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Certificate of Analysis

Kit (V654A), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-733, 14-733-K, 14-733M
Parent Lot # D9BN033U

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 V654A mutation. Co-ordinates encompass the cytoplasmic domain (544–976) according in Swiss Prot P10721. V654A is an acquired resistance mutation which has been found in patients with gastrointestinal stromal tumours (GISTs) undergoing treatment with Imatinib. The V654A mutation is strongly correlated with imatinib resistance and rapid progression of GISTs. (Chen LL *et al*., Cancer Research, (2004); **64**:5913-5919). Expressed in Sf21 insect cells. Purified using glutathione agarose. Purity 88.4% by SDS-PAGE and Coomassie blue staining. MW = 76.7kDa.

Specific Activity (Parent lot# D9BN033U): 215U/mg, where one unit of Kit (V654A) activity is defined as 1nmol phosphate incorporated into 250µM (GGMEDIYFEFMGGKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.274mg/ml of enzyme in 50mM Tris/HCl pH 7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

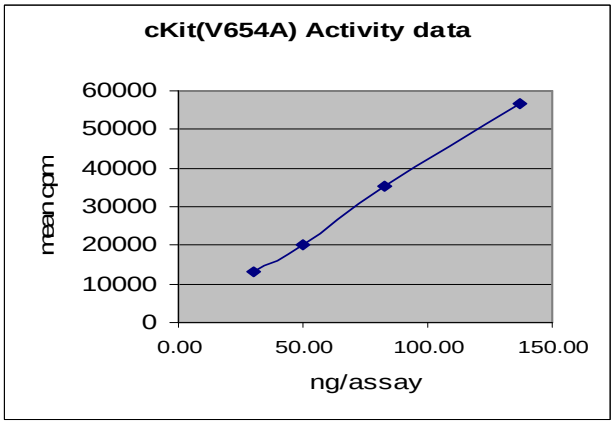
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

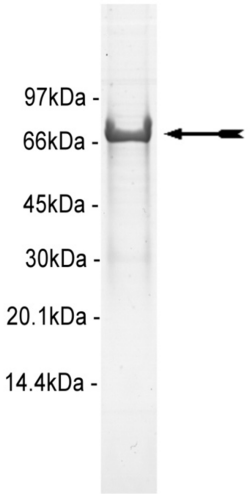
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 49.7–137ng 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 Kit (V654A) 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 active Kit (V654A).

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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 (V654A), active:** Dilute with 20mM MOPS/NaOH pH 7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 49.7–137ng 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 assay to wells.
2. Add 2.5µl of **(GGMEDIYFEFMGGKKK)**.
3. Add **2.5µl (49.7–137ng) Kit (V654A), active**.
4. Add 5µl of dH_2O.
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 **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 (V654A) Sequence Information

Protein	human Kit (V654A)
Tags	*N*-terminal GST
Native sequence	t237 of recombinant sequence is equivalent to T544 of native human Kit
Accession number	GenBank X06182

Recombinant Kit (V654A) 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 KVVEEINGNN YVYIDPTQLP YDHKWEFPRN RLSFGKTLGA GAFGKVVEAT
301 AYGLIKSDAA MTVAVKMLKP SAHLTEREAL MSELKVLSYL GNHMNIANLL 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 (V654A) 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
 661 ctggaagttc tgttccaggg gcccgaattc atgctagcag gggtctctga gtatgagctt
 721 cccgaagacc ctcgctggga gctgcctcgg gacagactgg tcttaggcaa acccctggga
 781 gagggctgct ttgggcaggt ggtgttggca gaggctatcg ggctggacaa ggacaaaccc
 841 aaccgtgtga ccaaagtggc tgtgaagatg ttgaagtcgg acgcaacaga gaaagacttg
 901 tcagacctga tctcagaaat ggagatgatg aagatgatcg ggaagcataa gaatatcatc
 961 aacctgctgg gggcctgcac gcaggatggt cccttgtatg tcatcatgga gtatgcctcc
1021 aagggcaacc tgcgggagta cctgcaggcc cggaggcccc cagggctgga atactgctac
1081 aaccccagcc acaacccaga ggagcagctc tcctccaagg acctggtgtc ctgcgcctac
1141 caggtggccc gaggcatgga gtatctggcc tccaagaagt gcatacaccg agacctggca
1201 gccaggaatg tcctggtgac agaggacaat gtgatgaaga tagcagactt tggcctcgca
1261 cgggacattc accacatcga ctactataaa aagacaacca acggccgact gcctgtgaag
1321 tggatggcac ccgaggcatt atttgaccgg atctacaccc accagagtga tgtgtggtct
1381 ttcggggtgc tcctgtggga gatcttcact ctgggcggct ccccataccc cggtgtgcct
1441 gtggaggaac ttttcaagct gctgaaggag ggtcaccgca tggacaagcc cagtaactgc
1501 accaacgagc tgtacatgat gatgcgggac tgctggcatg cagtgccctc acagagaccc
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```
1561 accttcaagc agctggtgga agacctggac cgcatcgtgg ccttgacctc caaccaggag
1621 taa
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Reviewed and approved by site quality representative.