Product Data Sheet

Rac1 G15A Agarose Beads (Active Rac-GEF)

CATALOG NUMBER: STA-432 **STORAGE:** -20ºC

QUANTITY AND CONCENTRATION: 800 µL of 50% Agarose slurry, 1 mg/mL Rac1 G15A in 1X PBS, 50% Glycerol

SHELF LIFE: 1 year from receipt under proper storage conditions; avoid multiple freeze thaw cycles

Background

Small GTP-binding proteins (or GTPases) are a family of proteins that serve as molecular regulators in signaling 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 influences molecular events by cycling between an inactive GDP-bound form and an active GTP-bound form. Cycling between the GDP-bound and GTP-bound state is regulated primarily by two distinct families of proteins: guanine nucleotide exchange factors (GEFs) activate Rho proteins by catalyzing the exchange of GDP for GTP, the GTPase activating proteins or GAPs negatively regulate GTPase function by stimulating GTP hydrolysis.

Similar to Ras mutants, constitutively active or dominant negative Rho GTPase mutants have been used to bind to Rho-GAP and effectors or to Rho-GEFs, respectively. A nucleotide-free GTPase has also been shown to form a high affinity binary complex with Rho-GEFs. Rac1 G15A Agarose beads to selectively isolate and pull-down the active form of Rac-GEFs from purified samples or endogenous lysates. Subsequently, the precipitated Rac-GEF is detected by western blot analysis using an anti-Rac-GEF antibody.

Presentation

Rac1 G15A Agarose beads, in color, are easy to visualize, minimizing potential loss during washes and aspirations of active Rac-GEF pulldown (Figure 1).

Figure 1: Rac1 G15A Beads in Color

Purity and Activity

Purity >90% by SDS-PAGE and Coomassie blue staining. Specifically interacts and precipitaes active Rac-GEF from cell lysate.

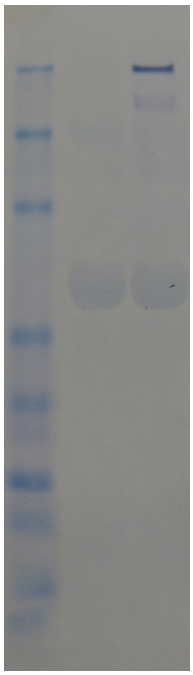

Figure 2: Tiam1 Activation Assay. 293 cells were transfected with active Tiam1 (ΔN Tiam1). Active Tiam1 in lysate was pulled down with Rac1 G15A agarose beads. *Lane 1*, Mock Transfection Control. *Lane 2*, ΔN Tiam1 Transfection.

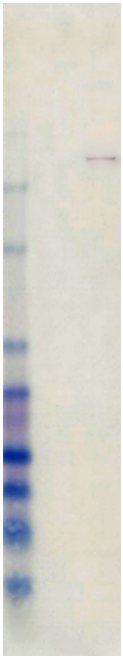

Figure 3: Tiam1 Activation Assay in MDA-231 Cells. Active Tiam-1 in 2 mg of MDA-231 lysate was pulled down with Rac1 G15A agarose beads and probed with anti-Tiam1 antibody according the Assay Protocol.

References

1. Arthur, W.T., Ellerbroek, S.M., Der, C.J., and Burridge K. (2002) *J. Biol. Chem.* **277**, 42964-42972
2. Garcia-Mata R., Wennerberg, K., Arthur, W.T., Noren, N.K., Ellenbroek, S.M., and Burridge K. (2006) *Methods Enzymol.* **406**, 425-437.

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