mouse Monoclonal Antibody

Catalog #: 4335-MC-01K-AC

Contents:			
	4335-MC-1K	Anti-PAR monoclonal	Size: 1000 µl
	4500-10-P	PARP treated protein control	10 x 10 µl

Description: Trevigen's monoclonal antibody (cat# 4335-MC-1K) is specific for poly(ADP-ribose) (PAR) polymer. It can be used to detect ribosylated proteins by ELISA, Western blot, immunocytochemistry *in situ,* and for immunopurification. It is supplied with a positive control for ELISA and Western blot consisting of a poly-ADP-ribosylated PARP-1 protein (PARP-PAR).

Storage Conditions: Store the anti-PAR monoclonal at -20°C (manual defrost freezer). Store the PARP-treated protein control at -80°C.

Physical State: This antibody is provided as purified immunoglobulin from mouse ascites in 1X PBS containing 50% glycerol at 1 mg/ml. PARP-PAR is provided at 75 µM in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA.

Immunogen: Purified ADP-ribose polymers between 2 and 50 units long

Ig Class: IgG_3

Specificity: The antibody is specific for PAR polymers 2 to 50 units long, but does not recognize structurally related RNA, DNA, ADP-ribose monomers, NAD, or other nucleic acid monomers.

Applications: ELISA, Western analysis, immunoprecipitation, and immunopurification. For Western blots, an antibody dilution of 1:1000 is recommended.

References:

1. Kupper, J.H., G. de Murcia, and A. Burkle. 1990. Inhibition of poly(ADP-ribosyl)ation by overexpressing the poly(ADP-ribose) polymerase DNA binding domain in mammalian cells. J Biol Chem **265**: 18721-18724.
2. Malik, N., M. Miwa, T. Sugimura, P. Thraves, and M. Smulson. 1983. Immunoaffinity fractionation of the poly(ADP-ribosyl)ated domains of chromatin. PNAS USA **80**:2554-2558.
3. Smulson, M.E. et al.. 1983. In ADP-Ribosylation, DNA Repair and Cancer (Miwa, M., O. Hayaishi, S. Shall, M. Smulson, and T. Sugimura, eds.), pp. 49-70. Tokyo: Japan Scientific Societies Press.
4. Kawamitsu, H., H. Hoshino, H. Okada, M. Miwa, H. Momoi, and T. Sugimura. 1984. Monoclonal antibodies to poly(adenosine diphosphate ribose) recognize different structures. Biochem **23**:3771- 3777.
5. 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**:280-283.

 E3/30/16v2

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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 an Immobilon-FL or 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 Trevigen's anti-PAR monoclonal antibody. Empirical determination of the optimal monoclonal antibody concentration will be required for optimal results.

Wash the membrane at room temperature for 5 minutes with 4 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-mouse conjugated to horseradish peroxidase, or IR800-conjugated secondary antibody (Licor).

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

Wash the membrane for 15 minutes with 4 changes of TBS-0.1% Tween.

Develop peroxidase reaction using e.g. chemiluminescence, or as described in the figure 2 legend.

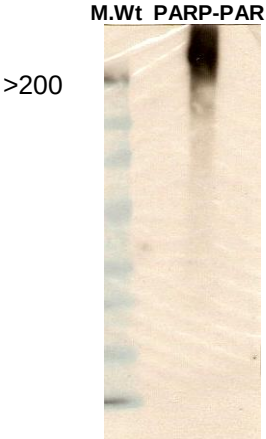

Figure 1. **Western blot analysis of parylated PARP** (cat# 4500-10-P). 15 µl of a 1:10 dilution of PARP-PAR in sample buffer were separated on a 4-20% Tris-Glycine SDS-PAGE gel and blotted on to PVDF membrane.

Ribosylated proteins were detected with 1:1000 dilution of anti-PAR followed by a 1:5000 dilution of HRP conjugated goat anti-mouse IgG and visualized using chemiluminescence.

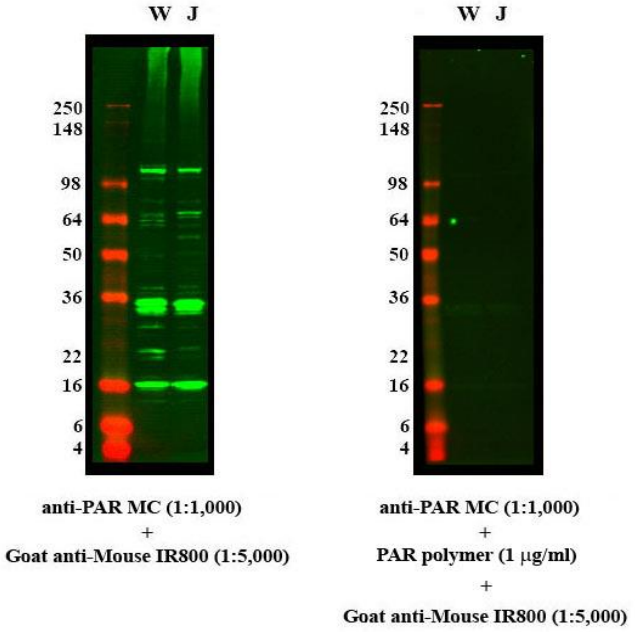

Figure 2. **Western blot analysis of Wehi and Jurkat cells using Trevigen's anti-PAR monoclonal antibody.** Cells were lysed in Tris-Glycine SDS sample buffer at the concentration 1 x 10^7 cells/ml and 10 µl of each lysate were loaded per well of 4-20% Tris-Glycine gel. Proteins were transferred onto an Immobilon FL membrane and ribosylated proteins were detected with Trevigen's anti-PAR antibody (cat# 4335-MC-100) followed by IR800-conjugated secondary antibody (Licor). For an absorption control (right panel), the anti-PAR antibody was pre-incubated with PAR polymer (cat# 4336-100-01). Membranes were scanned using an Odyssey Infrared Imaging System (Licor).

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(Manual Defrost Freezer)
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