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Product Specification

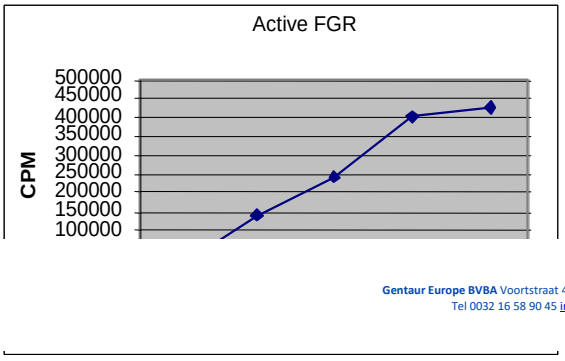
FGR, active
(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7724
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 282 nmol/min/mg

Quality Control Analysis
Activity assessment
FGR 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, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the Poly(Glu-Tyr) using the following assay conditions:

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

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



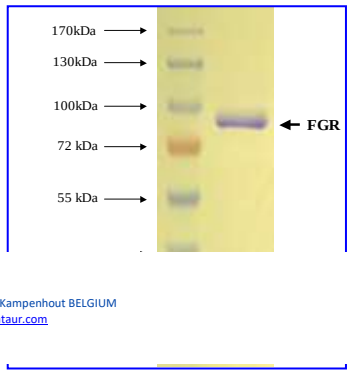


Fig. 1 FGR activity assay

Fig. 2 FGR protein gel

Purity assessment
2 µg of FGR protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the FGR product, and the band was at ~86 kDa (Fig. 2)

Product Description
Recombinant full length human FGR containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is NM_005248.
This material is sold for research purposes only.

Specific Activity
282 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 (0.83 µCi/assay).

Formulation
Recombinant proteins 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
Fgr is a protooncogene that is a unique member of the tyrosine kinase gene family. It is localized to the distal portion of the short arm of human chromosome 1 at p36.1-36.2 by in situ hybridization (1). Certain lymphomas (but not sarcomas or carcinomas) express fgr-related messenger RNA. This transcript is detected in Burkitt's lymphoma cell lines naturally infected with Epstein-Barr virus (EBV), but not in EBV-negative Burkitt's lymphoma cells (2). Normal umbilical cord or peripheral blood lymphocyte lines established in vitro by EBV infection also contain detectable c-fgr mRNA. Moreover, a 50-fold increase of the steady-state c-fgr mRNA concentration is observed when uninfected Burkitt's lymphoma cell lines are deliberately infected with EBV demonstrating the induction of a proto-oncogene in response to infection by a DNA tumour virus. Fgr expression is limited to normal peripheral blood granulocytes, monocytes, and alveolar macrophages, all of which contain 50 to 100 copies of c-fgr mRNA per cell (3). The c-fgr RNA molecules in these cells consisted of partially spliced transcripts containing intron 7 and completely spliced molecules capable of encoding the predicted p55 c-fgr protein. The level of fgr transcripts begin to increase 2 to 4 h after TPA addition, peak at 8 h, and subsequently declined suggesting transient transcriptional activation of fgr during TPA-induced differentiation. Cycloheximide also causes accumulation of c-fgr transcripts in U937 cells. Thus, c-fgr gene is expressed in a tissue- and development-specific fashion and constitutive expression of c-fgr in U937 cells is regulated by a labile transcriptional repressor.

References
1. Tronick SR, Popescu NC, Cheah MS, Swan DC, Amsbaugh SC, Lengel CR, DiPaolo JA, Robbins KC. Isolation and chromosomal localization of the human fgr protooncogene, a distinct member of the tyrosine kinase gene family. Proc Natl Acad Sci U S A. 1985 Oct;82(19):6595-9.
2. Cheah MS, Ley TJ, Tronick SR, Robbins KC. fgr proto-oncogene mRNA induced in B lymphocytes by Epstein-Barr virus infection. Nature. 1986 Jan 16-22;319(6050):238-40.
3. Ley TJ, Connolly NL, Katamine S, Cheah MS, Senior RM, Robbins KC. Tissue-specific expression and developmental regulation of the human fgr proto-oncogene. Mol Cell Biol. 1989 Jan;9(1):92-9.

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