CREATIVE BIOMART®
Assay Kit

Ral Activation Kit

Product Information

Cat.No. Kit-0760

Product Overview

Ral Activation Kit provides a rapid, cost-effective and reliable tool for the detection and semi-quantitative analysis of the cellular activation state of Ral GTPases. The kit exploits the selective interaction of the Ral-binding domain (RalBD) of the Ral-effector RIP1 (also known as RLIP or RalBP1) with the active, Ral-GTP conformation. Recombinant, GST-tagged RalBD is added to cell extracts to pull out Ral-GTP, which is consequently detected by Western blotting. The kit detects active, GTP-bound RalA and RalB. However, the antibody included in the kit allows detection of RalA only. The kit also includes recombinant RalA 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), Ral GTPases cycle between an active GTP-bound state and an inactive GDP-bound conformation. Cellular Ral-GDP/GTP levels are tightly controlled by two groups of proteins: 1. Guanine nucleotide exchange factors (GEFs) catalyze GTP uptake by Ral in response to upstream signals, which can include e.g. other active Ras-family GTPases,2. GTP hydrolase activating proteins (GAPs) which promote conversion of Ral-bound GTP to GDP. RalA and RalB play an important role in the control of exocytosis and have been proposed to be major players in ras-dependent oncogenic transformation, probably by regulating cell survival pathways in cancer cells. 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 Ral but it is a tedious and time consuming procedure that requires over-expression of heterologous tagged versions of Ral and working with large amounts of radioactivity.An alternative non-radioactive technique exploits

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the selective interaction of GTPase binding domains with the active, GTP-bound cognate GTPase conformation. For Ral activity determinations recombinant, GST-tagged RalBD is added to cell extracts to pull out Ral-GTP, which is consequently detected by Western blotting.

Applications

Functional Studies more details

Target Species

Reacts with: Human

Kit Components

Components: Identifier; 100X Protease Inhibitor Mix; 5X Lysis Buffer Stock; C-terminally truncated RalA protein (50% Glycerol Solution); GDP (10 mM in water); GDP (100 mM in water); Glutathione-Sepharose Slurry; GMPpNHp (10 mM in water); GST-RalBD; Magnesium Chloride (1 M); Ral Nucleotide Loading Solution (NLS); RalA Monoclonal Antibody:

Compatible Sample Types

Cell culture extracts

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