BioVision
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# Active RAF1(EE)

Recombinant protein expressed in Sf9 cells

**Catalog # 7726-5**

*Lot # C294-1*

## 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.

## Storage and Stability

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.

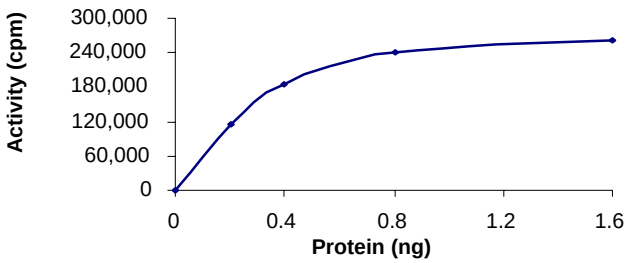
## Scientific Background

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).

## References

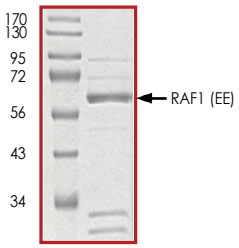
1. Rapp, U. et al: Structure and biological activity of v-raf, a unique oncogene transduced by a retrovirus. *Proc. Nat. Acad. Sci.* 80: 4218-4222, 1983.
2. Li, P. et al: Raf-1: a kinase currently without a cause but not lacking in effects. *Cell* 64: 479-482, 1991.

## Specific Activity




The specific activity of RAF1(EE) was determined to be **~6,000 nmol /min/mg** in a coupled assay as per activity assay protocol.

## Purity




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

# RAF1(EE), Active

Recombinant protein expressed in Sf9 cells

| | |
|---|---|
| Catalog Number | 7726-5 |
| Quantity | 5µg |
| Specific Activity | ~6,000 nmol/min/mg |
| Specific Lot Number | C294-1 |
| Purity | >85% |
| Format | 5µg in 50µl |
| Concentration | 0.1µg/µl |
| Stability | 1yr At –70°C from date of shipment |
| Storage & Shipping | 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. Product shipped on dry ice. |

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: 7726-5)

Active RAF1(EE) (0.1μg/μl) diluted with Kinase Dilution Buffer III and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active RAF1(EE) for optimal results).

### Kinase Dilution Buffer III

Kinase Assay Buffer I diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

### Kinase Assay Buffer I

Buffer components: 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.

### [$^{32}$P]-ATP Assay Cocktail

Prepare 250μM [$^{32}$P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution, 100μl [$^{32}$P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer I. Store 1ml aliquots at –20°C.

### 10mM ATP Stock Solution

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I. Store 200μl aliquots at –20°C.

### Substrate

Unactive MEK1 and ERK1 were activated in a coupled reaction. Myelin Basic Protein (MBP) diluted in distilled $H_2O$ to a final concentration of 1mg/ml was subsequently used as a substrate for the activated ERK1.

## Assay Protocol

**Step 1.** Thaw the Active RAF1(EE), Kinase Assay Buffer, Unactive ERK1 and Unactive MEK1 on ice. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active RAF1(EE)
- **Component 2.** 2μl of Unactive MEK1 (0.2μg/μl)
- **Component 3.** 3μl of Unactive ERK1 (0.2μg/μl)
- **Component 4.** 5μl of Kinase Dilution Buffer

**Step 2.** Start the reaction by the addition of 5 μl ATP (250μM) and incubate in a water bath at 30°C for 25 minutes.

**Step 3.** After the 25 minute incubation period, remove 5μl and add to the following reaction components bringing the initial reaction volume up to 20μl on ice:

- **Component 1.** 5μl of reaction mixture
- **Component 2.** 10μl distilled $H_2O$ on ice
- **Component 3.** 5μl of MBP substrate on ice(1 mg/ml)

**Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled $H_2O$.

**Step 5.** Initiate the reaction by the addition of 5μl [$^{32}$P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

**Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

**Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled $H_2O$) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

**Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

**Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

## Calculation of [P$^{32}$]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5μl [$^{32}$P]-ATP / pmoles of ATP (in 5μl of a 250μM ATP stock solution, i.e., 1250 pmoles)

## Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of $^{32}$P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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