

Certificate of Analysis

Raf1, active (recombinant enzyme expressed in sf9)

Quantity: 20µg

Cat Number: Z02210

Lot Number: P40450911

Description:

Recombinant human Raf1, N-terminal GST-tagged, residues 306-end, expressed in insect cell sf9. Purity 60% by SDS-PAGE and Commassie blue staining. Calculated M.W=65 kDa.

Accession: EMBL X03484

Specific Activity:

37761 nmol/min/mg, where one nmol/min of Raf-1 activity = 1 nmol/min of MAPK1 activity which in turn is defined as 1 nmol phosphate incorporated into 0.33 mg/ml MBP per minute at 30 °C with a final ATP concentration of 100 µM. Note the activity is determined using a triple linked assay which involves the activation of MAP2K1 by Raf-1, followed by the subsequent activation of MAPK1 by the activated MAP2K1.

Protein Concentration:

0.26 mg/ml, as determined by Bradford protein assay with BSA as a standard.

Storage and Handling:

Store at -80 °C. At first use, aliquot and store at -80 °C to avoid multiple freeze-thaws.

Storage Buffer:

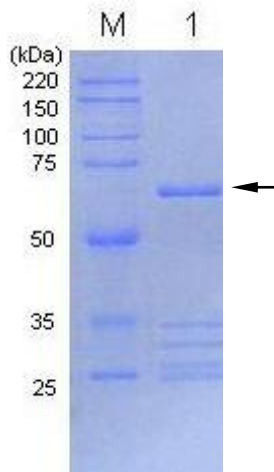
50mM Tris.HCl (pH8.0), 150mM NaCl, 10% glycerol, 0.1mM EGTA, 0.03% Triton X-100, 2mM DTT

Quality Assurance:

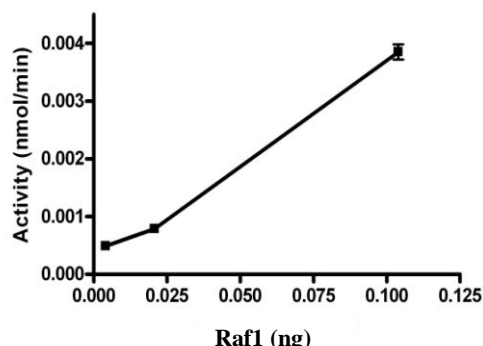
The SDS-PAGE was run on 12% Tris-Glycine Novex gels and stained with Commassie Blue.

Lane M: protein marker

Lane 1: Recombinant protein (1.5µg of Raf1)



Activity Graph:



The enzyme was assayed in 50 mM HEPES (pH 7.2), 10 mM MgCl₂, 1 mM Na₃VO₄, 1 mM DTT, 1 mM EGTA, 0.1 % Brij-35, 0.042 mg/ml MAP2K1, 0.045 mg/ml MAPK1, 0.33 mg/ml MBP and 100 µM ATP, for 30 minutes at 30 °C. The basal activity of MAP2K1 and MAPK1 was also assayed in the absence of RAF1 and subtracted from the actual response to yield the result. The specific activity was measured with a HTRF® Transcreener® ADP kit.

Certified by:

Catherine

Date: 07/15/2009

If the use of this product results in a publication, please feel free to cite us in the materials and methods of the publication as follows: The protein was expressed and purified by GenScript Corp., New Jersey, USA

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