CREATIVE BIOMART®
Assay Kit

Rap Activation Kit

Product Information

Cat.No. Kit-0761

Product Overview

Rap Activation Kit provides a rapid, cost-effective and reliable tool for the detection and semi-quantitative analysis of the cellular activation state of Rap GTPases. The kit exploits the selective interaction of the Ras-interaction domain (RA) of the Ras/Rap-effector RalGDS with the active, Rap-GTP conformation. Recombinant, GST-tagged RalGDS-RA is added to cell extracts to pull out Rap-GTP, which is consequently detected by Western blotting. The kit detects active, GTP-bound Rap1A, Rap1B, Rap2A and Rap2B. However, the antibody included in the kit allows detection of Rap1 isoforms only. The kit also includes recombinant Rap1A protein that, once preloaded with GDP or GMPpNHp (all included in the kit), can be used to "spike" the cell extracts thus serving as an internal control of signal specificity.

Size

1 kit

Description

As all other members of the Ras superfamily of small guanine nucleotide binding proteins (GTPases), Rap GTPases cycle between an active GTP-bound state and an inactive GDP-bound conformation. Cellular Rap-GDP/GTP levels are tightly controlled by two groups of proteins: 1. Guanine nucleotide exchange factors (GEFs) catalyze GTP uptake by Rap in response to multiple second messenger signals like Ca2+, diacylglycerol or cAMP2. GTP hydrolase activating proteins (GAPs) which promote conversion of Rap-bound GTP to GDP. Members of the Rap subfamily of GTPases, prominently Rap1A, Rap1B, Rap2A and Rap2B were originally thought to act as antagonists of Ras signalling. However it is now evident that Rap proteins have a function of their own in numerous cell types. Recent findings illustrate that Rap proteins regulate cell-cell and cell-matrix adhesion via the regulation of integrin, cadherin and other cell surface adhesion receptor function.Traditionally, GTPase activity measurements have involved metabolic labelling of cells with inorganic [32P]-phosphate followed by isolation of the GTPase and chromatographic analysis of bound guanine nucleotides. This methodology does provide quantitative data for GDP and GTP levels on

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Rap but it is a tedious and time consuming procedure that requires overexpression of heterologous tagged versions of Rap and working with large amounts of radioactivity. An alternative non-radioactive technique exploits the selective interaction of GTPase binding domains with the active, GTP-bound cognate GTPase conformation. For Rap activity determinations recombinant, GST tagged RalGDS-RA is added to cell extracts to pull out Rap-GTP, which is consequently detected by Western blotting.

Applications

Functional Studies more details

Kit Components

Components: Identifier; 100X Protease Inhibitor Mix; 5X Lysis Buffer Stock; GDP (10 mM in water); GDP (100 mM in water); Glutathione-Sepharose Slurry; GMPpNHp (10 mM in water); GST-RalGDS-RA (2-5 µg/µl in PBS); His-Rap-1A Protein (1-5 µg/µl in 50 % glycerol containing storage solution); Magnesium Chloride (1 M); Rap Nucleotide Loading Solution (NLS); Rap1 Polyclonal Antibody:

Compatible Sample Types

Cell culture extracts

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