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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 Ni^{2+}/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.

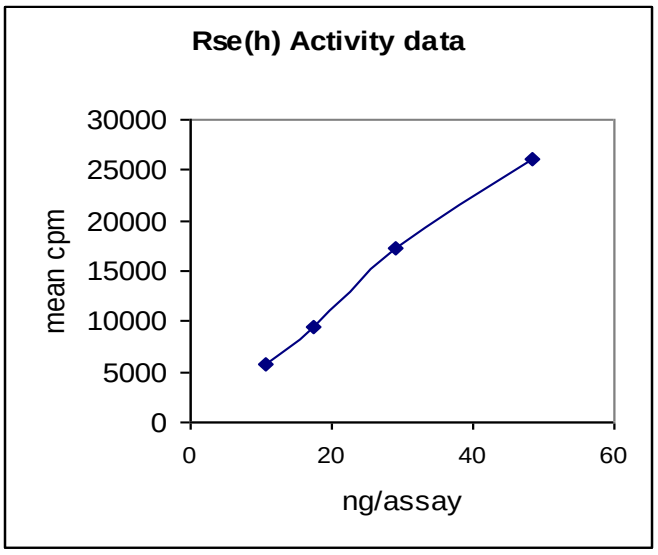
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

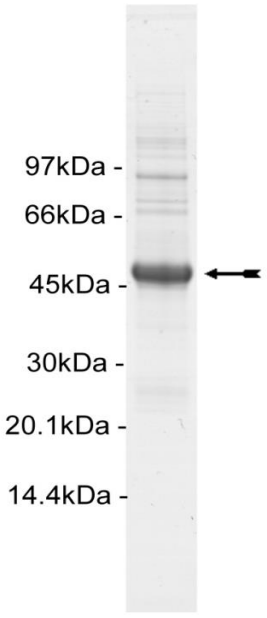
Quality Control Testing

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 1.25µl of stock per assay point.
3. **cdc2 substrate peptide:** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl 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. **[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 **cdc2 peptide**.
3. Add **2.5µl (10.6–48.4ng) Rse, 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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Rse Sequence Information

Protein human Rse

Tags *N*-terminal 6His

Native sequence L10 of the recombinant protein is equivalent to L451 of human Rse

Accession number GenBank D17517

Recombinant Rse amino acid sequence:

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   1 MHHHHHHEFL RKRRKETRFG QAFDSVMARG EPAVHFRAAR SFNRERPERI EATLDSLGIS
  61 DELKEKLEDV LIPEQQFTLG RMLGKGEFGS VREAQLKQED GSFVKVAVKM LKADIIASSD
 121 IEEFLREAAC MKEFDHPHVA KLVGVSLRSR AKGRLPIPMV ILPFMKHGDL HAFLLASRIG
 181 ENPFNLPLQT LIRFMVDIAC GMEYLSSRNF IHRDLAARNC MLAEDMTVCV ADFGLSRKIY
 241 SGDYYRQGCA SKLPVKWLAL ESLADNLYTV QSDVWAFGVT MWEIMTRGQT PYAGIENAEI
 301 YNYLIGGNRL KQPPECMEDV YDLMYQCWSA DPKQRPSFTC LRMELENILG QLSVLSASQD
 361 PLYINIERAE EPTAGGSLEL PGRDQPYSGA GDGSGMGAVG GTPSDCRYIL TPGGLAEQPG
 421 QAEHQPESPL NETQRLLLLQ QGLLPHSSC
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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 gagaaaggcc cgagcgcatc gaggccacat tggacagctt gggcatcagc
 181 gatgaactaa aggaaaaact ggaggatgtg ctcatcccag agcagcagtt caccctgggc
 241 cggatgttgg gcaaaggaga gtttggttca gtgcgggagg cccagctgaa gcaagaggat
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1261 caggcagagc accagccaga gagtcccctc aatgagacac agaggctttt gctgctgcag
1321 caagggctac tgccacacag tagctgttag
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Reviewed and approved by site quality representative.