Af1521 Macrodomain Magnetic Resin Catalog #2426

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

THE FOLLOWING INFORMATION IS INTENDED ONLY AS A GUIDE. THE USER MUST VALIDATE THE EXPERIMENTAL CONDITIONS FOR SUITABILITY OF THEIR INTENDED PURPOSE.

Background:

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

The Tulip Biolabs, Inc. Cat. #2426 Af1521 Macrodomain Magnetic Resin is highly purified Af1521 macrodomain protein covalently bound to super-paramagnetic silica-based beads (resin). It is intended for the isolation and study of mono- and poly-ADP-ribosylated (MARylated/PARylated) proteins. With this MAR/PAR affinity resin, MARylated and PARylated proteins can be isolated directly from cell or tissue lysates and analyzed by immunoblotting or other methods. A negative control magnetic resin, Cat. #2427, is also available for the determination of non-specific binding.

Description:

Cat. #2426 Af1521 Macrodomain Magnetic Resin is highly purified Af1521 macrodomain fusion protein expressed in *E. coli*, and covalently bound to super-paramagnetic silica-based beads (resin). Typical conditions for the elution of the affinity bound proteins to the resin, for example SDS-PAGE sample buffer, will not dissociate the Af1521 macrodomain affinity protein from the resin. The magnetic resin beads are small, average 1µm diameter, and remain suspended in solution for a few minutes after mixing allowing for convenient and accurate pipetting.

The resin is useful for affinity purification (pulldown) of MARylated and PARylated proteins. Tulip Biolabs Cat. #2302 (non-magnetic) and #2305 (magnetic) macrodomain beads are composed of the same affinity protein except non-covalently bound to glutathione beads.

Supplied As:

Each vial contains 0.5mg purified Af1521 macrodomain fusion protein covalently bound to 1µm superparamagnetic resin beads (orange dyed) in 0.5 mL buffer (50 mM Tris pH 8, 150 mM NaCl, 1 mM EDTA, 0.5mM TCEP, and 0.02% sodium azide).

Purity:

Af1521 macrodomain fusion protein purity is >95% by SDS-PAGE.

Storage and Stability:

To prevent desiccation of the resin, briefly centrifuge the tube after each use to consolidate any resin adhering along the walls/lid of the vial. Store the vial upright at 4˚C. Stable for 6 months from date of shipment when stored at 4˚C. DO NOT FREEZE!

Applications and Suggested Quantities:

Just before use, gently vortex the vial for ~30 sec to resuspend the resin. Use 20µL (20µg) suspended resin slurry to affinity purify/pull-down mono- and poly-ADP-ribose modified proteins in 0.15-1mg cell or tissue lysate. Analyze proteins by Western blotting with protein-specific antibodies to probe the immunoblot; mass spectrometry protein analysis; or other methods as desired. Each 0.5mL vial is typically sufficient for analysis of ~25 samples.

Note that contaminating proteins may be co-eluted with the #2426 Af1521 macrodomain mag resin depending on the experimental conditions. Therefore, additional methods are required to confirm the identity and modification of the isolated proteins. A negative control magnetic resin, Cat. #2427, is available from Tulip Biolabs to help assess non-specific binding.

Tulip BioLabs Related Mag Resin Products:

PARP14m3 Mag Resin (MARylation), Cat. #2414
WWE Domain Mag Resin (PARylation), Cat. #2438
Neg Control Mag Resin (non-specific), Cat. #2427

Tulip BioLabs Other Related Products:

PAR Affinity Resin Set (Macrodomain), Cat. #4301
Af1521 Macrodomain Mag Resin, Cat. #2305
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

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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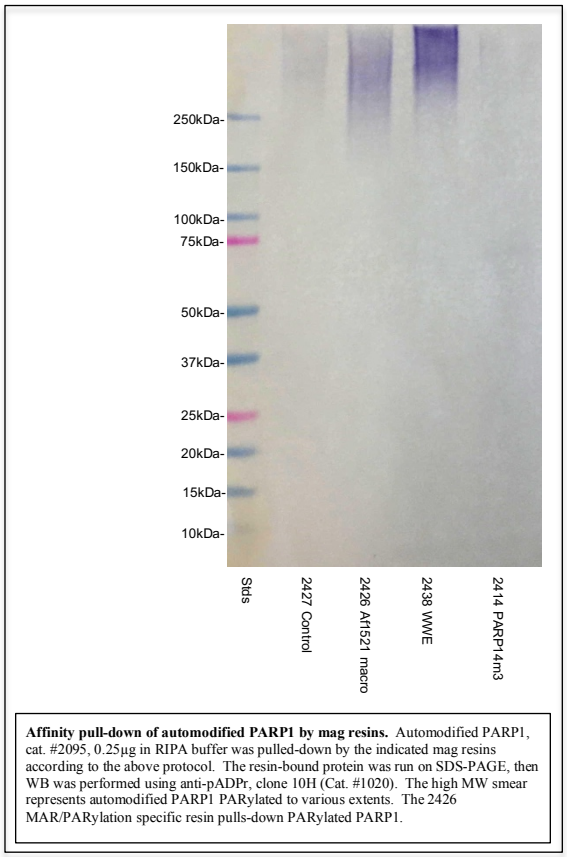
SUGGESTED GENERAL PROTOCOL for Af1521 Macrodomain Mag Resin, Cat. #2426

MATERIALS REQUIRED

Lysis/RIPA buffer (50mM Tris, pH7.5, 0.4M NaCl, 1mM EDTA, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 1mM DTT)
Cell/tissue extract containing ~0.15 to 1mg total protein per sample, at or below 2mg/mL concentration
Microcentrifuge tubes
Magnetic separator
SDS-PAGE sample buffer

PROCEDURE

1. Resuspend the Af1521 macrodomain mag resin by gently vortexing the product tube for approximately 30 seconds to obtain a homogenous suspension.
2. Pipette transfer 20µL of the resin suspension to 1mL of Lysis/RIPA buffer in a microfuge tube (cut the end of the tip if necessary). Then, briefly vortex the tube to thoroughly mix the transferred beads.
3. Place the tube in a magnetic separator (user supplied), wait 2-10 minutes to allow consolidation of the resin, then carefully remove the Lysis/RIPA buffer using a pipettor leaving the orange resin in the tube. Repeat the washing step by adding 1mL fresh Lysis/RIPA buffer to the resin, briefly vortexing, and removing excess buffer.
4. Add clarified cell/tissue extract in Lysis/RIPA buffer to the microfuge tube containing the washed resin. Suggested extract protein amount is 0.15 to 1mg in a total buffer volume of 0.5mL.
5. Incubate the magnetic resin/extract mixture on a Nutator, rotator, or similar device for several hours to overnight at 4˚C to allow binding of the target proteins to the resin.
6. Wash resin with 1mL of Lysis/RIPA buffer 3-times as in step 3. Perform a final wash in PBS (or desired buffer), then carefully remove all of the residual buffer without disturbing the resin.
7. Add 75µL 1X SDS-PAGE sample buffer to each tube, agitate, then incubate at 90˚C for 1 min to dissociate the proteins from the resin. Note that the Af1521 macrodomain affinity protein will remain bound to the beads.
8. Run samples on SDS-PAGE, and perform Western blotting. Probe immunoblot using desired antibodies to detect affinity purified proteins. Or, use preferred methods of detection/analysis.



Affinity pull-down of automodified PARP1 by mag resins. Automodified PARP1, cat. #2095, 0.25µg in RIPA buffer was pulled-down by the indicated mag resins according to the above protocol. The resin-bound protein was run on SDS-PAGE, then WB was performed using anti-pADPr, clone 10H (Cat. #1020). The high MW smear represents automodified PARP1 PARylated to various extents. The 2426 MAR/PARylation specific resin pulls-down PARylated PARP1.

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