

Certificate of Analysis

c-Kit (D816H), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-726, 14-726-K, 14-726M

Parent Lot # 1590305

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 c-Kit, amino acids 544–end containing the mutation D816H. Expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose followed by gel filtration. The D816H mutation has been found in patients with gastrointestinal stromal tumours and mastocytosis/mast cell leukaemia. This mutation confers constitutive activity and confers resistance to Gleevec®. (Kemmer *et al.*, American Journal of Pathology, (2004);164:305-313). Purity 57.2% by SDS-PAGE and Coomassie blue staining. MW = 76.7kDa.

Specific Activity (Parent lot# 1590305): 563U/mg, where one unit of c-Kit (D816H) activity is defined as 1nmol phosphate incorporated into 250µM (GGMEDIYFEFMGGKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.628mg/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.

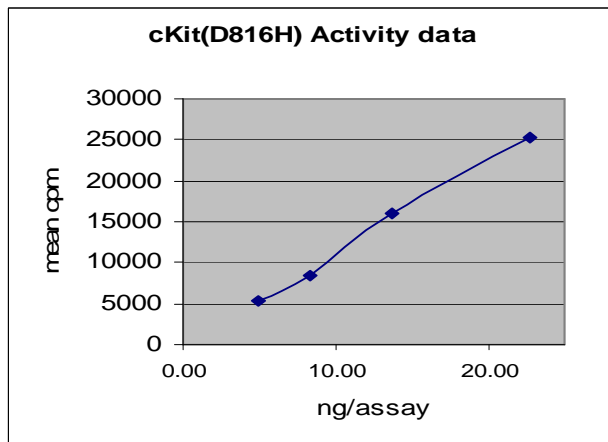
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

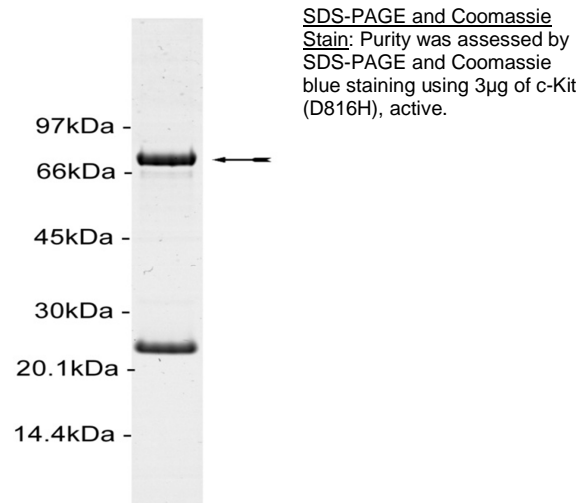
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 5–23ng 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 product identity as c-Kit (D816H) with the translated native sequence listed on page three.



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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 250 μ M. Make up a 2.5mM stock. Add 2.5 μ l of stock per assay point.
3. **c-Kit (D816H), active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 5–23ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(GGMEDIYFEFMGGKKK)**.
3. Add **2.5 μ l (5–23ng), c-Kit (D816H), active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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c-Kit (D816H) Sequence Information

<u>Protein</u>	Human c-Kit (D816H)
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	T237 of the recombinant protein is equivalent to T544 of human c-Kit (D816H)
<u>Accession number</u>	GenBank X06182

Recombinant c-Kit (D816H) amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRLEAIPQID  KYLKSSKYIA  WPLQGQWQATF  GGDHPPKSD  LEVLFQGPPEF  KGLRRQTYKY
241  LQKPMYEVQW  KVVVEINGNN  YVYIDPTQLP  YDHKWEFPRN  RLSFGKTLGA  GAFGKVVEAT
301  AYGLIKSDAA  M TVAVKMLKP  SAHLTEREAL  MSELKVL SYL  GNHMNIVNLL  GACTIGGPTL
361  VITEYCCYGD  LLNFLRRKRD  SFICSKQEDH  AEALYKNLL  HSKESSCSDS  TNEYMDMKPG
421  VSYVVPKAD  KRRSVRIGSY  IERDVT PAIM  EDELALDLE  DLLSFSYQVA  KGMAFLASKN
481  CIHRDLAARN  ILLTHGRITK  ICDFGLARHI  KNDSNYVVKG  NARLPVKWMA  PESIFNCVYT
541  FESDVWSYGI  FLWELFSLGS  SPYPGMPVDS  KFYKMIKEGF  RMLSPEHAPA  EMYDIMKTCW
601  DADPLKRPTF  KQIVQLIEKQ  ISESTNHIYS  NLANCSPNRQ  KPVDHSVRI  NSVGSTASSS
661  QPLLVDHDDV

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Recombinant c-Kit (D816H) nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgatg  agcgcatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttcca  atcttcctta  ttatatgat
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421  acatatataa  atggtgatca  tgtaacccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggacc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
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661  ctggaagttc  tgttccaggg  gcccgaattc  aaaggcctac  gtcgacaac  ctacaaatat
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1621  tttgaaagtg  acgtctggtc  ctatgggatt  tttctttggg  agctgttctc  tttaggaagc
1681  agcccctatc  ctggaatgcc  ggtcgattct  aagttctaca  agatgatcaa  ggaaggcttc
1741  cggatgctca  gccctgaaca  cgcacctgct  gaaatgatg  acataatgaa  gacttgctgg

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1861 atttcagaga gcaccaatca tatttactcc aacttagcaa actgcagccc caaccgacag
1921 aagcccgtgg tagaccattc tgtgcgatc aattctgtcg gcagcaccgc ttctctctcc
1981 cagcctctgc ttgtgcacga cgatgtctga
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