WWE Affinity Resin Set Catalog #4306

One Set Contains 1 each of the following…

WWE Affinity Resin **Catalog #2334**
WWE Neg Control Resin **Catalog #2335**

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

Background:

The Tulip Biolabs, Inc. WWE Affinity Resin and Negative Control Resin are designed for the isolation and study of poly-ADP-ribosylated (PARylated) proteins. Through the use of this highly specific PAR affinity resin, PARylated proteins are isolated from cell or tissue lysates without the use of anti-PAR antibodies. The resin-bound proteins can be eluted from the affinity resin, and analyzed by immunoblotting or other methods.

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. The Tulip Biolabs, Inc. WWE Affinity Resin Cat. #2334 is a GST fusion protein of WWE PAR-binding domain of human RNF146, amino acid residues 100-175, bound to a glutathione resin. The negative control resin #2335 is identical to the #2334 except for a single amino acid substitution that effectively abolishes PAR binding.

Description:

- **Cat. #2334: WWE Affinity Resin** is highly purified GST-WWE fusion protein expressed in *E. coli*, and bound to glutathione beads. The WWE domain sequence is human RNF146 (Iduna) amino acid residues 100-175. It is useful for affinity purification (pulldown) of PARsylated proteins.

- **Cat. #2335: WWE Negative Control Resin** is identical to the #2334 resin except for a single amino acid substitution R163A, which abolishes PAR binding. The negative control resin is useful to control for non-specific binding, and its use is optional.

Supplied As:

Each vial contains 0.5mg purified GST-WWE fusion protein bound to 50-75µL packed volume of glutathione beads in 0.5 mL buffer containing 10 mM sodium phosphate, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 0.02% sodium azide.

Purity:

GST-WWE 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 resin to affinity purify/pull-down 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 0.5mL vial is sufficient for analysis of ~25 samples.

Additional Notes:

The #2335 Neg Control Resin has been shown to bind to purified highly automodified PARP1 under certain conditions.

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

Tulip BioLabs Other Related Products:

WWE Domain Mag Resin, Cat. #2438
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.
Anti-poly(ADP-ribose) polymer, IgY, chicken polyclonal antibody, Cat. #1023.
Anti-PARP1, whole protein, IgY, chicken polyclonal antibody, Cat. #1051.

Specific References:

L. Zhong *et al.* (2015) *PLoS ONE* **10**:e0122948

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

Tulip BioLabs, Inc.
P.O. Box 334, West Point, PA 19486 USA
Tel/Fax 610.584.2706 info@tulipbiolabs.com
www.tulipbiolabs.com

SUGGESTED GENERAL PROTOCOL for WWE Affinity resins, Catalog #2334 and #2335

MATERIALS REQUIRED

Lysis buffer (e.g.: 50mM Tris, pH 8, 200mM NaCl, 1mM EDTA, 1% Triton X-100, 10% glycerol, 1 mM DTT, 0.1% SDS, and protease inhibitors)
Cell/tissue extract containing ~0.15 to 1mg total protein per sample
Microcentrifuge tubes
Microcentrifuge
SDS-PAGE sample buffer

PROCEDURE

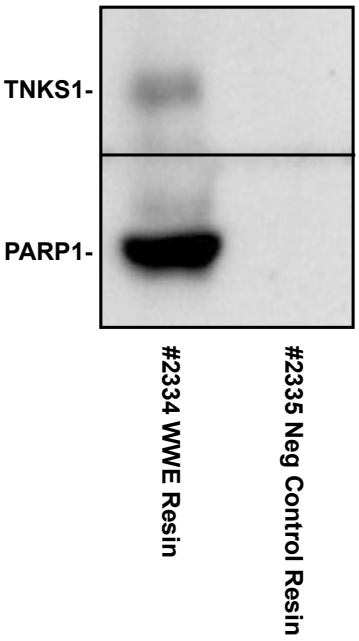
1. Resuspend the WWE affinity and neg control resins by gently inverting the product tubes several times to obtain a homogenous suspension of resin.
2. Use a wide-bore pipette or a cut pipette tip to transfer 20µL of the suspension to ~0.5mL of lysis buffer in a microfuge tube.
3. Sediment resin at 10k x g in a microfuge for 20 sec. Carefully remove most of the lysis buffer, leaving the resin (barely visible) undisturbed in the tube. NOTE: Position the tubes in the microfuge with the hinge oriented outward in order to ascertain the location of the sedimented resin.
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 0.5mL.
5. Incubate the reaction for several hours or overnight at 4˚C on a Nutator or similar device.
6. Sediment, 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 10 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) to detect affinity purified proteins. Compare results to negative control resin samples to assess non-specific binding, which should be minimal.

HEK293 CELLS EXAMPLE EXPERIMENTAL RESULTS
(NOTE: Refer to Suggested General Protocol)

PROCEDURE:

1. HEK293 cells were grown to confluence on 10 cm plates.
2. Cells were harvested at 4˚C in 1 mL Lysis buffer (50mM Tris, pH 8, 200mM NaCl, 1mM EDTA, 1% Triton X-100, 10% glycerol, 1 mM DTT, 0.1% SDS, and protease inhibitors).
3. After clarification by centrifugation at 15k x g for 10 min, cell lysates (~0.25mL) were incubated with WWE affinity resin (#2334) or neg control resin (#2335) (20µL suspended resin; see Suggested General Protocol) with agitation at 4˚C overnight.
4. Resins were sedimented with associated proteins in a microfuge at 15k x g for 10 sec, and supernatant discarded.
5. Resin was washed 4-times with 500µL Lysis buffer.
6. 100µL SDS-PAGE sample buffer was added to each tube then samples boiled for 10 min.
7. SDS-PAGE and Western blotting of samples were performed. Immunoblots were probed with anti-PARP1 and anti-TNKS antibodies, and detected using ECL.

RESULTS:



Isolation of PARsylated PARP1 and TNKS1 using WWE resin. WWE and neg control resins were used to pull down PARsylated proteins from clarified extracts of confluent HEK293 cells. The resin bound proteins were Western blotted and probed with either anti-TNKS1 or PARP1.

Tulip BioLabs, Inc.
P.O. Box 334, West Point, PA 19486 USA
Tel/Fax 610.584.2706 info@tulipbiolabs.com
www.tulipbiolabs.com