Datasheet for ABIN1741736

WWE Affinity Resin Set

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Overview

Quantity:	1 set
Target:	RNF146
Reactivity:	Human
Host:	Escherichia coli (E. coli)
Antibody Type:	Recombinant
Conjugate:	GST tag
Application:	Pull-Down Assay (Pull-Down)

Product Details

Specificity:	The WWE Affinity Resin, ABIN1741734 is highly purified GST-RNF146(100-175) fusion protein construct expressed in E. coli, and bound to glutathione beads. WWE Negative Control Resin, ABIN1741735 is identical to the ABIN1741734 resin except for R163A substitution, which effectively abolishes PAR binding. The negative control resin is useful to control for non-specific binding, and its use is optional. Both products will be delivered each in 0.5 mL (0.5 mg fusion protein) supplied as a slurry containing approx. 50 µL resin.
Characteristics:	Set contains one ABIN1741734 and ABIN1741735.
Purification:	Affinity chromatography
Purity:	> 95 %
Material not included:	Lysis buffer (e.g.: 50 mM Tris, pH 8, 200 mM NaCl, 1 mM EDTA, 1 % Triton X-100, 10 % glycerol, 1 mM DTT, 0.1 % SDS, and protease inhibitors)

Product Details

	Cell/tissue extract containing approx. 0.15 to 1 mg total protein per sample Microcentrifuge tubes Microcentrifuge SDS-PAGE sample buffer

Target Details

Target:	RNF146
Alternative Name:	RNF146 (RNF146 Products)
Sub Type:	Cocktail
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.
Molecular Weight:	8 kDa + GST

Application Details

Application Notes:	20 µL=20 µg per reaction (for each)
Comment:	280.00
Protocol:	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 µL of the suspension to approx. 0.5 mL of lysis buffer in a Microfuge tube. 3. Sediment resin at 10k x g in a Microfuge for 20 s. 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 1 mg in a total buffer volume of 0.5 mL. 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 0.5-1 mL lysis buffer, as in step 3. On the final wash, carefully remove residual buffer without disturbing the resin. 7. Add 75 µL 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

Application Details

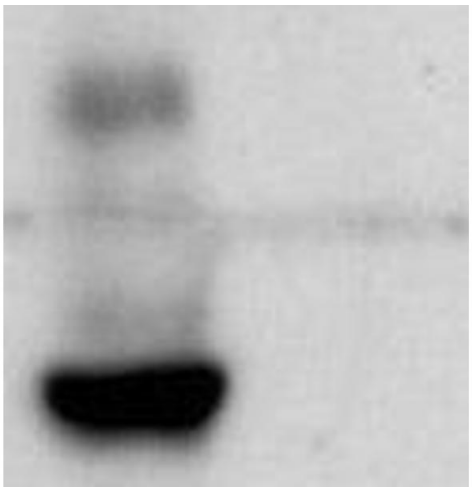
	protein-specific antibodies, for example anti-PARP1 (ABIN1741708) to detect affinity purified proteins. Compare results to negative control resin samples to assess non-specific binding, which should be minimal.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	10 mM sodium phosphate, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 % Triton X-100, and 0.02 % sodium azide
Preservative:	Sodium azide
Precaution of Use:	This product contains sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Handling Advice:	Do not freeze!
Storage:	4 °C
Storage Comment:	Stable for 6 months from date of shipment when stored at 4 ˚C.

Publications

Product cited in:	Zhong, Yeh, Hao, Pourtabatabaei, Mahata, Shao, Chessler, Chi: "Nutritional energy stimulates NAD+ production to promote tankyrase-mediated PARsylation in insulinoma cells." in: **PLoS ONE**, Vol. 10, Issue 4, pp. e0122948, (2015) (PubMed).



Western Blotting

Image 1. 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.