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Certificate of Analysis

Arg (mouse), active

(Recombinant enzyme expressed in Sf21 cells)
Item # 14-460, 14-460-K, 14-460M
Parent Lot # 25575U

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 mouse Arg residues 27–end. Expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 28% by SDS-PAGE and Coomassie blue staining. MW = 124.5kDa.

Specific Activity (Parent lot# 25575U): 2184U/mg, where one unit of Arg (mouse), active activity is defined as 1nmol phosphate incorporated into 50µM substrate peptide (EAIYAAPFAKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 3mg/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.

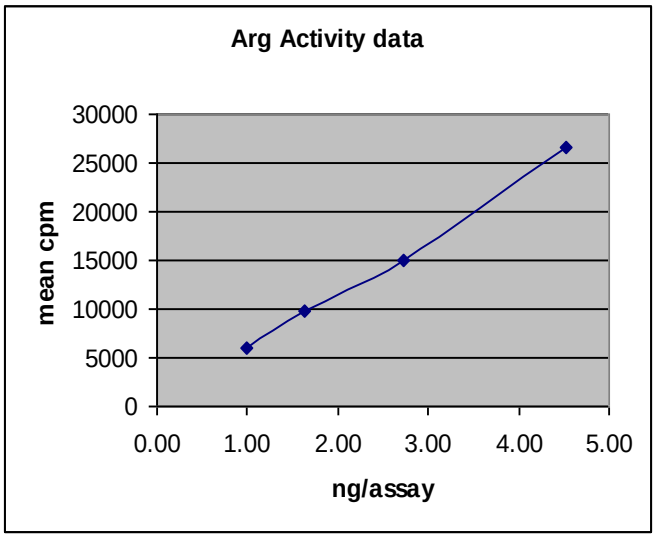
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

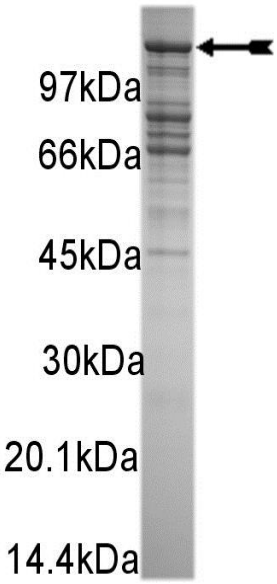
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.99–4.5ng of this lot of enzyme phosphorylated 50µM substrate peptide (EAIYAAPFAKKK) 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 (mouse) with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Arg (mouse) (27–end), active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Substrate Peptide (EAIYAAPFAKKK):** Use at a final assay concentration of 50µM. Prepare a 500µM stock. Add 2.5µl of stock per assay point.
3. **Arg (mouse), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.99–4.5ng 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 (0.99–4.5ng) Arg (mouse), 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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Mouse Arg (27–end) Sequence Information

Protein mouse Arg (27–end)

Tags *N*-terminal 6His

Native sequence E32 of the fusion protein is equivalent to E27 of mouse Arg

Accession number GenBank XM_136360. The recombinant protein also contains 2 residues which are in conflict with GenBank XM_136360. These are M982V, reported in GenBank NM_005158, NM_007314, M35296, and an A inserted at position 562, reported in GenBank U40827. The residue coordinates in the native sequence have been given.

Recombinant Mouse Arg (27–end) amino acid sequence:

```
   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF GEVLHRPFGC DAESQALNEA IRWSSKENLL
  61 GATESDPNLF VALYDFVASG DNTLSITKGE KLRVLGYNQN GEWSEVRSKN GQGWVPSNYI
 121 TPVNSLEKHS WYHGPVSRSA AEYLLSSLIN GSFLVRESES SPGQLSISLR YEGRVYHYRI
 181 NTTTDSKVYV TAESRFSTLA ELVHHHSTVA DGLVTTLHYP APKCNKPTVY GVSPIHDKWE
 241 MERTDITMKH KLGGGQYGEV YVGVWKKYSL TVAVKTLKED TMEVEEFLKE AAVMKEIKHP
 301 NLVQLLGVCT LEPPFYIVTE YMPYGNLLDY LRECSREEVT AVVLLYMATQ ISSAMEYLEK
 361 KNFIHRDLAA RNCLVGENHV VKVADFGLSR LMTGDTYTAH AGAKFPIKWT APESLAYNTF
 421 SIKSDVWAFG VLLWEIATYG MSPYPGIDLS QVYDLLEKGY RMEQPEGCPP KVYELMRACW
 481 KWSPADRPSF AETHQAFETM FHDSSISEEV AEELGRTASS SSVVPYLPRL PLLPSKTRTL
 541 RKQGENKENL DGGLDAAESL ASSSAPAGFI RSTQASSGSP ALPRKQRDKS PSSLLEDAKE
 601 TCFTRDRKGG FFSSFMKKRN APTPPKRSSS FREMENQPHK KYELTGNFSP VASLQHADGF
 661 SVAPSQQEPN LVPAKCYGGS FAQRNLCADD DSGGGGGSGT AGGGWSGITG FFTPRLIKKT
 721 LGLRAGKPTA SDDTSKPFPR SNSTSSMSSG LPEQDRMAMT LPRNCQRSKL QLERTVSTSS
 781 QPEENVDRAN DMLPKKSEEG AAPARERPKA KLLPRGATAL PLRAPDPAIT ESDSPGVGVA
 841 GVAAAPKGKE RNGGTRLGVA GVPEDGEQLG WSSPAKAVAV LPTTHNHKVP VLISPTLKHT
 901 PADVQLIGTD SQGNKFKLLS EHQVTSSGDK DRPRRVKPKC APPPPPVMRL LQHPSTCSDP
 961 EEEPTAPPAG QHTPETQEGG KKAAPGPVPS SGKPGRPVMP PPQVPLPTSS ISPAKMANGT
1021 AGTKVALRKT KQAAEKISAD KISKEALLEC ADLLSSAITE PVPNSQLVDT GHQLLDYCSG
1081 YVDSIPQTRN KFAFREAVSK LELSLQELQV SSTAAGVPGT NPVLNNLLSC VQEISDVVQR
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Recombinant Mouse Arg (27–end) nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc ggggaagttt tgcaccgccc gtttggctgt
 121 gatgctgaat ctcaggcact gaatgaagcg atcaggtgga gctccaagga gaacttgctg
 181 ggagccactg agagtgaccc taatctcttt gttgcacttt atgactttgt ggcaagtggt
 241 gataacacac tcagtatcac taaaggtgaa aagctgcgag tccttggtta taaccagaat
 301 ggcgagtgga gtgaagttcg ctccaagaat ggacagggtt gggtgccaag caactacatc
 361 actccagtta atagcctgga gaaacattcc tggtaccacg ggcctgtgtc ccgcagcgca
 421 gcagagtatc tcctcagtag cctaatcaac ggcagcttcc tggtccgaga gagtgagagc
 481 agccctgggc agctgtccat ctctctcagg tatgaggggc gtgtgtatca ctacaggatc
 541 aacaccacca cagacagcaa ggtgtacgtg acagctgaga gccgctttag caccttggca
 601 gagcttgttc accaccactc cacagttgct gatgggctag tgaccacgct gcactaccca
 661 gcaccgaagt gcaacaagcc aaccgtctat ggcgtgtctc ctatccatga caagtgggaa
 721 atggaacgga cagatatcac catgaagcac aaacttgggg gcggtcagta cggagaggtt
 781 tacgttggcg tctggaagaa gtacagcctt acagtggctg tgaaaacact gaaggaagac
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 961 tacatgccgt atgggaacct gcttgactat ctccgggagt gcagccgaga ggaggtgacc
1021 gccgtcgtgt tactttacat ggccacccag atctcctctg ccatggagta cttggagaag
1081 aagaacttca tccatagaga tcttgctgcc cggaactgcc tagtgggcga gaaccatgtg
1141 gtgaaagtgg ccgactttgg tttaagtaga ctgatgactg gagatacata cactgctcac
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1201 gctggagcca agttccctat taaatggaca gcacccgaga gtctggccta caataccttt
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1321 atgtcaccat atccaggtat tgacctatct caagtctatg acctactgga aaaaggatat
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1441 aagtggagcc ctgctgacag gccctctttt gctgaaaccc atcaagcttt tgaaacaatg
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3421 tag
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