Poly-ADP-ribose Affinity Resin Set — Catalog #4301

One Set Contains 1 each of the following…

Poly-ADP-ribose Affinity Resin — Catalog #2302

Poly-ADP-ribose Neg Control Resin — Catalog #2303

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

Background:

The Tulip Biolabs, Inc. Poly-ADP-ribose (PAR) Affinity Resin Set is designed for the isolation and study of intracellular poly-ADP-ribosylated (PARylated) proteins. Through the use of a highly specific PAR affinity resin, PARylated proteins are isolated from cell or tissue lysates without the use of anti-PAR antibodies. The resin bound proteins can be eluted from the affinity resin, and analyzed by immunoblotting or other methods.

Af1521 is a thermopohilic protein from *Archaeoglobus fulgidus,* and contains a conserved ~190 amino acid domain known as the macro domain. Macrodomains are found in a wide variety of organisms including bacteria, viruses, and vertebrates. Expressed and purified macrodomains from Af1521, Alc1, macroH2A and Bal/PARP9 proteins have been shown to bind polymeric ADP-ribose modified proteins with high specificity and affinity.

Description:

The Poly-ADP-ribose Binding Domain Resin Set, Cat. #4301 contains 1 each of the following:

- Poly-ADP-ribose Binding Domain Resin 1mL (1mg fusion protein supplied as a slurry containing ~75µL packed resin), Cat. #2302
- Poly-ADP-ribose Neg Control Resin 1mL (1mg fusion protein supplied as a slurry containing ~75µL packed resin), Cat. #2303

PAR Affinity Resin, Cat. #2302 is highly purified GST-Af1521 macrodomain fusion protein construct expressed in *E. coli*, and bound to glutathione beads. It is useful for affinity purification (pulldown) of PARsylated proteins as well as PAR polymer. The Af1521 macrodomain protein is also reported to bind mono-ADP-ribosylated proteins and ADP-ribose.

PAR Negative Control Resin, Cat. #2303 is identical to the #2302 resin except for two gly to asp substitutions, which abolish PAR binding. The negative control resin is useful to control for non-specific binding, and its use is optional.

Supplied As:

Each vial contains 1mg purified GST-macrodomain-fusion protein bound to 50-75µL packed volume of glutathione beads in 1 mL buffer containing 0.1M Tris, pH 8, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 0.02% sodium azide.

Purity:

GST-macrodomain fusion protein purity >95% by SDS-PAGE.

Storage and Stability:

Stable for 6 months from date of shipment when stored at 4˚C. DO NOT FREEZE!

Applications and Suggested Quantities:

Use 20µL (20µg) suspended resin to affinity purify/pull-down poly-ADP-ribose modified proteins in 0.15-1mg cell and tissue extracts. Analyze by Western blotting using protein specific antibodies to probe the immunoblot. Each 1mL vial is sufficient for analysis of ~50 samples.

Please note: This information is intended as a guide. The optimal quantities 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.

Background References:

G.I. Karras *et al*. (2005) *EMBO J*. **24** 1911 [PMID: 15902274]
G. Timinszky *et al*. (2009) *Nature Struct. Molec. Biol*. **16** 923 [PMID: 19680243]
A.J. Gottschalk *et al*. (2009) *PNAS* **106** 13770 [PMID: 19666485]
N. Dani *et al*. (2009) *PNAS* **106** 4243 [PMID: 19246377]

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SUGGESTED GENERAL PROTOCOL for PAR Affinity resin set, Catalog #4301

MATERIALS REQUIRED

Lysis buffer (e.g.: 50mM Tris, pH 8, 200mM NaCl, 1mM EDTA, 1% Triton X-100, 10% glycerol, 1 mM DTT, 0.5% deoxycholate, and protease inhibitors)
Cell/tissue extract containing ~0.15 to 1mg total protein per sample
Microcentrifuge tubes
Microcentrifuge
SDS-PAGE sample buffer

PROCEDURE

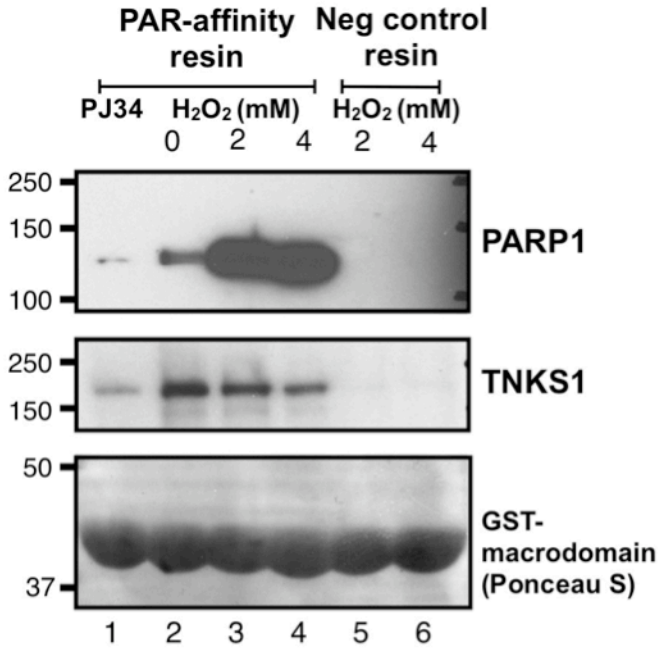
1. Resuspend the PAR affinity and neg control resins by gently inverting the product tubes several times to obtain a homogenous suspension of resin.
2. Use a wide-bore pipette or a cut pipette tip to transfer 20µL of the suspension to ~1mL of lysis buffer in a microfuge tube.
3. Sediment resin at 15k x g in a microfuge (highest speed setting) for 20 sec. Carefully remove most of the lysis buffer, leaving the resin (barely visible) undisturbed in the tube. NOTE: Position tubes in the microfuge with the hinge oriented outward in order to ascertain the location of the sedimented resin.
4. Add cell/tissue extract in lysis buffer to the microfuge tube containing the resin. Suggested extract protein amount is 0.15 to 1mg in a total buffer volume of 0.5mL.
5. Incubate the reaction for several hours or overnight at 4˚C on a Nutator or similar device.
6. Sediment then wash resin 3-times with 0.5-1mL lysis buffer, as in step 3. On the final wash, carefully remove residual buffer without disturbing the resin.
7. Add 75µL 1X SDS-PAGE sample buffer to each tube, agitate, then incubate at 95˚C for 10 min to dissociate GST-macrodomain from PARylated proteins and the resin.
8. Run samples on SDS-PAGE, and perform Western blotting. Probe immunoblot using desired protein-specific antibodies, for example anti-PARP1 (Cat. #1051), or anti-poly-ADP-ribose antibodies (#1020 or #1023) to detect affinity purified proteins. Compare results to negative control resin samples to assess non-specific binding, which should be minimal.

EXAMPLE RESULTS: MDCK CELLS STABLY EXPRESSING TNKS1
(NOTE: Refer to Suggested General Protocol)

PROCEDURE:

1. MDCK cells grown to confluence in 6 cm plates were treated for 1 hr with PJ-32 (50µM) to inhibit cellular PARPs or with H_2O_2 (2 and 5mM) to selectively activate PARP1 through DNA damage.
2. MDCK cells were harvested at 4˚C in 0.5mL lysis buffer (50mM Tris, pH 8, 200mM NaCl, 1mM EDTA, 1% Triton X-100, 10% glycerol, 1 mM DTT, 0.5% deoxycholate, and protease inhibitors).
3. After clarification by centrifugation at 15k x g for 10 min, lysates (~0.5mL) were incubated with PAR affinity resin or neg control resin (15µL suspended resin; see Suggested General Protocol) with agitation at 4˚C overnight.
4. Resins were sedimented with associated proteins in a microfuge at 15k x g for 20 sec, supernatant discarded.
5. Resin was washed 3-times with 500µL lysis buffer.
6. 100µL SDS-PAGE sample buffer was added to each tube and incubate dat 65˚C for 15 min to dissociate macrodomain fusion protein from affinity-precipitated proteins.
7. SDS-PAGE and Western blotting of samples were performed. Immunoblots were probed with anti-PARP1 and anti-TNKS1 antibodies, and detected using ECL. Blot was stained with Ponceau S to visualize the GST-fusion protein to confirm equal loading of resin/precipitates.

RESULTS:



Western blot of Poly-ADP-ribosylated PARP1 and TNKS1 in MDCK cells using PAR-affinity resins. MDCK cells were treated for 1hr with PARP inhibitor PJ-34 (lane 1), no inhibitor (lane 2), or PAR-induced with 2mM (lanes 3 and 5) or 4mM (lanes 4 and 6) H_2O_2. Cells were lysed and extracts incubated with PAR-affinity resin (lanes 1-4) or Neg Control resin (lanes 5-6). Western blots were probed with anti-PARP1 (upper panel) or anti-tankyrase1 (middle panel). The GST-fusion protein was visualized using Ponceau S to confirm equal loading of the PAR Affinity and Neg Control resins.

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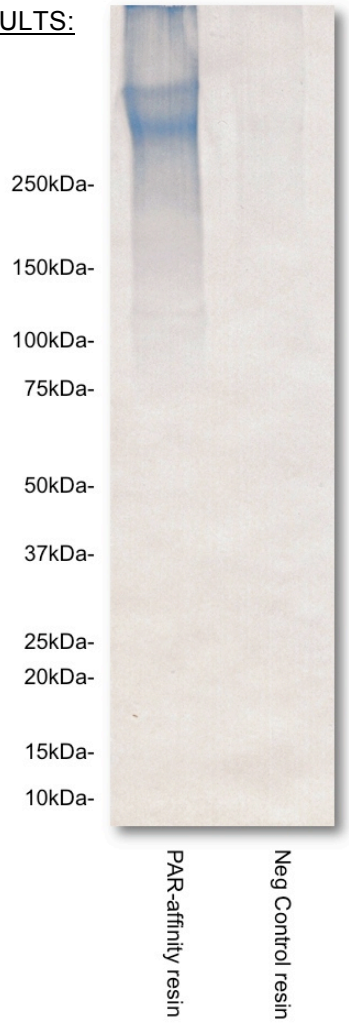
EXAMPLE RESULTS: PURIFIED POLY-ADP-RIBOSYLATED PARP1
(NOTE: Refer to Suggested General Protocol)

PROCEDURE:

1. Wash and sediment 20µL PAR affinity and neg control resins in 1.5mL microfuge tubes (see Suggested General Protocol).
2. Add 500µL TBST (20mM Tris, pH7.4, 0.15M NaCl, 0.05% Tween20) to each tube.
3. Add 400ng of purified poly-ADP-ribosylated PARP1 (Cat. #2095) to each tube. Resuspend resin by tapping tube and gentle mixing.
4. Incubate extract/resin for 2 hr at room temperature. Gently agitate tube periodically.
5. Sediment resin, then wash resin 3-times with 500µL TBST (see Suggested General Protocol). On the final wash, carefully remove residual buffer using a pipette (e.g.: yellow tip). NOTE: Be careful not to disturb resin.
6. Add 100µL SDS-PAGE sample buffer to each tube, agitate, and then incubate at 90˚C for 5 min to dissociate macrodomain fusion protein, affinity purified poly-ADP-ribosylated PARP1, and the resin.
7. Run samples on SDS-PAGE, and perform Western blotting. Use anti-poly-ADP-ribose, clone 10H, Cat. #1020 to blot according to product suggested protocol.

RESULTS:



Pull-down of Poly-ADP-ribosylated PARP1 by PAR-affinity resin. Purified poly-ADP-ribosylated PARP1 (Cat. #2095; 400ng) was incubated with PAR-affinity resin or Neg Control resin, washed, then dissociated with SDS-PAGE sample buffer. Samples were then immunoblotted with anti-poly-ADP-ribose, clone 10H (Cat. #1020). Note the PAR-affinity resin pulls-down poly-ADP-ribosylated PARP1, shown by a smear of high MW protein, >113kDa, whereas the negative control resin does not.

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