PARP14m3 Magnetic Resin Catalog #2414

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:

Macrodomains are evolutionarily conserved protein domains of 130 – 190 amino acids with high specificity binding to mono- and/or poly-ADP-ribose. By recognizing ADP-ribose units covalently attached to target proteins, macrodomains act as readers and modulators of mono- and poly-ADP-ribosylation pathways. Numerous macrodomain-containing proteins have been identified, including human PARP14 (ARTD8). PARP14 contains a WWE domain and three macrodomains (m1, m2, and m3). Expressed and purified PARP14 macrodomains m2 and m3 bind MARylated proteins with high-affinity, and do not interact with PARylated substrates.

The Tulip Biolabs, Inc. Cat. #2414 hPARP14m3 magnetic resin is a fusion protein of human PARP14 macrodomain 3 covalently bound to a super-paramagnetic silica-based resin. It is designed for the isolation and study of mono-ADP-ribosylated (MARylated) proteins. Through the use of this MAR affinity resin, MARylated proteins are isolated directly from cell or tissue lysates. The magnetic resin-bound proteins can be eluted from the affinity resin, and analyzed by immunoblotting or other methods.

Description:

Cat. #2414 hPARP14m3 Magnetic Resin is highly purified human PARP14 macrodomain 3 fusion protein expressed in *E. coli*, and covalently bound to superparamagnetic 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 PARP14m3 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 PARP14m3 mag resin is useful for affinity purification (pulldown) of MARylated proteins.

Supplied As:

Each vial contains 0.5mg purified hPARP14m3 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, 1mM TCEP, and 0.02% sodium azide).

Purity:

hPARP14m3 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 to affinity purify/pull-down mono-ADP-ribose modified proteins in 0.15-1mg cell or tissue lysate. Analyze proteins by Western blotting using protein-specific antibodies to probe the immunoblot, mass spec protein analysis, or other methods as desired. Each 0.5mL vial is sufficient for analysis of ~25 samples.

Note that contaminating proteins may be co-eluted with the #2414 PARP14m3 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 Other Related Mag Resin Products:

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

Tulip BioLabs Other Related Products:

PAR Affinity Resin Set (Macrodomain), Cat. #4301.
PARP1, Highly active, human, Cat. #2090.
PARP1, Automodified, human, Cat. #2095.
Anti-poly(ADP-ribose) polymer, clone 10H, mouse monoclonal antibody, Cat. #1020.

Specific References:

This product was developed at Tulip Biolabs, Inc.

General References:

A.H. Forst *et al.* (2013) Structure **21**, 462 doi.org/10.1016/j.str.2012.12.019
K. Feijs *et al.* (2013) Nat Rev Mol Cell Biol **14,** 443 doi.org/10.1038/nrm3601
J. Gregor *et al.* (2016) Annual Rev Biochem **85** 431 doi.org/10.1146/annurev-biochem-060815-014935

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SUGGESTED GENERAL PROTOCOL for PARP14m3 mag resin, Catalog #2414

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
Microcentrifuge tubes
Magnetic separator
SDS-PAGE sample buffer

PROCEDURE

1. Resuspend the PARP14m3 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.

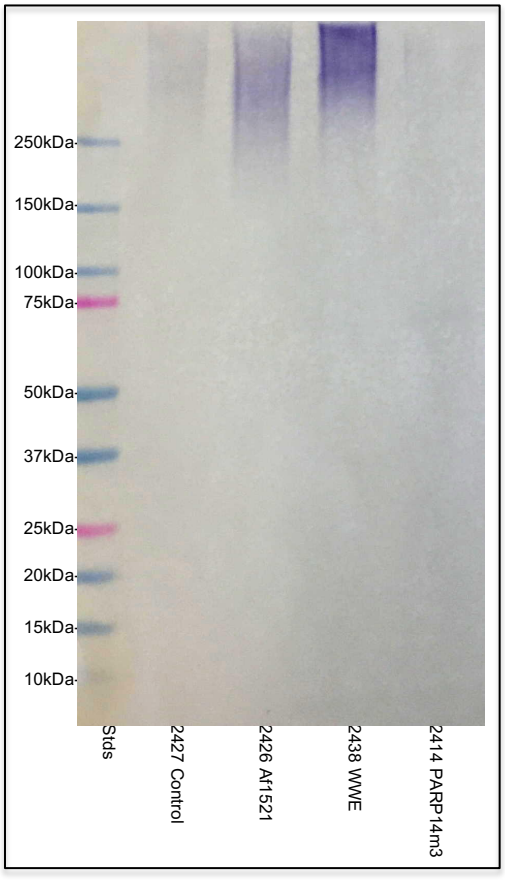

Figure. Pull-down of automodified PARP1 by mag resins. Automodified PARP1, cat. #2095, 0.25µg in RIPA buffer was pulled-down by the indicated mag resins according to the above protocol. The resin-bound protein was run on SDS-PAGE, then WB was performed using anti-pADPr, clone 10H (Cat. #1020). The high MW smear represents automodified PARP1 PARylated to various extents. The 2414 MARylation specific resin does not recognize PARylated PARP1, as expected.

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