

## Certificate of Analysis

### Ros, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-527, 14-527-K, 14-527M

Parent Lot # 1665632

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 Ros residues 1883–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA-agarose. Purity 81.6% by SDS-PAGE and Coomassie blue staining. MW = 53.4kDa.

**Specific Activity (Parent lot# 1665632):** 271U/mg, where one unit of Ros, active activity is defined as 1nmol phosphate incorporated into 250µM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 3.638mg/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.

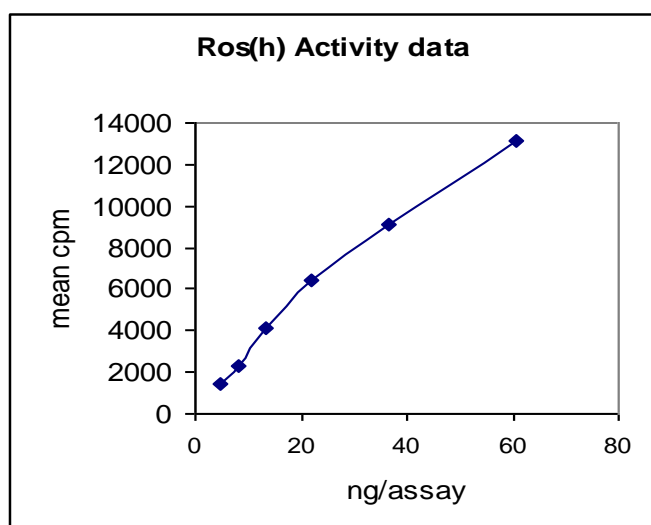
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

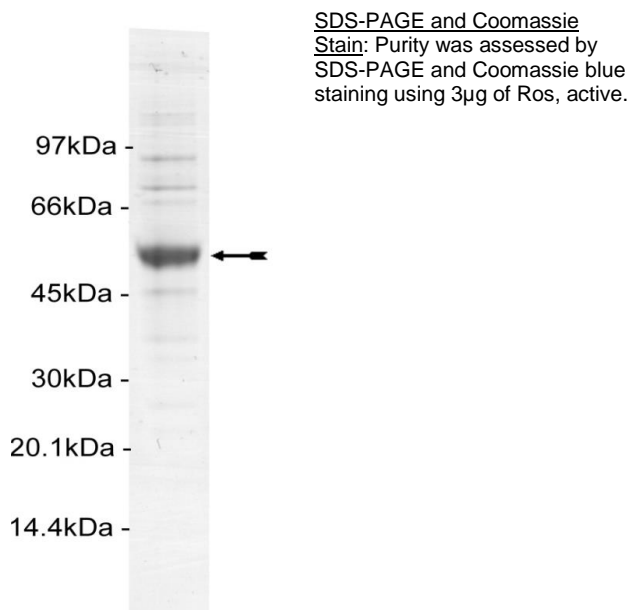
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 4.8–60.6ng of this lot of enzyme phosphorylated 250µM (KKKSPGEYVNIEFG) 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 Ros with the translated sequence listed on page three.



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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<sub>2</sub>):** Use at a final assay concentration of 10mM. Prepare a 200mM stock in dH<sub>2</sub>O and add 1.25µl of stock per assay point.
3. **(KKKSPGEYVNIEFG):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
4. **Ros, 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 4.8–60.6ng per assay point.
5. **[γ-<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[γ-<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-<sup>33</sup>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 **(KKKSPGEYVNIEFG)**.
3. Add 1.25µl of MnCl<sub>2</sub>.
4. Add **2.5µl (4.8–60.6ng) Ros, active**.
5. Add 3.75µl of dH<sub>2</sub>O.
6. Add 10µl of diluted [γ-<sup>33</sup>P] ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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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### Ros Sequence Information

<b><u>Protein</u></b>	human Ros
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	H9 of the recombinant protein is equivalent to H1883 of human Ros
<b><u>Accession number</u></b>	GenBank M34353. The recombinant protein contains the conflict V2262A with respect to GenBank M34353. This is reported in GenBank NM_002944 and Z98880.

#### Recombinant Ros amino acid sequence:

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1 MHHHHHRHR RLKNQKSAKE GVTVLINEDK ELAELRGLAA GVGLANACYA IHTLPTQEEI
61 ENLPAPPREK LTLRLLLGSG AFGEVYEGTA VDILGVGSGE IKVAVKTLKK GSTDQEKIEF
121 LKEAHLMSKF NHPNILKQLG VCLLNEPQYI ILELMGGDL LTYLRKARMA TFYGPLLTLV
181 DLVDLCVDIS KGCVYLERMH FIHRDLAARN CLVSVKDYTS PRIVKIGDFG LARDIYKNDY
241 YRKRGEGLLP VRWMAPESLM DGIFTTQSDV WSGFILIWEI LTLGHQPYPY HSNLDVLYNV
301 QTGGRLEPPR NCPDDLWNLM TQCWAQEPDQ RPTFHRIQNQ LQLFRNFFLN SIYQCRDEAN
361 NSGVINESFE GEDGDVICLN SDDIMPVALM ETKNREGLNY MVLATECGQG EEKSEGPLGS
421 QESESCGLRK EEKEPHADKD FCQEQVAYC PSGKPEGLNY ACLTHSGYGD GSD
  
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#### Recombinant Ros nucleotide sequence:

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1 atgcatcacc atcaccatca taggcataga agattaaaga atcaaaaaag tgccaaggaa
61 ggggtgacag tgcttataaa cgaagacaaa gagttggctg agctgcgagg tctggcagcc
121 ggagtaggcc tggctaatac ctgctatgca atacatactc ttccaacca agaggagatt
181 gaaaaatctt ctgccttccc tcgggaaaaa ctgactctgc gtctcttgct gggaaagtga
241 gcctttggag aagtgtatga aggaacagca gtggacatct taggagttgg aagtggagaa
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1141 tcagatgaca ttatgccagt tgctttaatg gaaacgaaga accgagaagg gttaaactat
1201 atggtacttg ctacagaatg tggccaaggt gaagaaaagt ctgagggtcc tctaggctcc
1261 caggaatctg aatcttggg tctgaggaaa gaagagaagg aaccacatgc agacaaagat
1321 ttctgccaag aaaaacaagt ggcttactgc ccttctggca agcctgaagg cctgaactat
1381 gcctgtctca ctcacagtgg atatggagat gggctctgatt aa
  
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