Datasheet for ABIN1741733

Poly-ADP-ribose Affinity Resin Set

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Overview

Quantity:	1 set
Target:	Af1521 Macrodomain
Reactivity:	Archaeoglobus fulgidus
Host:	Escherichia coli (E. coli)
Antibody Type:	Recombinant
Conjugate:	GST tag
Application:	Pull-Down Assay (Pull-Down)

Product Details

Specificity:	The PAR Affinity Resin, ABIN1741731 is highly purified GST-Af1521 macrodomain fusion protein construct expressed in E. coli, and bound to glutathione beads. The Af1521 macrodomain protein is reported to also bind mono-ADP-ribosylated proteins and ADP-ribose. PAR Negative Control Resin, Cat. ABIN1741732 is identical to the ABIN1741731 resin except for two gly to asp substitutions, which abolish PAR binding. The negative control resin is useful to control for non-specific binding, and its use is optional. Both products are supplied as 1 mL slurry containing approx. 75 µL resin (1 mg fusion protein)
Characteristics:	Set contains one ABIN1741731 and ABIN1741732.
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, 0.5 % deoxycholate, 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:	Af1521 Macrodomain
Sub Type:	Cocktail
Background:	Af1521 is a thermopohilic protein from Archaeoglobus fulgidus, and contains a conserved approx. 190 AA domain known as the macro domain. Macrodomains are found in a wide variety of organisms including bacteria, viruses, and vertebrates. Expressed and purified macrodomains from Af1521, Alc1, macroH2A and Bal/PARP9 proteins have been shown to bind polymeric ADP-ribose modified proteins with high specificity and affinity
Molecular Weight:	21 kDa + GST

Application Details

Application Notes:	20 µL=20 µg per reaction (for each)
Comment:	280.00
Protocol:	1. Resuspend the PAR 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. 1 mL of lysis buffer in a Microfuge tube. 3. Sediment resin at 15k x g in a Microfuge (highest speed setting) for 20 s. Carefully remove most of the lysis buffer, leaving the resin (barely visible) undisturbed in the tube. NOTE: Position 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.

Application Details

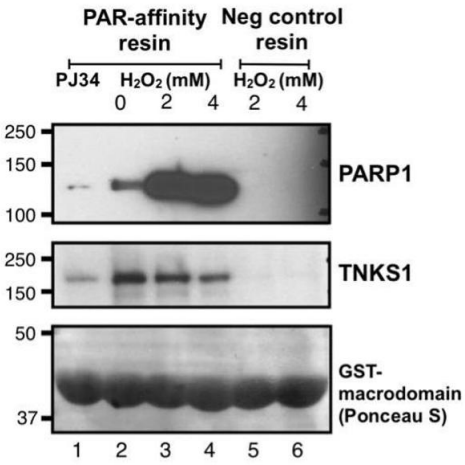
	8. Run samples on SDS-PAGE, and perform Western blotting. Probe immunoblot using desired protein-specific antibodies, for example anti-PARP1 (ABIN1741708), or anti-poly-ADP-ribose antibodies (ABIN1741702 or ABIN1741707) to detect affinity purified proteins. Compare results to negative control resin samples to assess non-specific binding, which should be minimal.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	PBS with 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.

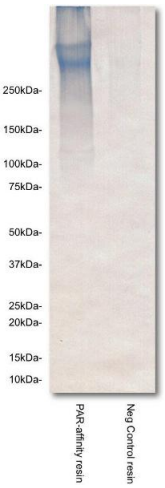
Publications

Product cited in:	Bozdagi, Sakurai, Dorr, Pilorge, Takahashi, Buxbaum: "Haploinsufficiency of Cyfip1 produces fragile X-like phenotypes in mice." in: **PLoS ONE**, Vol. 7, Issue 8, pp. e42422, (2012) (PubMed). Steffen, Faix, Resch, Linkner, Wehland, Small, Rottner, Stradal: "Filopodia formation in the absence of functional WAVE- and Arp2/3-complexes." in: **Molecular biology of the cell**, Vol. 17, Issue 6, pp. 2581-91, (2006) (PubMed).



Western Blotting

Image 1. Western blot of Poly-ADP-ribosylated PARP1 and TNKS1 in MDCK cells using PAR-affinity resins. MDCK cells were treated for 1hr with PARP inhibitor PJ-34 (lane 1), no inhibitor (lane 2), or PAR-induced with 2mM (lanes 3 and 5) or 4mM (lanes 4 and 6) H2O2. Cells were lysed and extracts incubated with PAR-affinity resin (lanes 1-4) or Neg Control resin (lanes 5-6). Western blots were probed with anti-PARP1 (upper panel) or anti-tankyrase1 (middle panel). The GST-fusion protein was visualized using Ponceau S to confirm equal loading of the PAR Affinity and Neg Control resins.



Western Blotting

Image 2. Pull-down of Poly-ADP-ribosylated PARP1 by PAR-affinity resin. Purified poly-ADP-ribosylated PARP1 (400ng) was incubated with PARaffinity resin or Neg Control resin, washed, then dissociated with SDS-PAGE sample buffer. Samples were then immunoblotted with anti-poly-ADP-ribose, clone 10H (Cat. #1020). Note the PAR-affinity resin pulls-down poly-ADPribosylated PARP1, shown by a smear of high MW protein, >113kDa, whereas the negative control resin does not.