CREATIVE BIOMART®
Assay Kit

Rac Activation Kit

Product Information

Cat.No. Kit-0757

Product Overview

Rac Activation Kit provides a rapid, cost-effective and reliable tool for detection and semi-quantitative analysis of the cellular activation state of Rac1 and Rac2 G-proteins. If combined with immunological detection of Cdc42, the kit can also detect activated Cdc42-GTP. Please note that this kit provides only an antibody for the detection of Rac1. Active Rac, i. e. Rac complexed to GTP, is purified out of cell extracts via a one-step batch affinity purification step employing GST-PAK-CRIB, an affinity probe that selectively interacts with the active, GTP-bound Rac conformation in loss of the inactive Rac-GDP complex. The kit further includes recombinant Rac1δC-term that serves dual roles: 1. It can be used as western blot control for immunodetection2. Upon loading with GDP or GTP-γ-S (also included in this kit), Rac1δC-GDP or Rac1DC-GTP-γ-S complexes can be used to "spike" cell extracts thus serving as internal assay controls of signal specificity.

Size

1 kit

Description

Traditionally, GTPase activity measurements involved metabolic labelling of cells with inorganic [32P]-phosphate followed by isolation of the GTPase and chromatographic analysis of bound guanine nucleotides. This methodology provides quantitative data for GDP and GTP levels on Rac but is a tedious and time consuming procedure that requires working with large amounts of radioactivity and is prone to various sources of errors.More recently an alternative non-radioactive technique has been described that exploits the selective interaction of the Cdc42/Rac interactive binding domain (CRIB) of the Rac-effector PAK1 with the active, Rac-GTP conformation. Recombinant, GST-tagged PAK-CRIB is added to cell extracts to pull out Rac-GTP, which is consequently detected by Western blotting. This approach has greatly accelerated and thus simplified semi-quantitative Rac activity determinations.

Applications

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Functional Studies more details

Target Species

Reacts with: Mouse, Human

Kit Components

Components: 1 kit; 100X Protease Inhibitor Mix: 1 x 650µl; 5X Lysis Buffer Stock: 1 x 30ml; GDP (10 mM in water): 1 x 100µl; GDP (100 mM in water): 1 x 100µl; Glutathione-Sepharose Slurry: 1 x 5ml; GST-PAK-CRIB: 1 x 1.5mg; GTP-gamma-S (10 mM in water): 1 x 100µl; Magnesium Chloride (1 M): 1 x 100µl; Rac nucleotide loading solution (NLS): 1 x 2ml; Rac1 rabbit polyclonal antibody: 1 x 50µl; Rac1deltaC (residues 1-178) in 50 % glycerol solution: 1 x 25µg

Compatible Sample Types

Cell culture extracts

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