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Certificate of Analysis

Arg, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-521, 14-521-K, 14-521M
Parent Lot # 25777U

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 6His-tagged recombinant human Arg residues 38–end. This is expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 32.7% by SDS-PAGE and Coomassie staining. MW = 121.9kDa.

Specific Activity (Parent lot# 25777U): 1948U/mg, where one unit of Arg, active activity is defined as 1nmol phosphate incorporated into 50µM Abltide (EAIYAAPFAKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.775mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.03% Brij-35. Frozen solution.

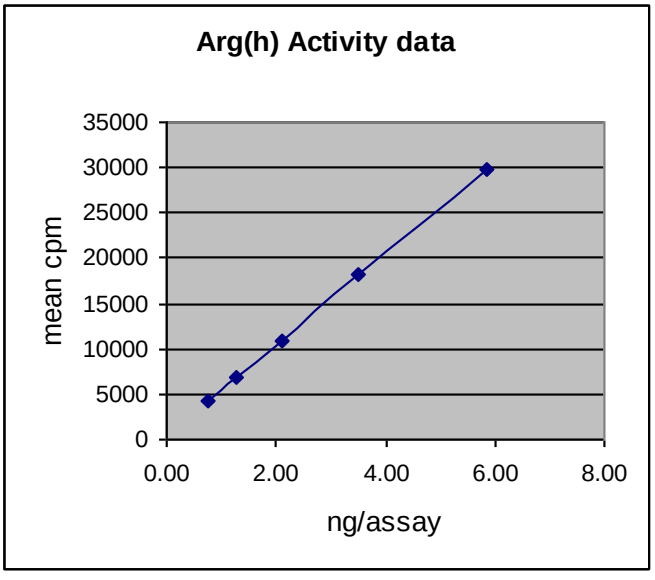
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

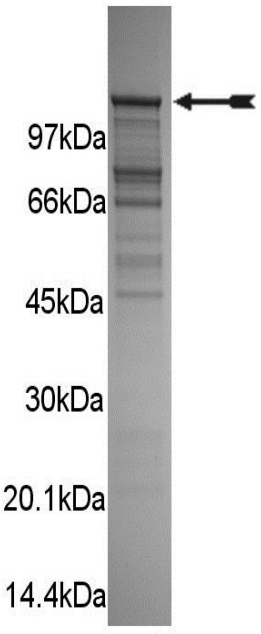
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 2.12–5.84ng of this lot of enzyme phosphorylated 50µM Abltide 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 Arg 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 Arg, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Abltide (EAIYAAPFAKKK):** Use at a final assay concentration of 50µM. Prepare a 500µM stock and add 2.5µl of stock per assay point.
3. **Arg, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2.12–5.84ng 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.
2. Add 2.5µl of **(EAIYAAPFAKKK)**.
3. Add **2.5µl (2.12–5.84ng) Arg, 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 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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Arg Sequence Information

Protein	human Arg (38–end)
Tags	*N*-terminal 6His
Native sequence	E10 of the recombinant protein is equivalent to E28 of human Arg
Accession number	GenBank NM_005158

Recombinant Arg amino acid sequence:

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   1 MHHHHHHEFE ALHRPYGCDV EPQALNEAIR WSSKENLLGA TESDPNLFVA LYDFVASGDN
  61 TLSITKGEKL RVLGYNQNGE WSEVRSKNGQ GWVPSNYITP VNSLEKHSWY HGPVSRSAAE
 121 YLLSSLINGS FLVRESESSP GQLSISLRYE GRVYHYRINT TADGKVYVTA ESRFSTLAEL
 181 VHHHSTVADG LVTTLHYPAP KCNKPTVYGV SPIHDKWEME RTDITMKHKL GGGQYGEVYV
 241 GVWKKYSLTV AVKTLKEDTM EVEEFLKEAA VMKEIKHPNL VQLLGVCTLE PPFYIVTEYM
 301 PYGNLLDYLR ECNREEVTAV VLLYMATQIS SAMEYLEKKN FIHRDLAARN CLVGENHVVK
 361 VADFGLSRLM TGDTYTAHAG AKFPIKWTAP ESLAYNTFSI KSDVWAFGVL LWEIATYGMS
 421 PYPGIDLSQV YDLLEKGYRM EQPEGCPPKV YELMRACWKW SPADRPSFAE THQAFETMFH
 481 DSSISEEVAE ELGRAASSSS VVPYLPRLPI LPSKTRTLKK QVENKENIEG AQDATENSAS
 541 SLAPGFIRGA QASSGSPALP RKQRDKSPSS LLEDAKETCF TRDRKGGFFS SFMKKRNAPT
 601 PPKRSSSFRE MENQPHKKYE LTGNFSSVAS LQHADGFSFT PAQQEANLVP PKCYGGSFAQ
 661 RNLCNDDGGG GGGSGTAGGG WSGITGFFTP RLIKKTLGLR AGKPTASDDT SKPFPRSNST
 721 SSMSSGLPEQ DRMAMTLPRN CQRSKLQLER TVSTSSQPEE NVDRANDMLP KKSEESAAPS
 781 RERPKAKLLP RGATALPLRT PSGDLAITEK DPPGVGVAGV AAAPKGKEKN GGARLGMAGV
 841 PEDGEQPGWP SPAKAAPVLP TTHNHKVPVL ISPTLKHTPA DVQLIGTDSQ GNKFKLLSEH
 901 QVTSSGDKDR PRRVKPKCAP PPPPVMRLLQ HPSICSDPTE EPTALTAGQS TSETQEGGKK
 961 AALGAVPISG KAGRPVMPPP QVPLPTSSIS PAKMANGTAG TKVALRKTKQ AAEKISADKI
1021 SKEALLECAD LLSSALTEPV PNSQLVDTGH QLLDYCSGYV DCIPQTRNKF AFREAVSKLE
1081 LSLQELQVSS AAAGVPGTNP VLNNLLSCVQ EISDVVQR
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Recombinant Arg nucleotide sequence:

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   1 atgcatcatc accatcacca tgaattcgaa gctttgcatc gtccctatgg ttgtgatgtt
  61 gaaccccagg cactaaatga ggctatcagg tggagctcca aggagaactt gctcggagcc
 121 actgagagtg accctaatct cttcgttgca ctttatgatt ttgtagcaag tggtgataac
 181 acactcagca tcactaaagg tgaaaagcta cgagtccttg gttacaacca gaatggtgag
 241 tggagtgaag ttcgctctaa gaatgggcag ggctgggtgc caagcaacta catcacccca
 301 gtgaacagcc tggaaaaaca ctcctggtac catggacctg tgtcacgcag tgcagctgag
 361 tatctgctca gcagtctaat caatggcagc ttcctggtgc gagaaagtga gagtagccct
 421 gggcagctgt ccatctcgct caggtacgag ggacgtgtat atcactacag gatcaatacc
 481 actgcagatg gcaaggtgta tgtgactgct gagagccgct tcagcacctt ggcagagctt
 541 gtacaccatc actccacagt ggctgatggg ctggtgacaa cattacacta cccagcaccc
 601 aagtgtaata agcctacagt ctatggtgtg tcccccatcc acgacaaatg ggaaatggag
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Reviewed and approved by site quality representative.