

# Certificate of Analysis

**HRI, active**  
**(Recombinant enzyme expressed in Sf21 insect cells)**  
**Item # 16-013, 16-013-K, 16-013M**  
**Parent Lot # 223812**

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 GST-tagged, recombinant, human HRI amino acids 140-end, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose and size exclusion chromatography. Purity 85% by SDS-PAGE and Coomassie blue staining. MW = 83 kDa.

**Specific Activity (Parent lot# 223812):** 61 U/mg, where one unit of HRI activity is defined as 1 nmol phosphate incorporated into 300  $\mu$ M RSRRSRSRSRSRSR per minute at 30°C with a final ATP concentration of 100  $\mu$ M.

**Formulation:** 0.55 mg/ml of enzyme in 50 mM Tris/HCl pH 7.5, 300 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 1 mM benzamidine, 0.2 mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

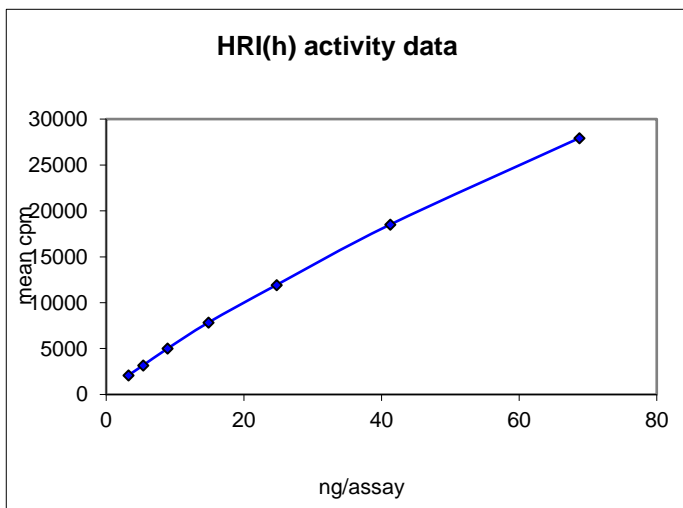
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

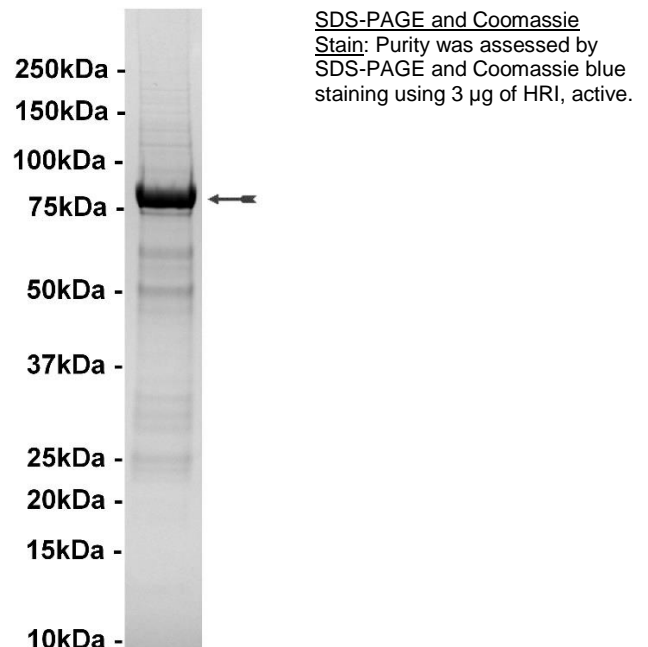
**FOR IN VITRO RESEARCH USE ONLY**  
**NOT FOR USE IN HUMANS OR ANIMALS**

## Quality Control Testing

**Kinase Assay:** 3.2–68.8 ng of this lot of enzyme phosphorylated 300  $\mu$ M RSRRSRSRSRSRSR 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 HRI with the translated sequence listed on page three.



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### Kinase Assay Protocol

#### Stock Solutions:

- 1. 5 x Reaction Buffer:** 250 mM Tris/HCl pH 7.5, 0.2 mM EGTA.
- 2. RSRSRSRSRSRSRSR:** Use at a final assay concentration of 300  $\mu$ M. Prepare a 3 mM stock and add 2.5  $\mu$ l of stock per assay point.
- 3. HRI, active:** Dilute with 25 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 3.2–68.8 ng per assay point.
- 4. [ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x MgAc/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25 mM MgAc and 0.25 mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 – 800 cpm/pmol as required).

#### Assay Procedure (96 well plate format):

1. Add 5  $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 2.5  $\mu$ l of 3 mM RSRSRSRSRSRSRSR.
3. Add **2.5  $\mu$ l (3.2–68.8 ng) HRI, active.**
4. Add 5  $\mu$ l of dH<sub>2</sub>O.
5. Add 10  $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 30 minutes at 30°C.
7. Stop the reaction by adding 5  $\mu$ l of 3% phosphoric acid.
8. Transfer a 10  $\mu$ l aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 75 mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4 ml 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  $\mu$ l of 30% phosphoric acid.

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## HRI, active Sequence Information

<b>Protein</b>	Human HRI
<b>Tags</b>	N-terminal GST
<b>Native sequence</b>	C231 of the recombinant protein is equivalent to C140 of human HRI
<b>Accession number</b>	GenBank NM_014413.2

### Recombinant HRI amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPOID  KYLKSSKYIA  WPLQGWOATF  GGGDHPPKSD  LEVLFQGP EF  CEDISRIQKI
241  RSREVALEAQ  TSRYLNEFEE  LAILGKGGYG  RVYKVRNKLD  GQYYAIKKIL  IKGATKTVCM
301  KVLREVKVLA  GLQHPNIVGY  HTAWIEHVHV  IQPRADRAAI  ELPSLEVLSD  QEEDREQCGV
361  KNDESSSSSI  IFAEPTPEKE  KRFGESDTEN  QNNKSVKYTT  NLVIRESGEL  ESTLELQENG
421  LAGLSASSIV  EQQLPLRRNS  HLEESFTSTE  ESSEENVNFL  GQTEAQYHLM  LHIQMQLCEL
481  SLWDWIVERN  KRGREYVDES  ACPYVMANVA  TKIFQELVEG  VFYIHNMGIV  HRDLKPRNIF
541  LHGPDQQVKI  GDFGLACTDI  LQKNTDWTNR  NGKRTPTHTS  RVGTCLYASP  EQLEGSEYDA
601  KSDMYSLGVV  LLELFQPFGT  EMERAEVLTG  LRTGQLPESL  RKRCPVQAKY  IQHLTRRNSS
661  QRPSAIQLLQ  SELFQNSGNV  NLTLQMKIIE  QEKEIAELKK  QLNLLSQDKG  VRDDGKDGCV
721  G

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### Recombinant HRI nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
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1321  cacctagagg  agagtttcac  atccaccgaa  gaatcttcg  aagaaaatgt  caactttttg
1381  ggtcagacag  aggcacagta  ccacctgatg  ctgcacatcc  agatgcagct  gtgtgagctc
1441  tcgctgtggg  attggatag  cgagagaaac  aagcggggcc  gggagtatgt  ggacgagctc

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## Certificate of Analysis

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2101 cagctaaacc tcctttctca agacaaaagg gtgagggatg acggaaagga tgggggcgtg
2161 ggatga
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