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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
F06-16HG-20	20 µg
F06-16HG-50	50 µg

FGFR3 (d613-653), Unactive

Recombinant human protein expressed in Sf9 cells

Catalog # F06-16HG

Lot # M2695-3

Product Description

Recombinant human FGFR3 (d613-653) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_000142.

Gene Aliases

ACH, CEK2, JTK4, CD333, HSFGFR3EX

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 25% glycerol

Storage and Stability

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.

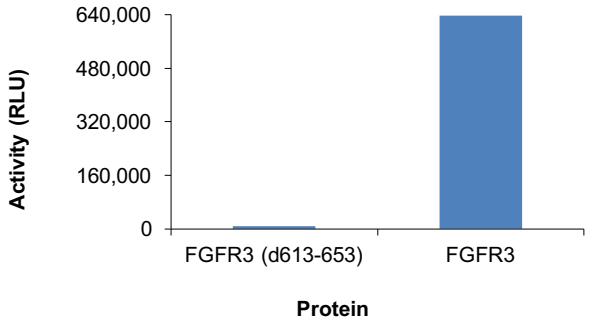
Scientific Background

Fibroblast growth factor receptor 3 (FGFR3) is part of a family of fibroblast growth factor receptors that share similar structures and functions. FGFR3 plays a role in several important cellular processes, including regulation of cell growth and division, determination of cell fate, formation of blood vessels, wound healing and embryo development (1). FGFR3 is involved in the development and maintenance of bone and brain tissue. Mutations in FGFR3 have been implicated in causing bladder cancer, cancer of white blood cells (multiple myeloma) and cervical cancer (2).

References

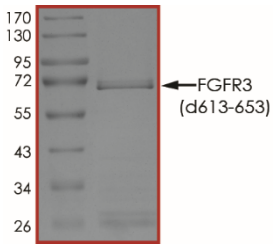
1. Chen, L. and Deng, C.X. Roles of FGF signaling in skeletal development and human genetic diseases. Front Biosci. 2005; 1(10):1961-1976.
2. Mhawech-Fauceglia, P. et al. 2006. FGFR3 and p53 protein expressions in patients with pTa and pT1 urothelial bladder cancer. Eur. J. Surg. Oncol. 2006; 32(2):231-237

Specific Activity



The specific activity of FGFR3 (d613-653) was determined to be **0.16 nmol/min/mg** as per activity assay protocol.

Purity



The purity of FGFR3 was determined to be **>95%** by densitometry, approx. MW **68kDa**.

FGFR3 (d613-653), Unactive

Recombinant human protein expressed in Sf9 cells

Catalog #	F06-16HG
Specific Activity	0.16 nmol/min/mg
Lot #	M2695-3
Purity	>95%
Concentration	0.05 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: F06-16HG)

Unactive FGFR3 (d613-653) (0.05μg/μl) diluted with Kinase Dilution Buffer X (1x) (Catalog #: K20-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of FGFR3 (d613-653) for optimal results).

Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM MgCl2 and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250μM.

Kinase Dilution Buffer X (1x) (Catalog #: K20-09)

Kinase Assay Buffer III (Catalog #: K03-09) with 0.1M $MnCl_2$ diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50μM.

ADP-GloTM Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM
ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

Substrate (Catalog #: P61-58)

Poly (4:1 Glu, Tyr) synthetic peptide substrate diluted in distilled H2O to a final concentration of 1mg/ml.

Cofactor: 2.5M $MnCl_2$ (Catalog #: M40-09-25)

Diluted to a working concentration of 0.1M in distilled H2O.

Assay Protocol

The FGFR3 assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the FGFR3 reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

Step 1. Thaw the FGFR3, Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15 μL enzyme dilution at the desired concentration, with Kinase Dilution Buffer X (1x), in a pre-chilled 96-well plate.

Step 2. Prepare a substrate/ATP mixture as follows (25 μM example):

Component	Amount (μL)	Component	Amount (μL)
10μM ATP Solution	1.25	Substrate at 1mg/mL	50
Kinase Assay Buffer III (5x)	46.75	0.1M $MnCl_2$	2

Step 3. Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5μL:

Component 1. 3μl of diluted FGFR3 (d613-653) (Catalog # F06-16HG).
Component 2. 2μl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

Step 4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer X (1x).

Step 5. Incubate at ambient temperature for 40 minutes.

Step 6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5μl of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.

Step 7. Then add 10μl of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.

Step 8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).

Step 9. Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)

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