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The recombinant human RAF1 protein (307-end) kinase containing N-terminal GST tag

Catalog No.	CRR103A	**Quantity:**	5 µg
	CRR103B		20 µg

Description: The recombinant human RAF1 protein (307-end) containing N-terminal GST tag was expressed by baculovirus in Sf 9 cells.

Raf-1 is a MAP kinase kinase kinase (MAP3K), which functions downstream of the Ras family of membrane associated GTPases to which it binds directly. The activated Raf-1 can phosphorylate to 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.

Concentration: 0.1 mg/ml

Gene Accession No: NM_002880

Source: Sf9 insect cells

Formulation: Recombinant protein in storage buffer (50 mM Tris-HCl + 150 mM NaCl + 0.25 mM DTT + 0.1 mM EGTA + 25% glycerol; pH 7.5).

Purity: 1 µg of protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the RAF1 product, the band was at ~64 kDa (Fig. 2).

Specific Activity: 105 nmol/min/mg: 105 nmol phosphate incorporated into MBP per minute per mg protein at 30°C using a final concentration of 50 µM ATP (0.83 µCi/assay). See QA/QC section for details.

Storage & Stability: Store product frozen at or below -70ºC. Stable for 1 year at -70ºC as undiluted stock. Aliquot to avoid repeated thawing and freezing.

QA/QC: Raf1 protein (100 ng/µl concentration) was diluted to 20 ng/µl with assay dilution buffer (4 mM MOPS + 2.5 mM β-glycerophosphate + 1 mM EGTA + 0.4 mM EDTA + 30 mM $MgCl_2$ + 0.05 mM DTT; pH 7.2), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the MBP in the following assay condition:

- 10 µl diluted Raf1 protein
- 10 µl MBP (1 mg/ml stock)
- 5 µl [^{32}P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30ºC and the reaction started by the addition of [^{32}P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

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Fig. 1 Raf1 activity assay

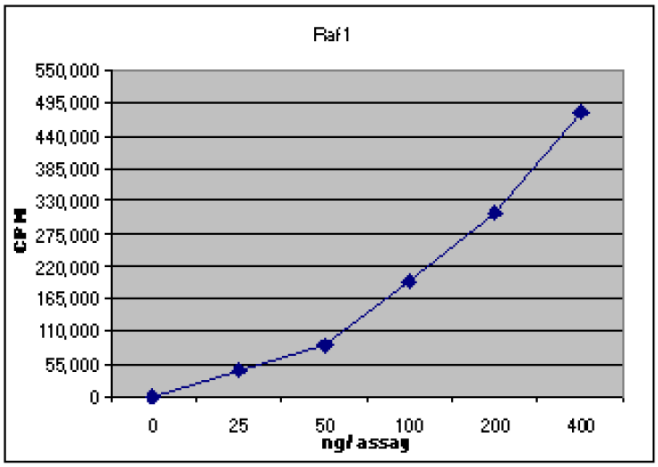
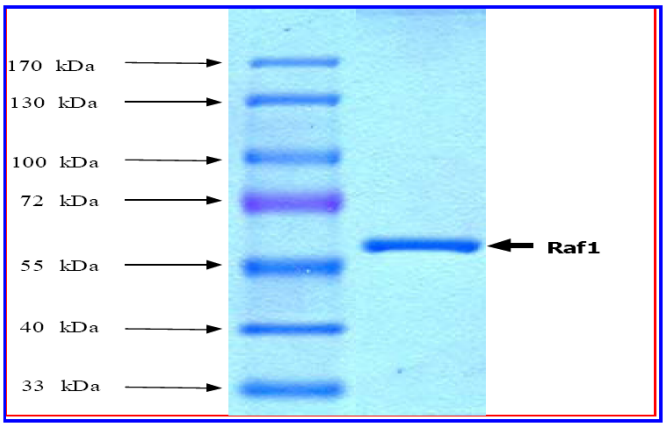


Fig. 2 Raf1 protein gel



Please note: always centrifuge vials before opening.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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