

c-KIT (V559D V654A), Active

Recombinant protein expressed in Sf9 cells

Catalog # K06-12RG

Lot # M2937-7

Product Description

Recombinant human c-KIT (V559D V654A) (544-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_000222](#).

Gene Aliases

PBT, SCFR, CD117

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.

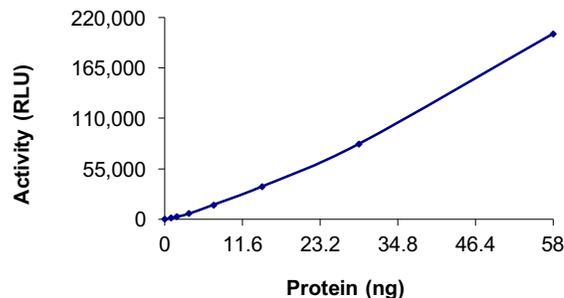
Scientific Background

c-KIT is a proto-oncogene and a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). c-KIT was first identified as the cellular homolog of the feline sarcoma viral oncogene v-kit. c-KIT together with its ligand regulates growth and activation of a variety of hemopoietic and non-hemopoietic cells. Mutations in c-KIT are associated with gastrointestinal stromal tumors, mast cell disease, acute myelogenous leukemia, and piebaldism. Recently, deregulation of the KIT receptor TK by the prevalent activation loop mutation D816V has served as a focal point in therapeutic strategies aimed at curbing neoplastic mast cell growth (2).

References

- Berger, S A.: Signaling pathways influencing SLF and c-kit-mediated survival and proliferation. *Immunol Res.* 2006;35(1-2):1-12.
- Gotlib, J.: KIT mutations in mastocytosis and their potential as therapeutic targets. *Immunol Allergy Clin North Am.* 2006 Aug;26(3):575-92.

Specific Activity



The specific activity of c-KIT (V559D V654A) was determined to be **9.2 nmol/min/mg** as per activity assay protocol, and was equivalent to **25 nmol/min/mg** as per radiometric assay.

Purity



The purity of c-KIT (V559D V654A) was determined to be **>70%** by densitometry, approx. MW **73 kDa**.

c-KIT (V559D V654A), Active

Recombinant protein expressed in Sf9 cells

Catalog #	K06-12RG
Specific Activity	9.2 nmol/min/mg
Lot #	M2937-7
Purity	>70%
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 #: K06-12RG)

Active c-KIT (V559D V654A) (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 active c-KIT (V559D V654A) 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 MnCl₂ 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-Glo™ 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 MnCl₂ (Catalog #: M40-09-25)

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

Assay Protocol

The c-KIT (V559D V654A) assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the c-KIT (V559D V654A) 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 c-KIT (V559D V654A), 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 c-KIT (V559D V654A) (Catalog # K06-12RG).
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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