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Certificate of Analysis

Hck, activated

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-843, 14-843-K, 14-843M
Parent Lot # D8CN013N

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 Ni^{2+}/NTA agarose. Auto-activated on column by incubating with Mg/ATP, excess ATP and MgAc removed by multiple column wash steps.
Purity 80% by SDS-PAGE and Coomassie blue staining. MW = 34kDa.

Specific Activity (Parent lot# D8CN013N): 40900U/mg, where one unit of Hck, activated activity is defined as 1nmol phosphate incorporated into 250µM (GGMEDIYFEFMGGKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.824mg/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, 5mM β-glycerophosphate, 1mM Na_3VO_4. Frozen solution.

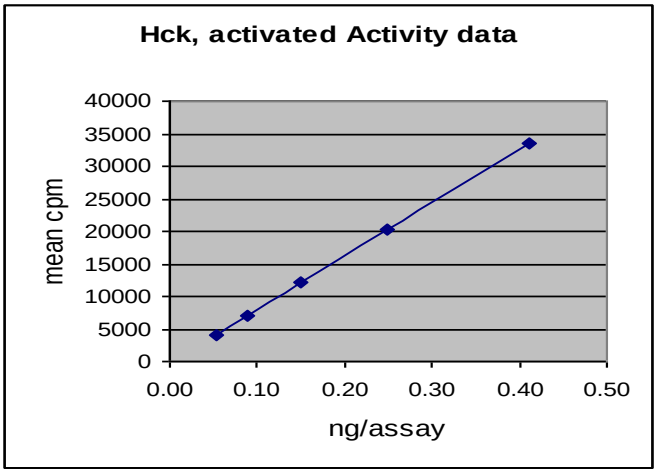
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

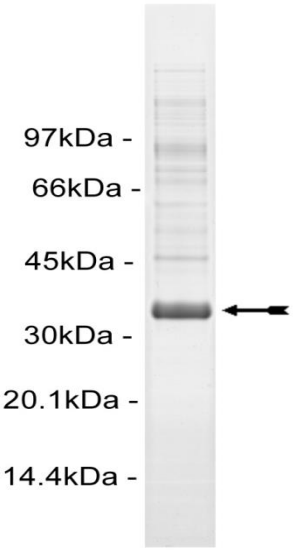
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.05–0.41ng of this lot of enzyme phosphorylated 250µM (GGMEDIYFEFMGGKKK) 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 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, activated.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA, other components.
2. **(GGMEDIYFEFMGGKKK):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **Hck, activated:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.05–0.41ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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 **(GGMEDIYFEFMGGKKK)**.
3. Add **2.5µl (0.05–0.41ng) activated Hck**.
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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Hck Sequence Information

Protein Human Hck

Tags N-terminal 6His

Native sequence E29 of the recombinant protein is equivalent to E230 of human Hck

Accession number GenBank NM_002110

Recombinant Hck amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMGSEK DAWEIPRESL KLEKKLGAGQ FGEVWMATYN
 61 KHTKVAVKTM KPGSMSVEAF LAEANVMKTL QHDKLVKLHA VVTKEPIYII TEFMAKGSLL
121 DFLKSDEGSK QPLPKLIDFS AQIAEGMAFI EQRNYIHRDL RAANILVSAS LVCKIADFGL
181 ARVIEDNEYT AREGAKFPIK WTAPEAINFG SFTIKSDVWS FGILLMEIVT YGRIPYPGMS
241 NPEVIRALER GYRMPRPENC PEELYNIMMR CWKNRPEERP TFEYIQSVLD DFYTAT
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Recombinant Hck nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatggg atctgagaaa gatgcctggg agatccctcg ggaatccctc
121 aagctggaga agaaacttgg agctgggcag tttggggaag tctggatggc cacctacaac
181 aagcacacca aggtggcagt gaagacgatg aagccaggga gcatgtcggt ggaggccttc
241 ctggcagagg ccaacgtgat gaaaactctg cagcatgaca agctggtcaa acttcatgcg
301 gtggtcacca aggagcccat ctacatcatc acggagttca tggccaaagg aagcttgctg
361 gactttctga aaagtgatga gggcagcaag cagccattgc caaaactcat tgacttctca
421 gcccagattg cagaaggcat ggccttcatc gagcagagga actacatcca ccgagacctc
481 cgagctgcca acatcttggt ctctgcatcc ctggtgtgta agattgctga ctttggcctg
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721 aaccctgaag tgatccgagc tctggagcgt ggataccgga tgcctcgccc agagaactgc
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Reviewed and approved by site quality representative.