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Certificate of Analysis

Kit (V560G), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-730, 14-730-K, 14-730M
Parent Lot # 33074U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal GST tagged, recombinant human Kit, amino acids 544–end containing the V560G mutation. Expressed in Sf21 insect cells. Purified using glutathione agarose. The V560G mutation has been found in patients with gastrointestinal stromal tumours and has been linked to poor prognosis. This mutation confers increased susceptibility to Gleevec®. (Frost MJ *et al*., American Cancer Therapeutics, (2002);**1**:1115-1124 & Cullinane C *et al*., Cancer Research, (2005);**65**:9633-9636) Purity 80.5% by SDS-PAGE and Coomassie blue staining. MW = 76.6kDa.

Specific Activity (Parent lot# 33074U): 116U/mg, where one unit of Kit (V560G) activity is defined as 1nmol phosphate incorporated into 250µM (GGMEDIYFEFMGGKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.831mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

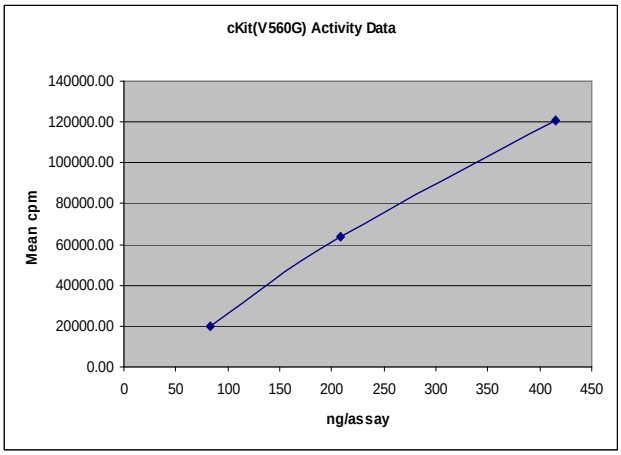
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

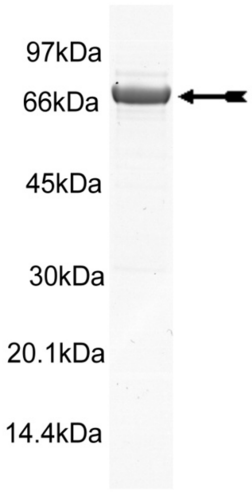
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 83.1–415.5ng of this lot of enzyme phosphorylated 250µM (GGMEDIYFEFMGGKKK) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as Kit (V560G) with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Kit (V560G), active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(GGMEDIYFEFMGGKKK):** Use at a final assay concentration of 250µM. Make up a 2.5mM stock. Add 2.5µl of stock per assay point.
3. **Kit (V560G), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 83.1–415.5ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per well.
2. Add 2.5µl of **(GGMEDIYFEFMGGKKK)**.
3. Add **2.5µl (83.1–415.5ng) Kit (V560G), active**.
4. Add 5µl dH_20.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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Kit (V560G) Sequence Information

Protein human Kit (V560G)

Tags *N*-terminal GST

Native sequence T237 of the recombinant sequence is equivalent to T544 of the native human Kit

Accession number GenBank X06182

Recombinant-Kit (V560G) amino acid sequence:

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   1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF KGLRRQTYKY
 241 LQKPMYEVQW KVGEEINGNN YVYIDPTQLP YDHKWEFPRN RLSFGKTLGA GAFGKVVEAT
 301 AYGLIKSDAA MTVAVKMLKP SAHLTEREAL MSELKVLSYL GNHMNIVNLL GACTIGGPTL
 361 VITEYCCYGD LLNFLRRKRD SFICSKQEDH AEAALYKNLL HSKESSCSDS TNEYMDMKPG
 421 VSYVVPTKAD KRRSVRIGSY IERDVTPAIM EDDELALDLE DLLSFSYQVA KGMAFLASKN
 481 CIHRDLAARN ILLTHGRITK ICDFGLARDI KNDSNYVVKG NARLPVKWMA PESIFNCVYT
 541 FESDVWSYGI FLWELFSLGS SPYPGMPVDS KFYKMIKEGF RMLSPEHAPA EMYDIMKTCW
 601 DADPLKRPTF KQIVQLIEKQ ISESTNHIYS NLANCSPNRQ KPVVDHSVRI NSVGSTASSS
 661 QPLLVHDDV
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Recombinant Kit (V560G) nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
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 721 ttacagaaac ccatgtatga agtacagtgg aaggttggtg aggagataaa tggaaacaat
 781 tatgtttaca tagacccaac acaacttcct tatgatcaca aatgggagtt tcccagaaac
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 901 gcttatggct taattaagtc agatgcggcc atgactgtcg ctgtaaagat gctcaagccg
 961 agtgcccatt tgacagaacg ggaagccctc atgtctgaac tcaaagtcct gagttacctt
1021 ggtaatcaca tgaatattgt gaatctactt ggagcctgca ccattggagg gcccaccctg
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1201 cattcaaagg agtcttcctg cagcgatagt actaatgagt acatggacat gaaacctgga
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1321 atagaaagag atgtgactcc cgccatcatg gaggatgacg agttggccct agacttagaa
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1741 cggatgctca gccctgaaca cgcacctgct gaaatgtatg acataatgaa gacttgctgg
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1801 gatgcagatc ccctaaaaag accaacattc aagcaaattg ttcagctaat tgagaagcag
1861 atttcagaga gcaccaatca tatttactcc aacttagcaa actgcagccc caaccgacag
1921 aagcccgtgg tagaccattc tgtgcggatc aattctgtcg gcagcaccgc ttcctcctcc
1981 cagcctctgc ttgtgcacga cgatgtctga
```

Reviewed and approved by site quality representative.