

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), active 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.

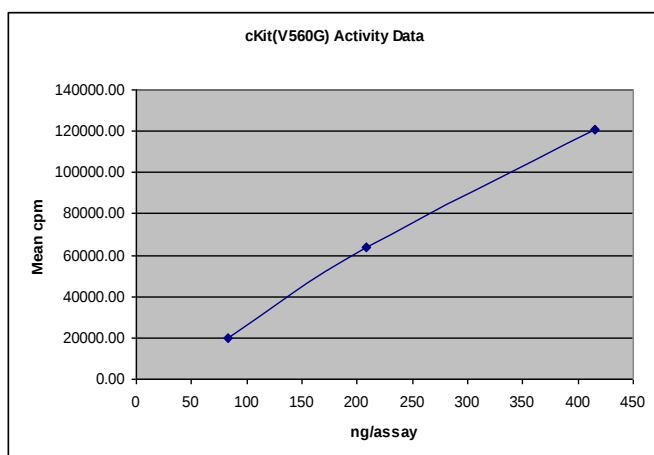
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

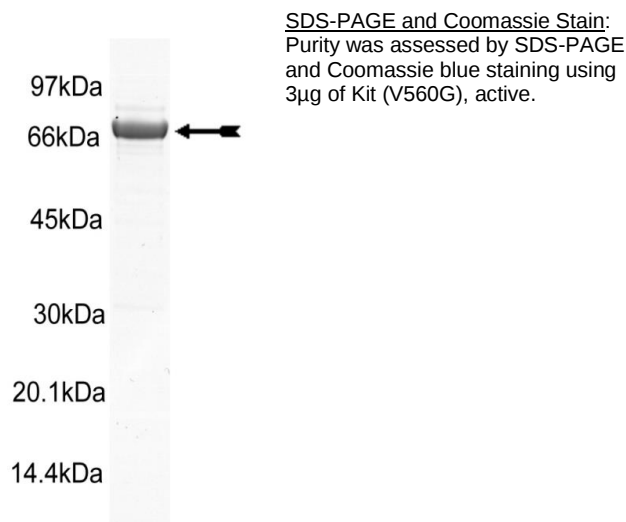
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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.



Certificate of Analysis

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 -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]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 dH₂O.
5. Add 10µl of diluted [γ -³³P]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.

Certificate of Analysis

Kit (V560G) Sequence Information

<u>Protein</u>	human Kit (V560G)
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	T237 of the recombinant sequence is equivalent to T544 of the native human Kit
<u>Accession number</u>	GenBank X06182

Recombinant-Kit (V560G) amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMa  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGPFE  KGLRRQTYKY
241 LQKPMYEVQW  KVGEERINGN  YVYIDPTQLP  YDHKWEFPRN  RLSFGKTLGA  GAFGKVVEAT
301 AYGLIKSDAA  MTVAVKMLKP  SAHLTEREAL  MSELKVLSYL  GNHMNIVNLL  GACTIGGPTL
361 VITEYCCYGD  LLNFLRRKRD  SFICSKQEDH  AEAALYKNLL  HSKESSCSDS  TNEYMDMKPG
421 VSYVVPKAD  KRRSVRIGSY  IERDVTPAIM  EDDELALDLE  DLLSFSYQVA  KGMAFLASKN
481 CIHRDLAARN  ILLTHGRITK  ICDFGLARDI  KNDSNYVVKG  NARLPVKWMA  PESIFNCVYT
541 FESDVWSYGI  FLWELFSLGS  SPYPGMPVDS  KFYKMIKEGF  RMLSPHAPHA  EMYDIMKTCW
601 DADPLKRPTF  KQIVQLIEKQ  ISESTNHIYS  NLANCSPNRQ  KPVDHHSVRI  NSVGSTASSS
661 QPLLHDDV

```

Recombinant Kit (V560G) nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatg  agcgcatga  aggtgataaa
121 tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgat
181 ggtgatgtta  aattaacaca  gtctatggcc  atcatagctt  atatagctga  caagcacaa
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
661 ctggaagtgc  tgttccaggg  gcccgaaatt  aaaggcctac  gtcgacaaac  ctacaaatat
721 ttacagaaac  ccattgtatg  agtacagtgg  aaggttggtg  aggagataaa  tggaaacaat
781 tatgtttaca  tagacccaac  acaacttcct  tatgatcaca  aatgggagtt  tcccagaaac
841 aggctgagtt  ttgggaaaa  cctgggtgct  ggagctttcg  ggaaggttgt  tgaggcaact
901 gcttatggct  taattaagtc  agatgcggcc  atgactgtcg  ctgtaaagat  gctcaagccg
961 agtgcccatt  tgacagaacg  ggaagccctc  atgtctgaac  tcaaagtcct  gatttacctt
1021 ggtaatcaca  tgaatattgt  gaacttactt  ggagcctgca  ccattggagg  gccaccctg
1081 gtcattacag  aatattgttg  ctatggtgat  cttttgaatt  ttttgagaag  aaaacgtgat
1141 tcatttatat  gttcaaagca  ggaagatcat  gcagaagctg  cactttataa  gaactctctg
1201 cattcaaagg  agtcttcctg  cagcgatagt  actaatgagt  acatggacat  gaaacctgga
1261 gtttcttatg  ttgtcccaac  caaggccgac  aaaaggagat  ctgtgagaat  aggtcctaac
1321 atagaaagag  atgtgactcc  cgccatcatg  gaggatgacg  agttggccct  agacttagaa
1381 gacttgctga  gcttttctta  ccagggtggc  aagggcattg  ctttcctcgc  ctccaagaat
1441 tgtattcaca  gagacttggc  agccagaaat  atcctcctta  ctcatggtcg  gatcacaaag
1501 atttgtgatt  ttggtctagc  cagagacatc  aagaatgatt  ctaattatgt  ggttaaagga
1561 aacgctcgac  tacctgtgaa  gtggatggca  cctgaaagca  ttttcaactg  tgtatacacg
1621 tttgaaagtg  acgtctggtc  ctatgggatt  tttctttggg  agctgttctc  ttttaggaagc
1681 agccccatc  ctggaatgcc  ggtcgattct  aagttctaca  agatgatcaa  ggaaggcttc
1741 cggatgctca  gccctgaaca  cgcacctgct  gaaatgtatg  acataatgaa  gacttgctgg

```

Certificate of Analysis

```
1801 gatgcagatc ccctaaaaag accaacattc aagcaaattg ttcagctaat tgagaagcag
1861 atttcagaga gcaccaatca tatttactcc aacttagcaa actgcagccc caaccgacag
1921 aagcccgtgg tagaccattc tgtgcggatc aattctgtcg gcagcaccgc ttctctctcc
1981 cagcctctgc ttgtgcacga cgatgtctga
```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 **Eurofins Pharma Discovery Services UK Limited** is an independent member of Eurofins Discovery Services.