

Af1521 Macrodomein Affinity Resin Set
Catalog #4301
One Set Contains 1 each of the following...
Af1521 Macrodomein Affinity Resin
Catalog #2302
Af1521 Macrodomein 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. Af1521 Macrodomein Affinity Resin Set is designed for the isolation and study of mono- (MARylated) and poly-ADP-ribosylated (PARylated) proteins. Through the use of this affinity resin, MARylated and PARylated proteins can be isolated from cell or tissue lysates. The resin bound proteins can be eluted from the affinity resin, and analyzed by immunoblotting or other methods.

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

Description:

The Af1521 Macrodomein Affinity Resin Set, Cat. #4301 contains 1 each of the following:

- Af1521 Macrodomein Affinity Resin 1mL (1mg fusion protein supplied as a slurry containing ~100µL packed resin), Cat. #2302
- Af1521 Macrodomein Neg Control Resin 0.5mL (0.5mg fusion protein supplied as a slurry containing ~75µL packed resin), Cat. #2303

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

Af1521 Macrodomein Negative Control Resin, Cat. #2303 is identical to the #2302 resin except for two gly to asp substitutions, which abolish MAR and PAR binding. The negative control resin is useful to control for non-specific binding, and its use is optional.

Supplied As:

Each vial contains either 1mg purified GST-macrodomein- fusion protein bound to approximately 100µL packed volume of glutathione beads in 1 mL

buffer and/or 0.5mg purified GST-macrodomein-negative control mutant fusion protein bound to approximately 75µL packed volume of glutathione beads in 0.5mL buffer. The buffer composition is phosphate buffered saline with 1 mM EDTA, 1% Triton X-100, and 0.02% sodium azide.

Purity:

GST-macrodomein 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 mono- and 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.
 Af1521 Macrodomein Mag Resin, Cat. #2305 (same as #2302 except mag resin).
 Af1521 Macrodomein Mag Resin, Cat. #2426 (covalently bound mag resin).

Original Reference:

This product was developed at Tulip Biolabs, Inc.

Product References:

S M Hoang *et al* (2020) *Nature Struct. Molec. Biol.* doi.org/10.1038/s41594-020-0512-7
 C.M. Daniels *et al.* (2014) *J Proteome Res* **13**: 3510
 J-P. Gagne *et al.* (2012) *Nucleic Acids Res* **40**: 7788

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 Af1521 Macrodomein 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

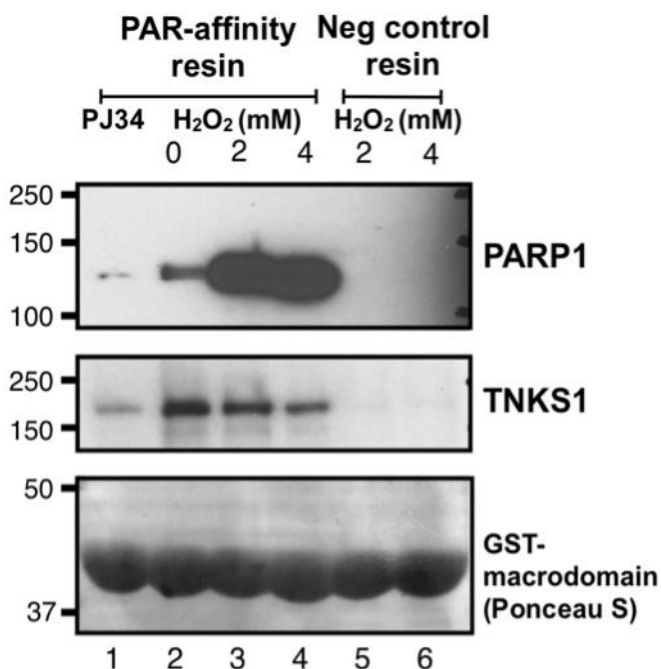
PROCEDURE

1. Resuspend the Macrodomein 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 20 μ L 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 rotisserie 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 75 μ L 1X SDS-PAGE sample buffer to each tube, agitate, then incubate at 95°C for 10 min to dissociate GST-macrodomein from MARYlated and PARYlated proteins from 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 (50 μ M) to inhibit cellular PARPs or with H₂O₂ (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 Af1521 Macrodomain Affinity resin or neg control resin (15 μ L 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 500 μ L lysis buffer.
6. 100 μ L SDS-PAGE sample buffer was added to each tube and incubate at 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.

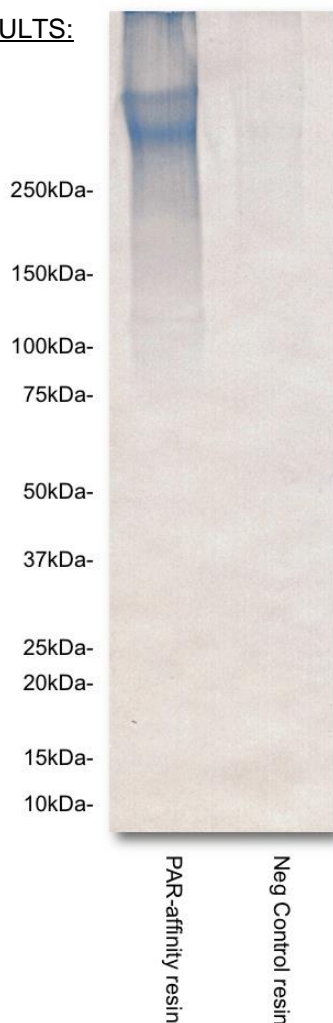
RESULTS:


Western blot of Poly-ADP-ribosylated PARP1 and TNKS1 in MDCK cells using Af1521 Macrodomain 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) H₂O₂. Cells were lysed and extracts incubated with Af1521 Macrodomain 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.

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

PROCEDURE:

1. Wash and sediment 20 μ L Af1521 Macrodmain Affinity and neg control resins in 1.5mL microfuge tubes (see Suggested General Protocol).
2. Add 500 μ L 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 500 μ L 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 100 μ L 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 Af1521 Macrodmain Affinity resin. Purified poly-ADP-ribosylated PARP1 (Cat. #2095; 400ng) was incubated with Af1521 Macrodmain 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