

Product Specification

BRK, active

(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7708-5
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 133 nmol/min/mg

Quality Control Analysis

Activity assessment

BRK protein (100 ng/µl concentration) was diluted to 20ng/µl in assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 0.2 mM EDTA, 5 mM of MnCl₂, 4 mM MgCl₂, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the Poly(Glu-Tyr) in the following assay condition:

- 10 µl diluted BRK protein
- 5 µl Poly(Glu-Tyr) (1mg/ml stock)
- 5 µl water
- 5 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30°C and the reaction started by the addition of [³²P] 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.

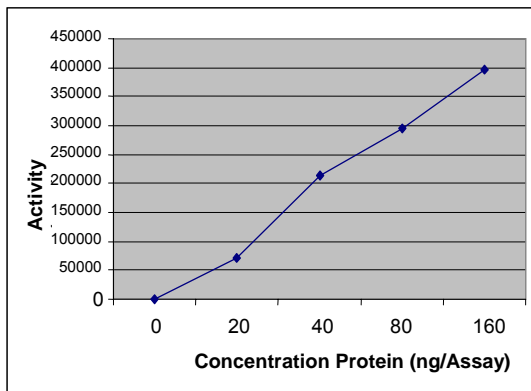


Fig. 1 BRK activity assay

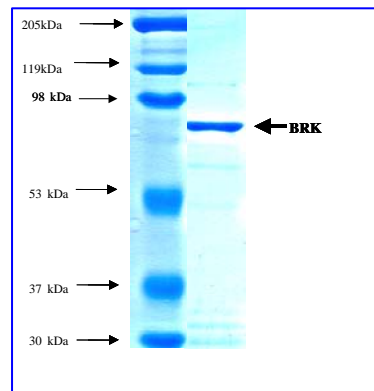


Fig. 2 BRK protein gel

Purity assessment

1 µg of BRK protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >95% purity of the BRK product, and the band was at ~80 kDa (Fig. 2).

Product Description

Recombinant full length human BRK containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_005975.

This material is sold for research purposes only.

Specific Activity

133 nmol phosphate incorporated into Poly(Glu-Tyr) per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 µM ATP and total of 0.83 µCi/µl P-32.

Formulation

Recombinant protein in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

BRK is another member of the non-receptor tyrosine kinases (PTKs) that contains an amino terminal SH3 and SH2 domains as well as the catalytic domain (1). Although BRK shows strongest sequence similarity to members of the Src family, there are several key structural and regulatory differences that place it on its own amongst non-receptor PTKs. The genomic structure of BRK consists of 8 exons, whose boundaries are distinct from other non-receptor PTK family members (2). Alternate splicing of the primary BRK transcript generates a distinct mRNA which encodes a truncated protein consisting of an SH3 domain and a novel C-terminal proline rich sequence. Brk transcript is expressed in the human breast tumor cell line and expression of a tumor derived Brk cDNA in mouse embryonic fibroblasts and human mammary epithelial cells supports anchorage independent growth, and in the latter potentiates the mitogenic response to epidermal growth factor. 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 (up to 43x). This expression pattern is mirrored in comparison of cell lines derived either from normal mammary epithelial cells or from carcinomas (3).

References

1. Mitchell PJ, Barker KT, Martindale JE, Kamalati T, Lowe PN, Page MJ, Gusterson BA, Crompton MR. 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, Barker KT, Shipley J, Crompton MR. 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.
3. Barker KT, Jackson LE, Crompton MR. BRK tyrosine kinase expression in a high proportion of human breast carcinomas. *Oncogene*. 1997 Aug 14;15(7):799-805.