Active Rac1 Pull-Down and Detection Kit

Cat. No. KITZ-031 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	GTPase Rac1 plays an important role in the organization of actin filament networks and in membrane ruffling. Rac1 (~22kDa) has been implicated in cell transformation, apoptosis and migration. Like other small GTPases, Rac1 is active when bound to GTP and is inactive when bound to GDP. Active Rac1 Pull-Down and Detection Kit is a simple and fast tool to monitor Rac1 small GTPase activation. The kit provides a GST-fusion protein containing the p21-binding domain (PBD) of human p21-activated protein kinase 1 (Pak1) along with glutathione agarose resin to specifically pull down active Rac1 and an anti-Rac1 antibody for Western blot detection. Also included are two control nucleotides, GTPγS and GDP, which can be used to generate positive and negative control lysates, respectively. Each kit is functionally tested to ensure component performance.
Size	30 pull-down reactions
Storage	4°C/-20°C
Shipping	Ice pack/Dry ice
Kit Components	Box 1 (these items ship together on dry ice; upon receipt store at -20°C): GST-human Pak1-PBD, 600µg, contains 1-2mg/mL in 25mM Tris•HCl, pH 7.2, 150mM NaCl and 10% glycerol; ~35kDa; GST-human Pak1-PBD interacts with Rac1 from human and mouse, and possibly from all mammalian species, store at -20°C Anti-Rac1 Antibody, 50µL (5 units), mouse monoclonal IgG2b; Anti-Rac1 antibody reacts with Rac1 of human, dog, rat, mouse and chicken; store at -20°C. Note: One

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	unit of Anti-Rac1 antibody is defined as the amount of antibody required to efficiently detect Rac1 in 20µg NIH3T3 whole cell lysate by Western blotting (8.5 × 7.5cm membrane). 100X GTPγS, 50µL, 10mM in sterile water, store at -20°C 100X GDP, 50µL, 100mM in sterile water, store at -20°C Box 2 (these items ship together with an ice pack; upon receipt store at 4°C): Glutathione Resin, 3.0mL, supplied as 50% slurry containing 0.05% sodium azide, store at 4°C 1X Lysis/Binding/Wash Buffer, 100mL, 25mM Tris•HCl, pH 7.2, 150mM NaCl, 5mM MgCl2, 1% NP-40 and 5% glycerol, store at 4°C 2X SDS Sample Buffer, 1.5mL, 125mM Tris•HCl, pH 6.8, 2% glycerol, 4% SDS (w/v) and 0.05% bromophenol blue, store at 4°C Spin Cups, 30 each, maximum volume 850µL, store at room temperature or 4°C Collection Tubes, 90 each, store at room temperature or 4°C
Materials Required but Not Supplied	Protease inhibitors Pierce BCA Protein Assay Reagent or Pierce 660nm Protein Assay β-mercaptoethanol or dithiothreitol (DTT) Polyacrylamide gel, 12% or 4-20% Nitrocellulose or PVDF membrane Tris-buffered saline (TBS; 25mM Tris•HCl, pH 7.5, 150mM NaCl) Tween-20 Detergent BSA, Fraction V Nonfat Dry Milk Pierce Goat Anti-Mouse IgG-Horseradish Peroxidase Conjugate West Pico Chemiluminescent Substrate CL-XPosure X-ray Film or a CCD camera 0.5M EDTA, pH 8.0 1M MgCl2

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	Sodium azide (NaN3)
	Electrophoresis apparatus
	Variable-speed Bench-top Microcentrifuge

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