Af1521 Macrodomain Covalent Resin Catalog #2506

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 mono- and poly-ADP-ribosylated (MARylated/PARylated) proteins with high specificity and affinity.

Description:

Tulip Biolabs Cat. #2506 Af1521 Macrodomain Covalent Resin is highly purified Af1521 macrodomain fusion protein expressed in *E. coli*, and covalently bound to 4% cross-linked agarose (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 protein from the resin.

The intended use of Cat. #2506 affinity resin is to isolate MARylated and PARylated proteins directly from cell or tissue lysates for analysis by immunoblotting or other methods.

Supplied As:

Each vial contains 0.5mg purified Af1521 macrodomain fusion protein covalently bound to 4% cross-linked agarose (~50µL packed beads) in 0.5 mL buffer (50 mM Tris pH 8, 150 mM NaCl, 1 mM EDTA, 1mM 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 ~10 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 specific antibodies to probe the immunoblot; mass spectrometry protein analysis; or other methods as desired. Each 0.5mg vial is typically sufficient for analysis of ~25 samples.

Note that contaminating proteins may be co-eluted with the #2506 Af1521 macrodomain covalent resin depending on the experimental conditions. Therefore, additional methods are required to confirm the identity and modification of the isolated proteins. A negative control covalent resin, Cat. #2507, is available from Tulip Biolabs to help assess non-specific binding.

Tulip BioLabs Af1521 Macrodomain Products:

Af1521 Macrodomain Mag Resin, Cat. #2426 *(same as Cat. #2506 except covalently bound to magnetic resin).*

Af1521 Macrodomain Affinity Resin, Cat. #2302 *(same as Cat. #2506 except non-covalently bound to glutathione resin).*

Negative Control Covalent Resin, Cat. #2507 *(negative control resin for Cat. #2506).*

Tulip BioLabs Other Related Products:

PARP14m3 Mag Resin, Cat. #2414 (MARylation).

WWE Domain Mag Resin, Cat. #2438 (PARylation)

***PARP1, Automodified, human,* Cat. #2095,** a useful positive control for PARylation.

***Anti-poly(ADP-ribose) polymer, clone 10H, mouse monoclonal antibody,* Cat. #1020**, for detection of PARylation.

***Anti-poly(ADP-ribose) polymer, IgY, chicken polyclonal antibody,* Cat. #1023**, for detection of PARylation.

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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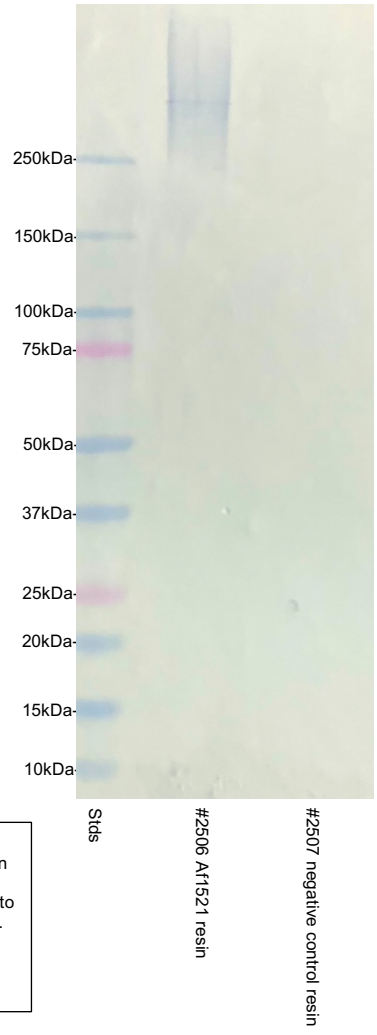
SUGGESTED GENERAL PROTOCOL
Cat. #2506 Af1521 Macrodomain Covalent Resin

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
SDS-PAGE sample buffer

PROCEDURE

1. Resuspend the Af1521 macrodomain covalent resin by gently vortexing the product tube for approximately 10 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 pipette tip if necessary). Then, briefly vortex the tube to thoroughly mix the transferred beads.
3. Centrifuge the tubes at 10k x g for 1min at 4˚C, then carefully remove the Lysis/RIPA buffer using a pipettor leaving the washed resin in the tube.
4. Add clarified cell/tissue extract solubilized 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 covalent resin/extract mixture on a Nutator, rotator, or similar device for >2 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 2-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. Automodified PARP1 (Cat. #2095), 0.25µg, in RIPA buffer was pulled-down by Cat. #2506 Af1521 Covalent resin according to the suggested protocol. The resin-bound protein was subjected to WB with detection using anti-pADPr, clone 10H (Cat. #1020). The high MW smear represents automodified PARP1 PARylated to various extents. The Cat. #2507 Negative Control resin shows no pull-down.

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