Af1521 Macrodomain Affinity Mag Resin Set Catalog #4319

One Set Contains 1 each of the following…

Af1521 Macrodomain Affinity Mag Resin Catalog #2305

Af1521 Neg Control Mag Resin Catalog #2306

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 Mag Resin Set is designed for the isolation and study of mono- (MARylated) and poly-ADP-ribosylated (PARylated) proteins. Through the use of this affinity magnetic 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. This is the magnetic bead version of the Tulip Biolabs Cat. #4301 Af1521 Macrodomain 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 macrodomains 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:

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

Af1521 Macrodomain Negative Control Resin, Cat. #2306 is identical to the #2305 magnetic resin except for two gly to asp substitutions in the Af1521 sequence, which abolish MAR and PAR binding. The negative control mag resin is useful to control for non-specific binding, and its use is optional.

Supplied As:

The Af1521 Macrodomain Affinity Resin Set, Cat. #4319 contains 1 each of the following:

- Af1521 Macrodomain Affinity Mag Resin 1mL (1mg fusion protein supplied as a slurry containing ~100µL packed magnetic resin), Cat. #2305
- Af1521 Neg Control Mag Resin 0.5mL (0.5mg fusion protein supplied as a slurry containing ~75µL packed resin), Cat. #2306

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 20µL (20µg) suspended magnetic 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 Macrodomain Mag Resin, Cat. #2305 (same as #2302 except mag resin).
Af1521 Macrodomain 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 Macrodomain Affinity resin set, Catalog #4319

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 or neodymium magnet
SDS-PAGE sample buffer

PROCEDURE

1. Resuspend the Macrodomain affinity and neg control mag 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 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 (or use neodymium magnet) 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 90˚C for 1 min to dissociate GST-macrodomain from MARylated/PARylated proteins and the mag 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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