WWE Domain Magnetic Resin Catalog #2438

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:

RNF146 (Iduna) is a RING-domain E3 ubiquitin ligase that positively regulates Wnt signalling. RNF146 directly interacts with poly(ADP-ribose) through its WWE domain. The WWE domain is a conserved globular domain found in multiple PARPs and E3 ligases. Expressed and purified WWE domain has been shown to bind polymeric ADP-ribose modified proteins with high specificity and affinity.

The Tulip Biolabs, Inc. Cat. #2438 WWE Domain Magnetic Resin is a fusion protein of WWE PAR-binding domain of human RNF146, covalently bound to super-paramagnetic silica-based beads (resin). It is intended for the isolation and study of poly-ADP-ribosylated (PARylated) proteins. With this PAR affinity resin, 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. #2438 WWE Domain Magnetic Resin is highly purified WWE Domain 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 WWE Domain affinity protein from the resin. The magnetic resin beads are small, with an average 1µm diameter, which remain suspended in solution for a few minutes after mixing, allowing for convenient and accurate pipetting. For comparison, Tulip Biolabs Cat. #2334 WWE domain resin is composed of the same affinity protein except non-covalently bound to glutathione beads (non-magnetic).

Supplied As:

Each vial contains 0.5mg purified WWE Domain fusion protein covalently bound to 1µm super-paramagnetic 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:

WWE Domain 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 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 #2438 WWE Domain 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
Af1521 Macrodomain Mag Resin (MAR/PARylation), Cat. #2426
Neg Control Mag Resin (non-specific), Cat. #2427

Tulip BioLabs Other Related Products:

WWE Affinity Resin Set Cat. #4306
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:

H.C. Kang *et al.* (2011) *PNAS* **108** 14103
Y. Zhang *et al.* (2011) *Nature Cell Biol.* **13** 623
M.G. Callow *et al.* (2011) *PLoS ONE* **6** e22595
S.A. Andrabi *et al.* (2011) *Nature Medicine* **17** 692
Z.D. Zhou *et al.* (2011) *Cell Adh. Migr.* **5** 463
Z. Wang *et al.* (2012) *Genes & Dev.* **26** 235

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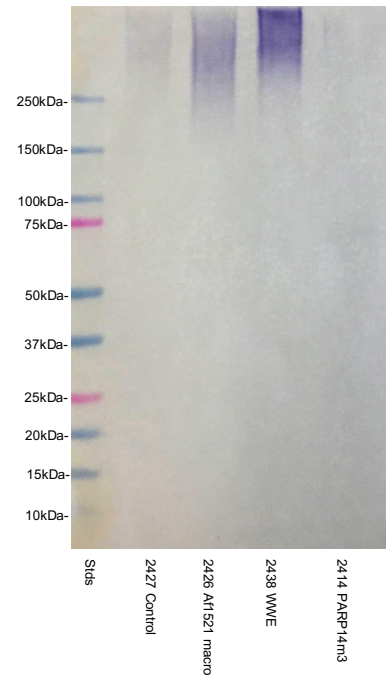
SUGGESTED GENERAL PROTOCOL for WWE Domain Mag Resin, Cat. #2438

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 WWE Domain mag 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 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 if desired 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 WWE Domain 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 2438 WWE domain resin pulls-down PARylated PARP1.

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