

WWE Affinity Resin Set

One Set Contains 1 each of the following...

WWE Affinity Resin
WWE Neg Control Resin
Catalog #4306
Catalog #2334
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:

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

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 20 μ L 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 75 μ L 1X SDS-PAGE sample buffer to each tube, agitate, then incubate at 95°C for 10 min to dissociate GST-macrodomein 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:

