

Certificate of Analysis

Rse, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-535, 14-535-K, 14-535M

Parent Lot # 1642049

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 Rse, residues 451–end. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 89.6% by SDS-PAGE and Coomassie blue staining. MW = 50.4kDa.

Specific Activity (Parent lot# 1642049): 460U/mg, where one unit of Rse, active activity is defined as 1nmol phosphate incorporated into 250µM cdc2 substrate peptide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.967mg/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.

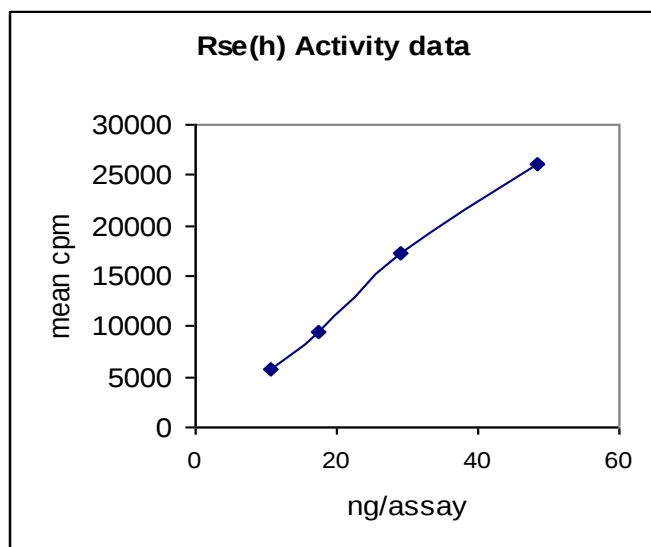
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

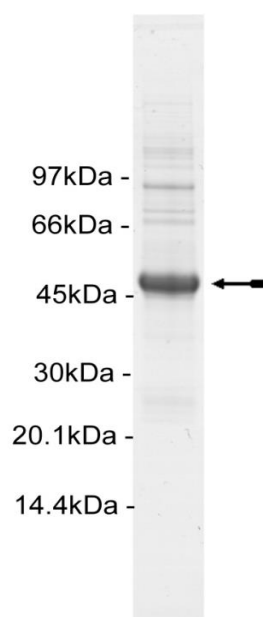
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 10.6–48.4ng of this lot of enzyme phosphorylated 250µM cdc2 substrate peptide 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 Rse 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 Rse, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Manganese Chloride (MnCl_2):** Use at a final assay concentration of 1mM. Prepare a 20mM stock and add 1.25 μl of stock per assay point.
3. **cdc2 substrate peptide:** Use at a final assay concentration of 250 μM . Prepare a 2.5mM stock and add 2.5 μl of stock per assay point.
4. **Rse, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 10.6–48.4ng per assay point.
5. **$[\gamma\text{-}^{33}\text{P}]\text{ATP}$:** 2.5 x magnesium acetate/ $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ cocktail: 25mM MgAc and 0.25mM ATP to which is added $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μl of 5 x reaction buffer per assay to wells.
2. Add 2.5 μl of **cdc2 peptide**.
3. Add **2.5 μl (10.6–48.4ng) Rse, active**.
4. Add 5 μl of dH_2O .
5. Add 10 μl of diluted $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μl of 3% phosphoric acid.
8. Transfer a 10 μl 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 μl of 30% phosphoric acid.

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Rse Sequence Information

<u>Protein</u>	human Rse
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	L10 of the recombinant protein is equivalent to L451 of human Rse
<u>Accession number</u>	GenBank D17517

Recombinant Rse amino acid sequence:

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1  MHHHHHHEFL  RKRRKETRF  QAFDSVMARG  EPAVHFRAAR  SFNRERPERI  EATLDSLGIS
61  DELKEKLEDV  LIPEQQFTLG  RMLGKGFEFG  VREAQLKQED  GSFVKVAVKM  LKADIIASSD
121  IEEFLREAAC  MKEFDHHPVA  KLVGVSLRSR  AKGRLLPIPMV  ILPFMKHGD  L  HAFLLASRIG
181  ENPFNLPLQT  LIRFMVDIAC  GMEYLSSRNF  IHRDLAARNC  MLAEDMTVCV  ADFGLSRKIY
241  SGDYRQGC  A  SKLPVKWLAL  ESLADNLYTV  QSDVWAFGVT  MWEIMTRGQT  PYAGIENAEI
301  YNYLIGGNRL  KQPPECMEDV  YDLMYQCWSA  DPKQRPSFTC  LRMELENILG  QLSVLSASQD
361  PLYINIERAE  EPTAGGSLEL  PGRDQPYSGA  GDGSGMGAVG  GTPSDCRYIL  TPGGLAEQPG
421  QAEHQPE  S  PL  NETQRLLLLQ  QGLLP  H  SSC

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Recombinant Rse nucleotide sequence:

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1  atgcatcatc  accatcacca  tgaattcctt  cgaaagagac  ggaaagagac  gcggtttggg
61  caagcctttg  acagtgtcat  ggcccgggga  gagccagccg  ttcacttccg  ggcagcccgg
121  tccttcaatc  gagaaaaggc  cgagcgcac  gagccacat  tggacagctt  gggcatcagc
181  gatgaactaa  aggaaaaact  ggaggatgtg  ctcattcccag  agcagcagtt  caccctgggc
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1261  caggcagagc  accagccaga  gagtcccttc  aatgagacac  agaggctttt  gctgctgcag
1321  caagggctac  tgccacacag  tagctgttag

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Reviewed and approved by site quality representative.

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