Rac1 Activation Assay Kit ab211161

Overview

Product name: Rac1 Activation Assay Kit
Sample type: Tissue, Adherent cells, Suspension cells
Species reactivity: Reacts with: Mouse, Rat, Human
Product overview: Rac1 Activation Assay Kit (ab211161) uses PAK1 PBD Agarose beads to selectively isolate and pull-down the active form of Rac from purified samples or endogenous lysates. Subsequently, the precipitated GTP-Rac is detected by western blot analysis using an anti-Rac1 specific monoclonal antibody, which reacts with the human, mouse and rat protein.

Features: 1) non radioactive assay format; 2) fast results: 1 hour assay plus electrophoresis/blotting time; 3) includes Cdc42 positive control; 4) pink colored agarose beads for easy identification during washing and aspiration steps.

Notes: Small GTP-binding proteins (or GTPases) are a family of proteins that serve as molecular regulators in signaling transduction pathways.

Rac1 (Ras-related C3 Botulinum Toxin Substrate 1), a 21 kDa protein, belongs to the family of Rho GTPases and regulates a variety of biological response pathways that include cell motility, cell division, gene transcription, and cell transformation. Like other small GTPases, Rac1 regulates molecular events by cycling between an inactive GDP-bound form and an active GTP-bound form. In its active (GTP-bound) state, Rac1 binds specifically to the p21-binding domain (PBD) of p21-activated protein kinase (PAK) to control downstream signaling cascades.

Diseases associated with Rac1 include amyotrophic lateral sclerosis type 2 and Newcastle disease.

Storage instructions: Store at -20°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>20 tests</th>
<th>5 tests</th>
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<tbody>
<tr>
<td>100X GDP</td>
<td>1 x 50µl</td>
<td>1 x 20µl</td>
</tr>
<tr>
<td>100X GTPyS</td>
<td>1 x 50µl</td>
<td>1 x 20µl</td>
</tr>
<tr>
<td>5X Assay/Lysis Buffer</td>
<td>1 x 30ml</td>
<td>4 x 2ml</td>
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**Function**

Plasma membrane-associated small GTPase which cycles between active GTP-bound and inactive GDP-bound states. In its active state, binds to a variety of effector proteins to regulate cellular responses such as secretory processes, phagocytosis of apoptotic cells, epithelial cell polarization and growth-factor induced formation of membrane ruffles. Rac1 p21/rho GDI heterodimer is the active component of the cytosolic factor sigma 1, which is involved in stimulation of the NADPH oxidase activity in macrophages (By similarity). Essential for the SPATA13-mediated regulation of cell migration and adhesion assembly and disassembly. Isoform B has an accelerated GEF-independent GDP/GTP exchange and an impaired GTP hydrolysis, which is restored partially by GTPase-activating proteins. It is able to bind to the GTPase-binding domain of PAK but not full-length PAK in a GTP-dependent manner, suggesting that the insertion does not completely abolish effector interaction.

**Tissue specificity**

Isoform B is predominantly identified in skin and epithelial tissues from the intestinal tract. Its expression is elevated in colorectal tumors at various stages of neoplastic progression, as compared to their respective adjacent tissues.

**Sequence similarities**

Belongs to the small GTPase superfamily. Rho family.

**Domain**

The effector region mediates interaction with DEF6.

**Post-translational modifications**

AMPylation at Tyr-32 and Thr-35 are mediated by bacterial enzymes in case of infection by H.somnus and V.parahaemolyticus, respectively. AMPylation occurs in the effector region and leads to inactivation of the GTPase activity by preventing the interaction with downstream effectors, thereby inhibiting actin assembly in infected cells. It is unclear whether some human enzyme mediates AMPylation; FICD has such ability in vitro but additional experiments remain to be done to confirm results in vivo.

**Cellular localization**

Cell membrane. Melanosome. Inner surface of plasma membrane possibly with attachment requiring prenylation of the C-terminal cysteine (By similarity). Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

**Images**

Rac1 positive control (recombinant human Rac1 from E. coli) by immunoblot.
Dot blot that demonstrates specificity of anti-Rac1 mouse antibody.

Rac1 activation assay. Lane 1: MW Standard. Lane 2: 293 cell lysate loaded with GDP and incubated with PAK1 PBD Agarose beads. Lane 3: 293 cell lysate loaded with GTPγS and incubated with PAK1 PBD Agarose beads.

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