abcam

Product datasheet

Raf-1 RBD Agarose Beads ab211176

Overview

Product name Raf-1 RBD Agarose Beads

Sample type Cell Lysate

Product overview **Background:** Small GTP-binding proteins (or GTPases) are a family of proteins that serve as molecular regulators in signalling transduction pathways. Ras, a 21 kDa protein, regulating a variety of biological response pathways that include cell growth, cell transformation and tumour invasion. Like other small GTPases, Ras regulates molecular events by cycling between an inactive GDP-bound form and an active GTP-bound form. In its active (GTP-bound) state, Ras binds specifically to the Ras-binding domain (RBD) of Raf1 to control downstream signalling cascades.

Use: Our Raf-1 RBD Agarose Beads are designed to pull down only the active form of Ras.

Description: Our Raf RBD Agarose beads are colored for easy visualization, minimizing potential loss during washes and aspirations during Ras-GTP pulldown.

Activity: Product specifically interacts and precipitates GTP-bound Ras from cell lysate.

Concentration: 800 µL of 50% Agarose slurry, 400 µg Raf1-RBD 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.

Components	400 µg
ab211176 - Raf-1 RBD Agarose Beads	1 x 400µg

Function Involved in the transduction of mitogenic signals from the cell membrane to the nucleus. Part of the Ras-dependent signaling pathway from receptors to the nucleus. Protects cells from apoptosis mediated by STK3.

Tissue specificity In skeletal muscle, isoform 1 is more abundant than isoform 2.

Involvement in disease Defects in RAF1 are the cause of Noonan syndrome type 5 (NS5) [MIM:611553]. Noonan syndrome (NS) is a disorder characterized by dysmorphic facial features, short stature, hypertelorism, cardiac anomalies, deafness, motor delay, and a bleeding diathesis. It is a genetically heterogeneous and relatively common syndrome, with an estimated incidence of 1 in 1000-2500 live births.
Defects in RAF1 are the cause of LEOPARD syndrome type 2 (LEOPARD2) [MIM:611554]. LEOPARD syndrome is an autosomal dominant disorder allelic with Noonan syndrome. The acronym LEOPARD stands for lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormalities of genitalia, retardation of growth, and deafness.

Sequence similarities Belongs to the protein kinase superfamily. TKL Ser/Thr protein kinase family. RAF subfamily. Contains 1 phorbol-ester/DAG-type zinc finger.

	Contains 1 protein kinase domain. Contains 1 RBD (Ras-binding) domain.
Post-translational modifications	Phosphorylated upon DNA damage, probably by ATM or ATR. Phosphorylation at Thr-269 increases its kinase activity. Phosphorylation at Ser-259 induces the interaction with YWHAZ and inactivates kinase activity. Dephosphorylation of Ser-259 by the complex containing protein phosphatase 1, SHOC2 and M-Ras/MRAS relieves inactivation, leading to stimulate RAF1 activity.
Cellular localization	Cytoplasm. Cell membrane. Colocalizes with RGS14 and BRAF in both the cytoplasm and membranes.

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