

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 Ni²⁺/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.

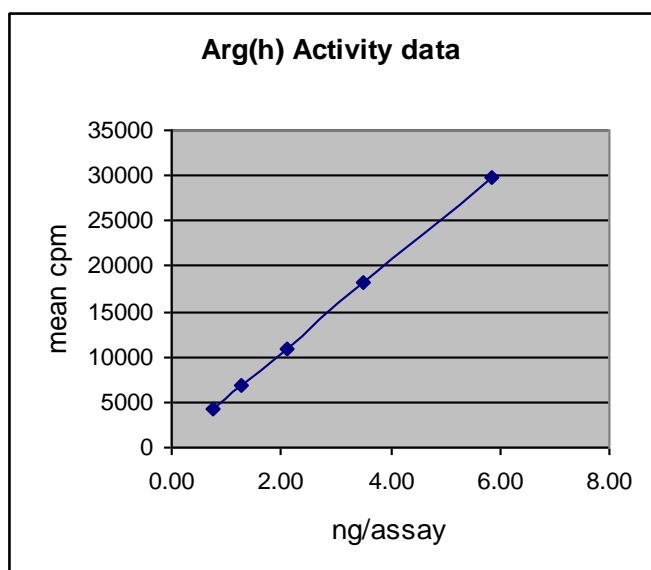
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

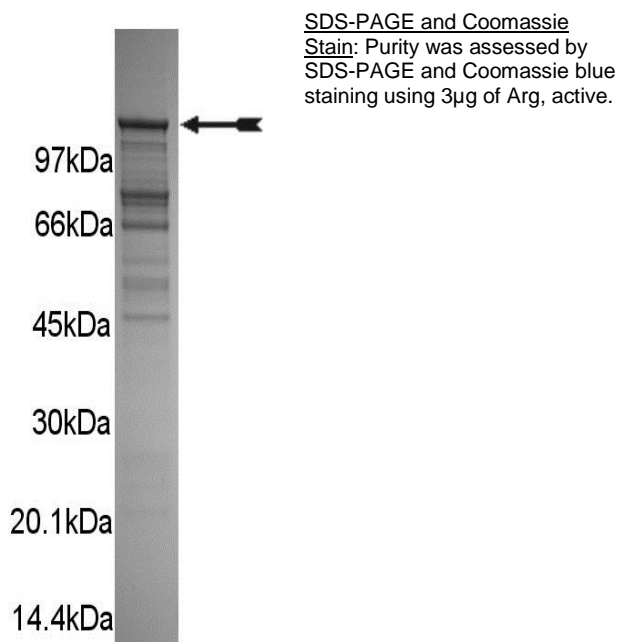
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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 defined 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. Abltide (EAIYAAPFAKKK):** Use at a final assay concentration of 50 μ M. Prepare a 500 μ M stock and add 2.5 μ l 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 -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.
2. Add 2.5 μ l of **(EAIYAAPFAKKK)**.
3. Add **2.5 μ l (2.12–5.84ng) Arg, 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.

Certificate of Analysis

Arg Sequence Information

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

Recombinant Arg amino acid sequence:

```

1 MHHHHHHEFE ALHRPYGCDV EPQALNEAIR WSSKENLLGA TESDPNLFVA LYDFVASGDN
61 TLSITKGEKL RVLGYNQNGE WSEVRSKNGQ GWVPSNYITP VNSLEKHSWY HGPVSRSAAE
121 YLLSSLINGS FLVRESESSP GQLSISLRYE GRVYHYRINT TADGKVYVTA ESRFSTLAEL
181 VHHHSTVADG LVTTLHYPAP KCNKP TVYGV SPIHDKWEME RTDITMKHKL GGGQYGEVYV
241 GVWKYSLTV AVKTLKEDTM EVEEFLKEAA VMKEIKHPNL VQLLGVCTLE PPFYIVTEYM
301 PYGNLLDYLR ECNREEVTAV VLLYMATQIS SAMEYLEKKN FIHRDLAARN CLVGENHVVK
361 VADFGLSRLM TGD TYTAHAG AKFPIKWTAP ESLAYNTFSI KSDVWAFGVL LWEIATYGMS
421 PYPGIDLSQV YDLLEKGYRM EQPEGCPPKV YELMRACWKW SPADRPSFAE THQAFETMFH
481 DSSISEEVAE ELGRAASSSS VVPYLPRLPI LPSKTRTLKK QVENKENIEG AQDATENSAS
541 SLAPGFIRGA QASSGSPALP RKQRDKSPSS LLEDAKETCF TRDRKGGFFS SFMKRNAPT
601 PPKRSSSFRE MENQPHKKYE LTGNFSSVAS LQHADGFSFT PAQQEANLVP PKCYGGSFAQ
661 RNLCDNDGGG GGGSGTAGGG WSGITGFFTP RLIKKTGLR AGKPTASDDT SKPFPRSNST
721 SSMSSGLPEQ DRAMTLP RN CQRSKQLER TVSTSSQPEE NVDRANDMLP KKSEESAAPS
781 RERP KAKLLP RGATALPLRT PSGDLAITEK DPPGVGVAGV AAAPKGKEKN GGARLGMAGV
841 PEDGEQGPWP SPAKAAPVLP TTHNHKVPVL ISPTLKHTPA DVQLIGTDSQ GNKFKLLSEH
901 QVTSSGDKDR PRRVKPKCAP PPPPVMRL LQ HPSICSDPTE EPTALTAGQS TSETQEGGKK
961 AALGAVPISG KAGRPVMP PP QVPLPTSSIS PAKMANGTAG TKVALRKTQ AAEKISADKI
1021 SKEALLECAD LLSSALTEPV PNSQLVDTGH QLLDYCSGYV DCIPQTRNKF AFREAVSKLE
1081 LSLQELQVSS AAAGVPGTNP VLNNLLSCVQ EISDVVQR

```

Recombinant Arg nucleotide sequence:

```

1 atgcatcatc accatcacca tgaattcgaa gctttgcatc gtccctatgg ttgtgatggt
61 gaaccccagg cactaaatga ggctatcagg tggagctcca aggagaactt gctcggagcc
121 actgagagtg accctaactc ctctggtgca ctttatgatt ttgtagcaag tgggtgataac
181 aactcagca tcactaaagg tgaaaagcta cgagtccttg gttacaacca gaatggtgag
241 tggagtgaag ttcgctctaa gaatggcgag ggctgggtgc caagcaacta catcacccca
301 gtgaacagcc tggaaaaaca ctctgggtac catggacctg tgtcacgcag tgcagctgag
361 tatctgctca gcagctctaat caatggcagc ttctggtgc gagaaagtga gactagccct
421 gggcagctgt ccatctcgtc caggtacgag ggacgtgtat atcactacag gatcaatacc
481 actgcagatg gcaagtgta tgtgactgct gagagccgct tcagacactt gccagactt
541 gtacaccatc actccacagt gggtgatggg ctggtgacaa cattacacta cccagcacc
601 aagtgtata agcctacagt ctatggtgtg tccccatcc acgacaaatg ggaaatggag
661 cgaacagata ttacatgaa gcacaaactt gggggcggtc agtatggaga ggtttacggt
721 ggcgtctgga agaaatacag cttacagtt gctgtgaaaa cattgaagga agataccatg
781 gaggtagaag aattcctgaa agaagctgca gtaatgaagg aatcaagca tcctaactctg
841 gtacaacttt taggtgtgtg tactttggag ccaccatttt acattgtgac tgaatacatg
901 ccatacggga atttgctgga ttacctccga gaatgcaacc gagaagaggt gactgcagtt
961 gtgctgctct acatggccac tcagatttct tctgcaatgg agtacttaga gaagaagaat
1021 ttcatccata gagatcttgc agctcgtaac tgcctagtgg gagaaaacca tgtggtaaaa
1081 gtggctgact ttggcttaag tagattgatg actggagaca cttatactgc tcatgctgga
1141 gccaaatttc ctattaagtg gacagcacca gagagtcttg cctacaatac cttctcaatt
1201 aaatctgacg tctgggcttt tggggatttg ttgtgggaaa ttgctaccta tggaatgtca
1261 ccatatccag gtattgacct gtctcaggtc tatgacctac tagaaaagg atatcgaatg
1321 gaacagcctg aggatgccc ccctaaggtt tatgaactta tgagagcatg ctggaagtgg

```

Certificate of Analysis

```
1381 agccctgccg ataggeccctc ttttgctgaa acacaccaag cttttgaaac catgttccat
1441 gactccagca tttctgaaga ggtagctgag gagcttggga gagccgcctc ctcgatcatc
1501 gttgttccat acctgccccg gctacctata cttccttcca agactcggac actgaagaaa
1561 caggtggaga acaaggagaa cattgaaggg gcacaagatg ccacagaaaa ttctgcttcc
1621 agtttagcac cagggttcat cagaggtgca caggcctcta gtggatcccc agcactgcct
1681 cgaaagcaaa gagacaagtc acccagcagc ctcttgggaag atgccaaaaga gacatgcttc
1741 accagggata ggaagggggg cttcttcagc tccttcatga agaagagaaa tgctcctaca
1801 cccccaaac gcagcagctc cttccgagaa atggagaatc agccccataa gaaatacгаа
1861 ctacagggta acttctcatc tgttgcttct ctacagcatg ctgatgggtt ctctttcact
1921 cctgcccagc aagaggcgaa tctggtgcca cccaagtgct atggggggag ctttgcacag
1981 aggaacctct gtaatgacga cggtggtggg ggtgggggca gtggcactgc tgggggtggg
2041 tggctctggca tcacaggctt ctttacacca cgcttaatca aaaagacact gggcttacga
2101 gcaggtaaac ccacagccag tgatgacact tccaagcctt ttccaaggtc aaactctaca
2161 tcttccatgt cctcagggtt tccagagcag gataggatgg caatgaccct tcccaggaac
2221 tgccagaggt ccaaactcca gctggaaagg acagtgtcca cctcttctca gccagaagag
2281 aatgtggaca gggccaatga catgcttcca aaaaaatcag aggaaagtgc tgctccaagc
2341 agggagagac caaaagccaa gttattgcc agaggagcca cagctcttcc tctcagaaca
2401 ccctctgggg atctagccat tacagagaag gaccctccag gggtgaggat ggctggagtg
2461 gcagctgccc ccaagggtaa agagaagaat ggtggggcac gacttgggat ggctggagtt
2521 ccagaggatg gagagcagcc gggctggcct tctccagcca aggctgcccc cgtectccca
2581 accactcaca accacaaagt gccagtcctt atctcaccca ctctgaaaca cactccagct
2641 gacgtgcagc tcattggcac agactctcag ggaataaat tcaagctctt atctgagcat
2701 caggtcacat cctctggaga caaggaccga ccccagcggg taaaacccaa gtgtgccccca
2761 cccccaccac cagtgatgag actactgcag catccgtcca tctgctcaga ccctacagaa
2821 gagccaactg ccctaactgc aggacagtcc acatcagaaa cacaggaagg aggaaagaag
2881 gcagctctgg gcgcagtgcc catcagtggg aaagctggga ggccagtgat gcctccacct
2941 caagtgcctc tgcccacatc ttccatctcg ccagccaaaa tggccaatgg cacagcaggt
3001 actaaagtgg ctctgagaaa aaccaaacag gccgctgaga aaatctcagc agacaaaatc
3061 agcaaagagg ccctgctgga atgtgctgac ctactgtcca gtgcactcac ggaacctgtg
3121 cccaacagcc agctggtaga cactggacac cagctgcttg actactgctc aggctatgtg
3181 gactgcatcc ctcaaactcg caacaaatth gccttccgag aggctgtgag caaactggaa
3241 ctgagcctgc aggagctaca ggtttcttca gcagctgctg gtgtgccccg gacaaaccct
3301 gtccttaata acttattgtc atgtgtacag gaaatcagtg atgtgggtgca gaggtag
```

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.