abcam

Product datasheet

PAK1 PBD Agarose Beads ab211177

2 Images

Overview

Product name PAK1 PBD Agarose Beads

Product overview

Background: Small GTP-binding proteins (or GTPases) are a family of proteins that serve as molecular regulators in signalling transduction pathways. Rac, a 21 kDa protein, belongs to the family of Rho GTPases regulating a variety of biological response pathways that include cell motility, cell division, gene transcription, and cell transformation. Like other small GTPases, Rac regulates molecular events by cycling between an inactive GDP-bound form and an active GTP-bound form. In its active (GTP-bound) state, Rac binds specifically to the p21-binding domain (PBD) of p21-activated protein kinase (PAK) to control downstream signaling cascades.

Use: Our PAK1 PBD Agarose Beads are designed to pull down only the active form of Rac or Cdc42.

Description: PAK1 PBD Agarose Beads, in color, are easy to visualize, minimizing potential loss during washes and aspirations of Rac-GTP pulldown.

Activity: Product specifically interacts and precipitates GTP-bound Rac or Cdc 42 from cell lysate.

Concentration: 800 µL of 50% Agarose slurry, 400 µg PAK1-PBD in 1X PBS, 50% Glycerol

Notes

Protocol for the pull down assay:

1. Aliquot 0.5 – 1 mL of cell lysate to a microcentrifuge tube.
2. Adjust the volume of each sample to 1 mL with 1X lysis buffer.
3. Thoroughly resuspend the agarose bead slurry by vortexing or titrating.
4. Quickly add 40 µL of resuspended bead slurry to each tube.
5. Incubate the tubes at 4°C for 1 hour with gentle agitation.
6. Pellet the beads by centrifugation for 10 seconds at 14,000 x g.

7. Aspirate and discard the supernatant, making sure not to disturb/remove the bead pellet.
8. Wash the bead 3 times with 0.5 mL of 1X lysis buffer, centrifuging and aspirating each time.
9. After the last wash, pellet the beads and carefully remove all the supernatant.
10. Resuspend the bead pellet in 40 µL of 2X reducing SDS-PAGE sample buffer.
12. Boil each sample for 5 minutes.
13. Centrifuge each sample for 10 seconds at 14,000 x g.

For best results with these beads, it is important to first determine the amount of cell lysate that is detectable on the blot before performing the pull down. We recommend running a lysate titration on a Western blot to determine the concentration that gives a good signal. For the GTPase assay, you will then want to add 100-fold that amount. For example, if you run 5, 10 and 20ug of lysate on a Western blot and 10ug gives a good signal, you will use 10ug x 100 = 1mg of lysate per pull down.

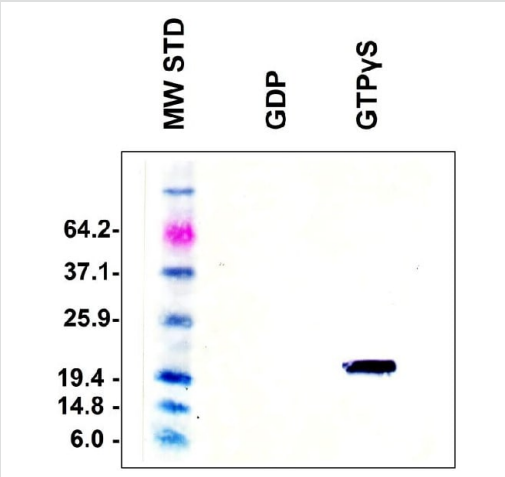
The activity level of the small GTPase in the sample will determine how much gets pulled down. The beads are designed to only pull down small GTPase in the GTP-bound (active) form. If the majority of the GTPase in the sample is in the GDP-bound form (inactive), it will not get pulled down, regardless of the amount of lysate loaded. The lysate can be preloaded with GTPγS and used as a positive control.

Sequence alignment of a specific small GTPase indicates that there is at most one or two amino acid variation between various species. Therefore, our beads may be used across many species.

Properties

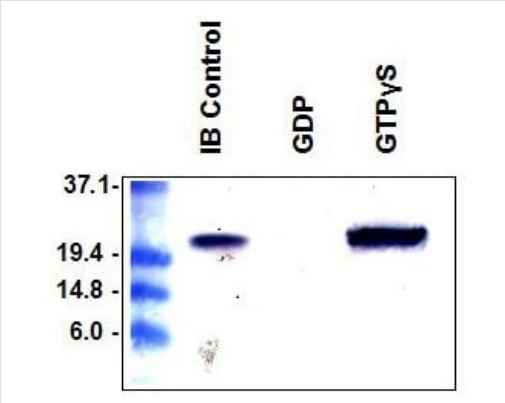
Storage instructions	Store at -20°C. Please refer to protocols.
Function	The activated kinase acts on a variety of targets. Likely to be the GTPase effector that links the Rho-related GTPases to the JNK MAP kinase pathway. Activated by CDC42 and RAC1. Involved in dissolution of stress fibers and reorganization of focal complexes. Involved in regulation of microtubule biogenesis through phosphorylation of TBCB. Activity is inhibited in cells undergoing apoptosis, potentially due to binding of CDC2L1 and CDC2L2.
Sequence similarities	Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. STE20 subfamily. Contains 1 CRIB domain. Contains 1 protein kinase domain.
Post-translational modifications	Autophosphorylated when activated by CDC42/p21 and RAC1.
Cellular localization	Cytoplasm. Cell junction > focal adhesion. Recruited to focal adhesions upon activation.

Images



Cdc42 Activation Assay

Lane 1, MW Standard. Lane 2, 293 cell lysate loaded with GDP and incubated with PAK PBD Agarose beads. Lane 3, 293 cell lysate loaded with GTPγS and incubated with PAK-1 PBD Agarose beads. Samples were immunoblotted with anti-Cdc42 antibody.



Rac Activation Assay

Lane 1, GTPase Immunoblot Positive Control. Lane 2, 293 cell lysate loaded with GDP and incubated with PAK PBD Agarose beads. Lane 3, 293 cell lysate loaded with GTPγS and incubated with PAK-1 PBD Agarose beads. Samples were immunoblotted with anti-Rac antibody.

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