Recombinant Human Active RAF-1 (Y340E Y341E, 306-end), CF

Catalog Number: 4540-KS-010
Lot Number: 1757176

SPECIFICATIONS AND USE

Source	Recombinant human RAF-1 (Y340E Y341E; amino acids 306-end) was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_002880.
Molecular Mass	The approximate molecular weight is 63 kDa
Purity	The purity was determined to be > 85% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 10 mM Glutathione, 0.1 mM EDTA, 0.1 mM PMSF, and 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of RAF-1 was determined to be 5600 nmol/min/mg in a coupled assay as per Activity Assay Protocol.
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

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 (1). The activated RAF-1 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).

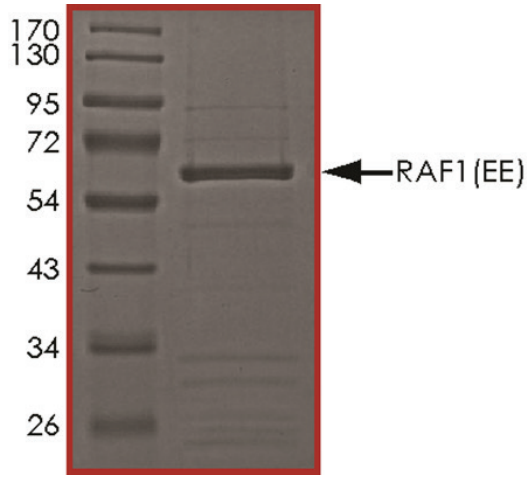

Figure 1: The approximate molecular weight is 63 kDa and the purity is > 70%.

Figure 2: The specific activity of RAF-1 was determined to be 5600 nmol/min/mg in a coupled assay as per Activity Assay Protocol.

REFERENCES

1. Rapp, U. *et al.* (1983) Proc. Natl. Acad. Sci. USA **80**:4218.
2. Li, P. *et al.* (1991) Cell **64**:479.

Global bio-techne.com info@bio-techne.com TEL +1 612 379 2956 **USA** TEL 800 343 7475 **Canada** TEL 855 668 8722
Europe | Middle East | Africa TEL +44 (0)1235 529449 **China** TEL +86 (21) 52380373

ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active RAF-1(EE) (0.1 µg/µL) diluted with Kinase Dilution Buffer III and assayed as outlined in Figure 2.
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Assay Buffer I - 25 mM MOPS pH 7.2, 12.5 mM β-glycerolphosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer I prior to use.

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

[^{33}P]-ATP Assay Cocktail - Prepare 250 µM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 µL of 10 mM ATP Stock Solution and100 µL [^{33}P]-ATP, 5.75 mL of Kinase Assay Buffer I. Store 1 mL aliquots at ≤ -20 °C.

10 mM ATP Stock Solution - Prepare ATP stock solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I. Store 200 µL aliquots at ≤ -20 °C.

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

ASSAY PROCEDURE

1. Thaw the Active RAF1 (EE), Kinase Assay Buffer I, 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 20 µL:

Reaction Component	Amount
Diluted Active RAF1	10 µL
Unactive MEK1 (0.2 µg/µL)	1 µL
Unactive ERK1 (0.2 µg/µL)	1 µL
Kinase Dilution Buffer	8 µL

2. Start the reaction by the addition of 5 µL ATP (250 µM) and incubate in a water bath at 30 °C for 15 minutes.
3. After the 15 minute incubation period, remove 5 µL and add to the following reaction components bringing the initial reaction volume up to 20 µL on ice.

Component 1	3 µL of diluted Active RAF-1
Component 2	2 µL of Substrate/ATP mix as prepared in the table above. This initiates the reaction.
Component 3	5 µL of MBP substrate on ice (1 mg/mL)

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 water.
5. Initiate the reaction by the addition of 5 µL [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25 µL and incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15 minute incubation period, terminate the reaction by spotting 20 µL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10 mL of phosphoric acid and make a 1L solution with distilled water) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
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 [P33]-ATP Specific Activity (SA) (cpm/pmol)
Specific Activity (SA) = cpm for 5 µL [^{33}P]-ATP / pmoles of ATP (in 5 µL of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)
Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)] * [(Reaction Volume) / (Spot Volume)