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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
M52-19G -05	5 µg
M52-19G -10	10 µg

TFG-MET (Tex5Mex15), Active

Recombinant human protein expressed in Sf9 cells

Catalog # M52-19G
Lot # M2888-9

Product Description

Recombinant human TFG (1-193a.a., exons1-5)-MET (1010a.a.-end, exons15-21) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number of TFG is NM_001195478 and MET is NM_000245.

Gene Aliases

TFG: HMSNP; SPG57; TF6
MET: HGFR, RCCP2

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.

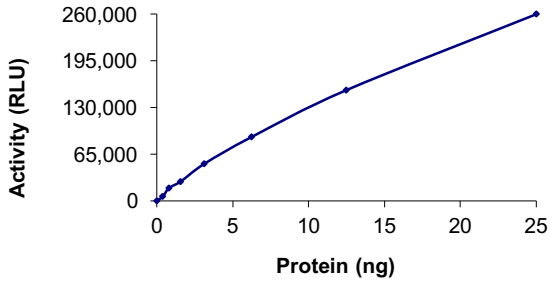
Scientific Background

MET is a proto-oncogene that encodes a transmembrane growth factor receptor which is a heterodimer of two disulphide linked chains of 50 kDa (alpha) and 145 kDa (beta). MET is widely expressed in the kidney, brain, lung, skin, and embryonic tissue (1). Hepatocyte growth factor (HGF) binds to MET and activates its tyrosine kinase activity. MET is overexpressed and activated in a variety of human cancers including pancreatic, colon, gastric, cervical and ovarian cancers and has been shown to be involved in tumor cell migration and invasion (2). The fusion protein of TFG-MET has been found in the spindle cell sarcoma (3).

References

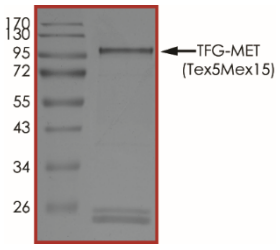
1. Giordano, S. et al: Biosynthesis of the protein encoded by the c-met proto-oncogene. Oncogene. 1989 Nov;4(11):1383-8.
2. Iyer, A. et al: Structure, tissue-specific expression, and transforming activity of the mouse met protooncogene. Cell Growth Differ. 1990 Feb;1(2):87-95.
3. Flucke U, et al: TFG-MET fusion in an infantile spindle cell sarcoma with neural features. Genes Chromosomes Cancer. 2017 Sep;56(9):663-667.

Specific Activity



The specific activity of TFG-MET (Tex5Mex15) was determined to be **59.6 nmol/min/mg** as per activity assay protocol.

Purity



The purity of TFG-MET (Tex5Mex15) was determined to be **>95%** by densitometry, approx. MW **102 kDa**.

TFG-MET (Tex5Mex15), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	M52-19G
Specific Activity	59.6 nmol/min/mg
Lot #	M2888-9
Purity	>95%
Concentration	0.1 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 #: M52-19G)

Active TFG-MET (Tex5Mex15) (0.1μg/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 active TFG-MET for optimal results).

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

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM $MgCl_2$ 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 12.5mM $MnCl_2$ 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) peptide substrate diluted in distilled H_2O to a final concentration of 0.2 mg/ml.

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

Diluted to a working concentration of 0.1M in distilled H_2O.

Assay Protocol

The TFG-MET assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the TFG-MET 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 Active TFG-MET, 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 Active TFG-MET (Tex5Mex15) (Catalog # M52-19G).
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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