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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
K06-12RG -05	5 µg
K06-12RG -10	10 µg

c-KIT (V559D V654A), Active

Recombinant protein expressed in Sf9 cells

Catalog # K06-12RG
Lot # G1247-2

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, 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.

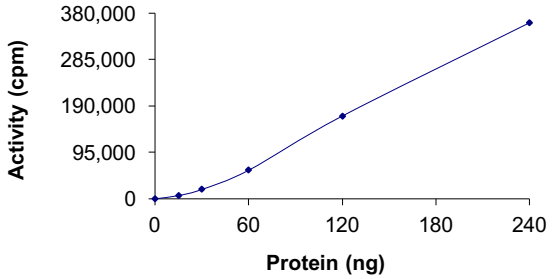
Scientific Background

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 lukemia, 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

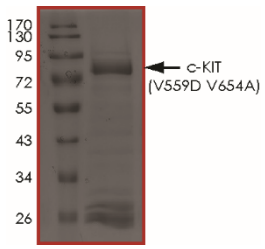
1. Berger, S A.: Signaling pathways influencing SLF and c-kit-mediated survival and proliferation. Immunol Res. 2006;35(1-2):1-12.
2. Gotlib, J.: KIT mutations in mastocytosis and their potential as therapeutic targets. Immunol Allergy Clin North Am. 2006 Aug;26(3):575-92.

Specific Activity



The specific activity of c-KIT (V559D V654A) was determined to be **24 nmol /min/mg** as per activity assay protocol.

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	24 nmol/min/mg
Lot #	G1247-2
Purity	>70%
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 #: K06-12RG)

Active c-KIT (V559D V654A) (0.1μg/μl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) 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 Dilution Buffer IV (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

Kinase Assay Buffer II (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 20mM $MgC1_2$, 25mM $MnC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: C50-58)

CREBtide synthetic peptide substrate (KRREILSRRPSYR) diluted in distilled H_2O to a final concentration of 1mg/ml.

Note: Poly (Glu:Tyr, 4:1) synthetic peptide (Catalog #P61-58) can also been used as a substrate for this target and it showed a better activity in the assay condition.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active c-KIT (V559D V654A), Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active c-KIT (V559D V654A) (Catalog #K06-12RG)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog # P61-58)
- **Component 3.** 5μl distilled H_2O (4°C)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5 μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

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

Calculation of [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}P]-ATP / pmoles of ATP (in 5 μl of a 250 μM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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