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Certificate of Analysis

c-Kit (D816V), active

(Recombinant enzyme expressed in Sf21 cells)
Item # 14-611, 14-611-K, 14-611M
Parent Lot # 33188U

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 mutation D816V. This mutation is thought to produce a constitutively active form of Kit and has been shown to be associated with mastocytosis, leukaemia and germ cell tumours. It is expressed by baculovirus, in Sf21 insect cells and purified using glutathione agarose. Purity 63% by SDS-PAGE and Coomassie blue staining. MW = 76.7kDa.

Specific Activity (Parent lot# 33188U): 9U/mg, where one unit of Kit (D816V) activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.89mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

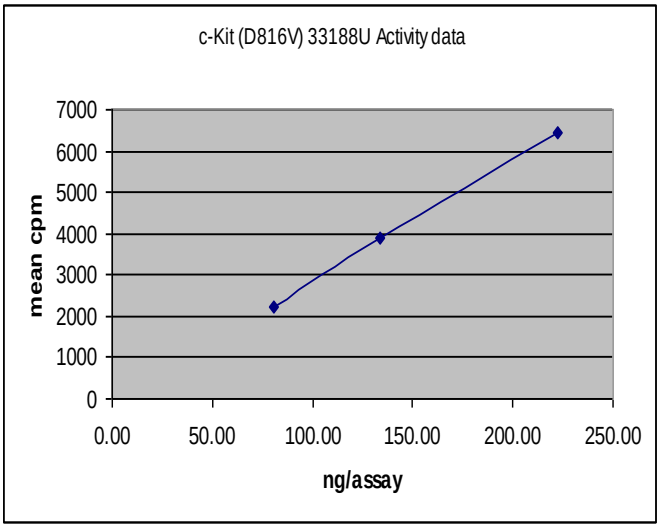
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

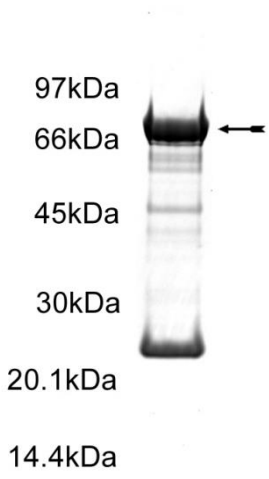
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 81–222ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) (4:1) in the assay described on page two. Assay background was 659cpm, and was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as Kit (D816V) with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Kit (D816V), active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Manganese Chloride:** Use at a final assay concentration of 10mM. Prepare a 200mM stock and add 1.25µl of stock per assay point.
3. **Poly(Glu, Tyr) (4:1):** Use at a final concentration of 0.1mg/ml. Make a 1mg/ml stock. Use 2.5µl of stock per assay point.
4. **Kit (D816V), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 81–222ng per assay point.
5. **[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 assay to wells.
2. Add 2.5µl of 1mg/ml **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (81–222ng) Kit (D816V), active**.
4. Add 1.25µl 200mM $MnCl_2$.
5. Add 3.75µl dH_20.
6. Add 10µl of diluted [γ-^{33}P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **Filtermat A**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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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GST-Kit (D816V) Sequence Information

Protein	Human Kit (D816V)
Tags	*N*-terminal GST
Native sequence	T237 of the recombinant protein is equivalent to T544 of human Kit
Accession number	GenBank X06182

Recombinant Kit(D816V) 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 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF KGLRRQTYKY
241 LQKPMYEVQW KVVEEINGNN 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 ICDFGLARVI 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(D816V) 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
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 961 agtgcccatt tgacagaacg ggaagccctc atgtctgaac tcaaagtcct gagttacctt
1021 ggtaatcaca tgaatattgt gaatctactt ggagcctgca ccattggagg gcccaccctg
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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.