CREATIVE BIOMART®
Assay Kit

Ras Activation Kit

Product Information

Cat.No. Kit-0762

Product Overview

Ras Activation Kit provides a rapid, cost-effective and reliable tool for the detection and semi-quantitative analysis of the cellular activation state of the three prototypical Ras proteins K-Ras, N-Ras and H-Ras (collectively referred to as Ras). Active Ras, i. e. Ras complexed to GTP, is purified from cell extracts via a one-step batch affinity purification step employing GST-RBD, an affinity probe that selectively interacts with the active, GTP-bound Ras conformation in loss of the inactive Ras-GDP complex. The kit further includes recombinant H-Ras. K-Ras and N-Ras proteins serve dual roles: 1. They may be used as Western Blot controls for Ras isoform-specific immunodetection2. Upon loading with GDP or GTP-γ-S (also included in the kit), Ras-GDP or Ras-GTP-γ-S proteins can be used to "spike" cell extracts thus serving as internal assay controls of signal specificity.

Size

1 kit

Description

Members of the Ras family of guanine nucleotide binding proteins (GTPases) are nodal regulators of numerous cell biological processes including proliferation, cell-adhesion and apoptosis. Ras cycles between an active GTP-bound state and an inactive GDP-bound conformation. Cellular Ras-GDP/GTP levels are tightly controlled by guanine nucleotide exchange factors (GEFs), which activate Ras by promoting GTP uptake, and GTP hydrolase activating proteins (GAPs), which accelerate conversion of Ras bound GTP to GDP. Traditionally GTPase activity measurements in vivo involved metabolic labelling of cells with inorganic [32P]-phosphate followed by isolation of the GTPase and chromatographic analysis of bound guanine nucleotides. This method provides quantitative data for GDP and GTP levels of Ras but is a tedious and time consuming procedure which 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 Ras-binding domain (RBD) of the Ras effector Raf with the active, Ras-GTP conformation. Recombinant, GST-tagged RBD is added to cell extracts to pull out Ras-GTP,

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which is consequently detected by Western blotting. This approach has greatly accelerated and thus simplified semi-quantitative Ras activity determinations.

Applications

Functional Studies more details

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-c-Raf-RBD: 1 x 1.5mg; GTP-gamma-S (10 mM in water): 1 x 100µl; His-H-Ras Protein (50% Glycerol Solution): 1 x 25µg; His-K-Ras Protein (50% Glycerol Solution): 1 x 25µg; His-N-Ras Protein (50% Glycerol Solution): 1 x 25µg; Magnesium Chloride (1 M): 1 x 100µl; Pan-Ras Monoclonal Antibody: 1 x 50µl; Ras Nucleotide Loading Solution (NLS): 1 x 2ml

Compatible Sample Types

Cell culture extracts

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