BRK (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP94

Lot No. A360-1
5 µg 0.1 µg/µl

Background:

BRK is a member of the non-receptor tyrosine kinases (PTKs) that contains an amino terminal SH3 and SH2 domain as well as the catalytic domain (1). BRK expression is low or undetectable in normal mammary tissue and benign lesions. However, approximately two-thirds of breast tumors express appreciable levels, and 27% of tumors over express BRK by fivefold or more (2).

Product Description:

Recombinant full-length human BRK was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_005975.

Gene Aliases:

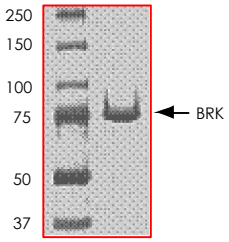
PTK6

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 >90% by densitometry. Approx. MW 80kDa.



Storage:

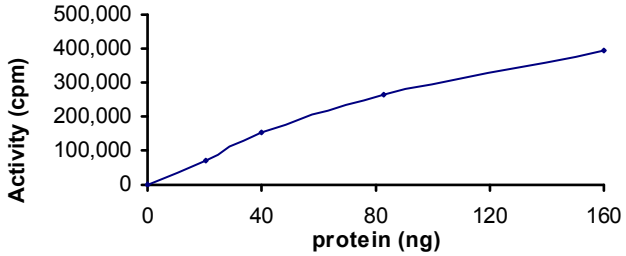
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

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

Specific Activity:

The specific activity was determined to be 133 nmol/min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 10 µL of 1 mg/ml the Substrate Solution, 5 µL of diluted kinase 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 the 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:

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H_2O to a final concentration of 1 mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, 12.5mM β-glycerol-phosphate, 20mM $MgC1_2$, 25mM $MnC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Mitchell, PJ. et al: Cloning and characterisation of cDNAs encoding a novel non-receptor tyrosine kinase, brk, expressed in human breast tumours. Oncogene. 1994 Aug;9(8):2383-90.

2.Mitchell, PJ. et al: Characterisation and chromosome mapping of the human non receptor tyrosine kinase gene, brk. Oncogene. 1997 Sep 18;15(12):1497-502.Erratum in: Oncogene 1998 Jul 9;17(1):129.

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