

## RAP 1A Human, Recombinant Expressed in *E. coli*

Product Number R 2152

### **Product Description**

Recombinant Rap 1A is a small Ras-like GTP-binding protein, tagged with GST. It has a molecular weight of approx. 41 kDa (native protein MW of 21 kDa plus 20 kDa GST). Rap 1A is expressed in *E. coli* and purified on nickel agarose. It has been used as a positive control in immunoblotting applications with Anti-Rap 1A and in protein binding assays.

Ras-related proteins, designated Rap 1A, Rap 1B, and Rap 2 are small GTPases of the Ras superfamily. The Ras-related proteins share ~ 50% amino acid identity with the classical Ras proteins and have common structural features. The C-terminal cysteine and the GTP-binding regions of the p21 RAS proteins are also present in the Rap proteins. This suggests that these proteins could bind GTP/GDP and have membrane localization. The most striking difference between the Rap and Ras proteins resides in their 61st amino acid. Both Rap proteins have a threonine instead of the glutamine at position 61 of the classical Ras proteins. RAP 1A contains a C-terminal CLLL motif for post-translational geranylgeranylation and a single site for PKA phosphorylation.

RAP proteins are potential modulators of the Ras/Raf/MAP kinases cascade that governs cell growth control. Rap 1A induces morphological reversion of a cell line transformed by a Ras oncogene. It also counteracts the mitogenic function of Ras, at least in part due to its ability to interact with Ras-Gaps and Raf in a competitive manner. A Rap 1A is a membrane bound protein localized in the endoplasmic reticulum, late endosomes, and lysosomes, indicating its potential role in the regulation of intracellular protein degradation.

#### Reagent

Recombinant Rap 1A is supplied as a solution in 50 mM Tris buffer, pH 7.5, containing 150 mM NaCl, 10 mM MgCl2, 1 mM DTT, and 10% glycerol.

# **ProductInformation**

#### **Precautions and Disclaimer**

A material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

## Storage/Stability

Store at -70 °C. For extended storage, freeze in working aliquots. Avoid repeated freezing and thawing to prevent denaturing of the protein. Do not store in the frost-free freezer. The protein is stable for at least 12 months when stored appropriately.

#### **Product Profile**

Rap 1A may be used as a control in immunoblotting with Anti-Rap 1A antibody. 10 to 500 ng of Rap 1A may be used per lane for this purpose, depending on the sensitivity of the antibody used. For binding assays, the recommended amount ranges from 50 ng to 5  $\mu$ g per test, depending on the assay conditions. In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

#### References

- 1. Takai, Y., et al., Small GTP-binding proteins. Physiol. Rev., 1, 153-208 (2001).
- McPhee, I., et al., Use of an activation-specific probe to show that Rap1A and Rap1B display different sensitivities to activation by forskolin in rat1 cells. FEBS Lett., 477, 213-218 (2000).
- 3. Hermann, C., et al., Differential interaction of the ras family GTP-binding proteins H-Ras, Rap 1A and R-Ras with the putative effector molecules Raf kinase and Ral-guanine nucleotide exchange factor. J. Biol. Chem., **271**, 6794-6800 (1996).
- 4. Yatani, A, et al., Rap1A antagonizes the ability of Ras and Ras-Gap to inhibit muscarinic K+ channels. J Biol Chem., **266**, 22222-22226 (1991).

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