

Certificate of Analysis

Hck, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-577, 14-577-K, 14-577M

Parent Lot # 1730858

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 Hck, amino acids 230–497, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 79.4% by SDS-PAGE and Coomassie blue staining. MW = 34.1kDa.

Specific Activity (Parent lot# 1730858): 9071U/mg, where one unit of Hck, active activity is defined as 1nmol phosphate incorporated into 250µM cdc2 substrate peptide (cat# 12-140) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.311mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

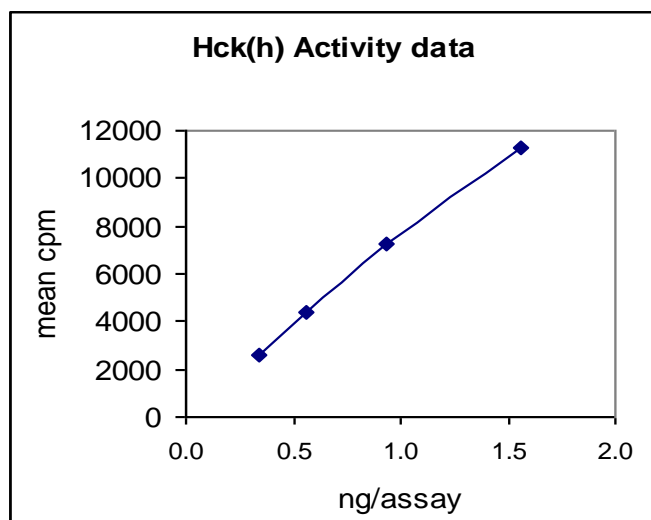
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

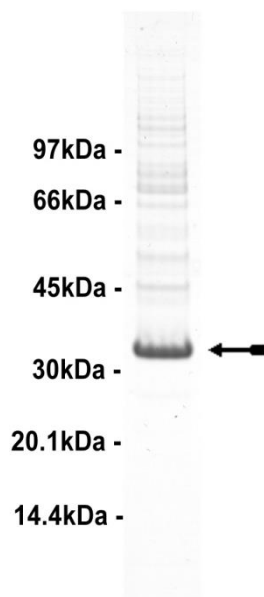
Quality Control Testing

Kinase Assay: 0.3–1.6ng 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 Hck 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 Hck, active.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **cdc2 peptide (KVEKIGEGTYGVVYK):** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
3. **Hck, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.3–1.6ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **cdc2 peptide**.
3. Add **2.5 μ l (0.3–1.6ng) Hck, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 μ l of 30% phosphoric acid.

Certificate of Analysis

Hck Sequence Information

<u>Protein</u>	human Hck
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	E29 of the recombinant protein is equivalent to E230 of human Hck
<u>Accession number</u>	GenBank NM_002110

Recombinant Hck amino acid sequence:

```

1 MSYYHHHHH DYDIPTTENL YFQGAMGSEK DAWIIPRESL KLEKKGAGQ FGEVWMATYN
61 KHTKVAVKTM KPGSMSVEAF LAEANVMKTL QHDKLVKLHA VVTKEPIYII TEFMAKGSLL
121 DFLKSDEGSK QPLPKLIDFS AQIAEGMAFI EQRNYIHRDL RAANILVSAS LVCKIADFGF
181 ARVIEDNEYT AREGAKFPIK WTAPEAINFG SFTIKSDVWS FGILLMEIVT YGRIPYPGMS
241 NPEVIRALER GYRMPRPENC PEELYNIMMR CWKNRPEERP TFEYIQSVLD DFYTAT
  
```

Recombinant Hck nucleotide sequence:

```

1 atgtcgact accatcacca tcacatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatggg atctgagaaa gatgcctggg agatccctcg ggaatccctc
121 aagctggaga agaaacttgg agctgggagc tttggggaag tctggatggc cacctacaac
181 aagcacacca agtgggcagt gaagacgatg aagccaggga gcatgtcggg ggaggccttc
241 ctggcagagg ccaacgtgat gaaaactctg cagcatgaca agctgggtcaa acttcatgcg
301 gtggtcacca aggagcccat ctacatcatc acggagttca tggccaaagg aagcttgctg
361 gactttctga aaagtgatga gggcagcaag cagccattgc caaaactcat tgacttctca
421 gccagattg cagaaggcat ggccttcatc gagcagagga actacatcca ccgagacctc
481 cgagctgcca acatcttggg ctctgcatcc ctgggtgtgta agattgctga ctttggcctg
541 gccgggtca ttgaggacaa cgagtacacg gctcggggaag gggccaagtt ccccatcaag
601 tggacagctc ctgaagccat caactttggc tccttcacca tcaagtcaga cgtctgggtc
661 tttggtatcc tgctgatgga gatcgtcacc tacggccgga tcccttacc agggatgtca
721 aaccctgaag tgatccgagc tctggagcgt ggataccgga tgcctcgccc agagaactgc
781 ccagaggagc tctacaacat catgatgcgc tgctggaaaa accgtccgga ggagcggccg
841 accttcgaat acatccagag tgtgctggat gacttctaca cggccacata g
  
```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.