RAF1(EE) (Human), Active

Recombinant protein expressed in Sf9 cells

Cat# CY-SPR01

Lot No. C294-1
5 µg 0.1 µg/µl

Background:

RAF1 is a MAP kinase kinase kinase (MAP3K), which functions downstream of the Ras family of membrane associated GTPases to which it binds directly (1). The activated RAF1 can phosphorylate and activate the dual specificity protein kinases MEK1 and MEK2, which in turn phosphorylate to activate the serine/threonine specific protein kinases ERK1 and ERK2. Activated ERKs are pleiotropic effectors of cell physiology and play an important role in the control of gene expression involved in the cell division cycle, apoptosis, cell differentiation and cell migration (2).

Product Description:

Recombinant human RAF1 (Y340E Y341E, 306-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_002880.

Gene Aliases:

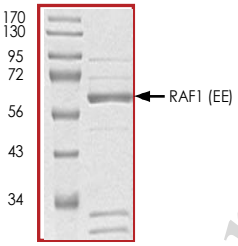
None

Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Purity & Molecular Weight:

The purity was determined to be >85% by densitometry. Approx. MW 63kDa.



Storage:

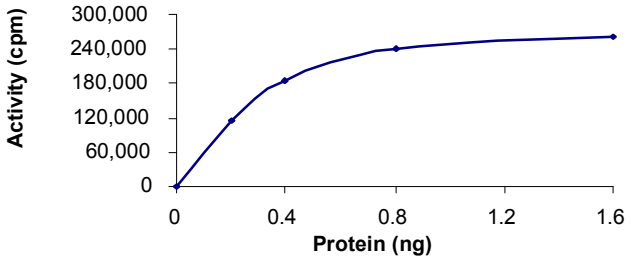
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

1 year at –70 °C from date of shipment.

Specific Activity:

The specific activity was determined to be ~6,000 nmol /min/mg in a coupled assay as per Activity Assay Protocol.



Activity Assay Protocol:

Assay 1st reaction of the RAF1 (EE) activity in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 2 µL of 0.2 µg/µL unactive MEK1 Substrate Solution, 3 µL of 0.2 µg/µL unactive ERK1 Substrate Solution, 10 µL of the diluted RAF1 (EE) and 5 µL of 250 µM ATP solution. Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Then assay 2nd reaction in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 5 µL of 1 mg/ml MBP Substrate Solution, 5 µL of 1st reaction mixture, 5 µL of distilled H_2O and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate 2nd reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

0.2 µg/µL of unactive MEK1 as 1st substrate for the RAF1 (EE) and 0.2 µg/µL of unactive ERK1 as 2nd substrate for the activated MEK1 and Myelin Basic Protein (MBP) diluted in distilled H_2O to a final concentration of 1 mg/ml was used as 3rd substrate for the activated ERK1.

5 X Kinase Assay Buffer:

25mM MOPS, pH 7.2, 12.5mM β -glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Rapp, U. et al: Structure and biological activity of v-raf, a unique oncogene transduced by a retrovirus. Proc. Nat. Acad. Sci. 80: 4218-4222, 1983.

2.Li, P. et al: Raf-1: a kinase currently without a cause but not lacking in effects. Cell 64: 479-482, 1991.

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