TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Anti-PAR Monoclonal Affinity Purified

Catalog #: 4335-AMC-050 Size: 50 µl

Description: Trevigen's Anti-poly (ADP-ribose) (PAR) mouse monoclonal affinity purified antibody (Cat# 4335-AMC-050) is specific for PAR polymer. It can be used to detect ribosylated proteins by ELISA, Western blot, immunocytochemistry *in situ,* and for immunopurification. The antibody was generated against purified ADP-ribose polymers between 2 and 50 units long.

Physical State and Storage Conditions: Affinity Purified monoclonal anti-PAR is provided at 1 mg/ml in 1X PBS containing 50% glycerol. It is stable for at least 1 year at -20 °C. It may be aliquoted to avoid repeated freeze-thawing, and can also be stored at -80 °C.

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

Ig Class: IgG_{3a}

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 from 1:1000 to 1:2000 is recommended.

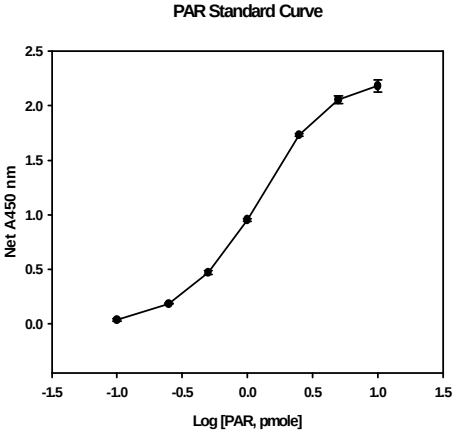

Figure 1: Typical capture ELISA results obtained using Anti-Poly (ADP-ribose) antibody (Cat# 4335-AMC-050) and PARP treated protein control (PARP-PAR) (Cat# 4500-10-P). Serial dilutions of PARP-PAR were applied in triplicate to an anti-PAR monoclonal pre-coated 96-well plate and following incubation for 1.5 hrs, rabbit polyclonal anti-PAR (Cat# 4336-BPC-100) was added and the plate was incubated at room temperature for 1.5 hrs. After washing, goat anti-rabbit IgG-HRP was added and incubated at room tempera-ture for 1 hr. Post washing, wells were developed using TACS-sapphire™ (Cat# 4822-96-08) for 5 minutes, and the reaction was stopped using phosphoric acid. The A_{450} was determined using a plate reader.

 P4/9/08v1

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Procedure for Quantification of PAR by Capture ELISA

1. Dilute anti-PAR monoclonal antibody (Cat# 4335-AMC-050) to 2 ng/µl in 50 mM sodium carbonate buffer, pH 9.6 and distribute 50 µl/well in a 96 well plate designed for immunoassays. Incubate overnight at 4 °C. Block the plate with 100 µl/well Blocking/antibody solution for 1 hr.
2. Wash the wells 4 times with 1X PBS + 0.1% Tween 20.
3. PARP-PAR Control (Cat# 4500-10-P) is provided at a concentration of 75 µM (or 75 pmol/µl). Serially dilute the PAR standard with Blocking/antibody solution to make at least 200 µl of each dilution just before use. The recommended final concentrations are 10 pmole/50 µl, 5 pmole/50 µl, 2.5 pmole/50 µl, 1 pmole/50 µl, 0.5 pmole/50 µl, 0.25 pmole/50 µl, and 0.1 pmole/50 µl. Include triplicate wells with no PAR for the background control. The standard curve requires 50 µl/well of each PAR dilution and each is performed in triplicate.
4. Add 50 µl/well of the serial dilutions of PARP-PAR Control and incubate at room temperature for 1.5 hr.
5. Wash the wells 4 times with 1X PBS + 0.1% Tween 20.
6. Dilute Rabbit anti-PAR polyclonal antibody (Cat# 4336-BPC-100) 1,000-fold with Blocking/antibody solution. Add 50 µl per well of diluted anti-PAR Polyclonal Antibody to all the wells. Incubate at room temperature for 1.5 hours.
7. Wash the wells 4 times with 1X PBS + 0.1% Tween 20.
8. Add 50 µl per well of Goat anti-Rabbit IgG-HRP conjugate (1:5,000 dilution). Incubate at room temperature for 1.5 hours.
9. Wash the wells 4 times with 1X PBS + 0.1% Tween 20.
10. Add 50 µl per well of TACS-Sapphire™ (Cat# 4822-96-08) HRP colorimetric substrate and incubate, **in the dark**, for 5-10 minutes. Stop the reactions by adding 50 µl per well of 0.2M HCl or 5% Phosphoric Acid and read the absorbance at 450 nm in a colorimetric plate reader.
11. Subtract the mean background A_{450} nm values from the mean A_{450} nm values of the PAR standards. Plot the net mean absorbance data of the PAR standards as described in Fig. 1. Determine the levels of PAR in your samples from the standard curve.

References:

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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.
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6. Putt, K.S. and P.J. Hergenrother. 2004. A nonradiometric, high-throughput assay for poly(ADP-ribose) glycohydrolase (PARG): application to inhibitor identification and evalution. Anal Biochem **333**:256-264.

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