Af1521 Macrodomain Affinity Magnetic Resin Catalog #2305

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

Background:

The Tulip Biolabs, Inc. Af1521 Macrodomain Affinity Magnetic resin is designed for the isolation and study of intracellular poly (PARylated) and mono (MARylated) -ADP-ribosylated proteins. Through the use of this highly specific PAR and MAR affinity resin, PARylated and MARylated proteins are isolated from cell or tissue lysates. The resin bound proteins can be eluted from the affinity resin, and analyzed by immunoblotting, mass spec, or other methods. This is the magnetic bead version of the Tulip Biolabs Cat. #2302 PAR Affinity Domain resin.

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.

Description:

The Af1521 Macrodomain Affinity Magnetic Resin, Cat. #2305, is highly purified GST-Af1521 macrodomain fusion protein construct expressed in *E. coli*, and bound to magnetic glutathione beads. The superparamagnetic beads have an approximate diameter of 5µm. It is useful for the affinity purification (pulldown) of PARylated and MARylated proteins as well as PAR polymer.

Supplied As:

Each vial contains 1mg purified GST-macrodomain-fusion protein bound to approximately 100µL packed volume of glutathione magnetic beads in 1 mL buffer. The buffer composition is phosphate buffered saline with 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 25µL (25µg) suspended resin to affinity purify/pull-down poly-ADP-ribose modified proteins in 0.15-1mg cell and tissue extracts. Analyze by mass spec or Western blotting using protein specific antibodies to probe the immunoblot. Each 1mL vial is sufficient for analysis of ~40 samples.

Please note: This information is intended as a guide. The optimal quantities must be determined by the user.

Tulip BioLabs Other Related Products:

WWE Affinity Resin Set, Cat. #4306
PAR Affinity Resin Set, Cat. #4301
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.

Product References:

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 WESTERN BLOTTING PROTOCOL for Af1521 Macrodomain Affinity Magnetic Resin, Catalog #2305

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
Magnetic stand
SDS-PAGE sample buffer

PROCEDURE

1. Resuspend the Af1521 macrodomain affinity magnetic resin by gently inverting the product tube several times to obtain a homogenous suspension of resin.
2. Use a wide-bore pipette or a cut pipette tip to transfer 25µL of the suspension to ~1mL of lysis buffer in a microfuge tube. Gently invert the tube a few times to resuspend the beads.
3. Sediment the magnetic resin by placing the tube in a magnetic stand (not supplied) for a few minutes. Withdraw and discard the supernatant.
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 0.5 to 1mL.
5. Incubate the reaction for several hours or overnight at 4˚C on a Nutator or similar device.
6. Sediment the beads 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 3 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.

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