eurofins | Discovery Services

Certificate of Analysis

Haspin, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-744, 14-744-K, 14-744M
Parent Lot # D9AN003U

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 Haspin, amino acids 471–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 62.8% by SDS-PAGE and Coomassie blue staining. MW = 41.1kDa.

Specific Activity (Parent lot# D9AN003U): 1340U/mg, where one unit of Haspin, active activity is defined as 1nmol phosphate incorporated into 500µM (RARTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.036mg/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.

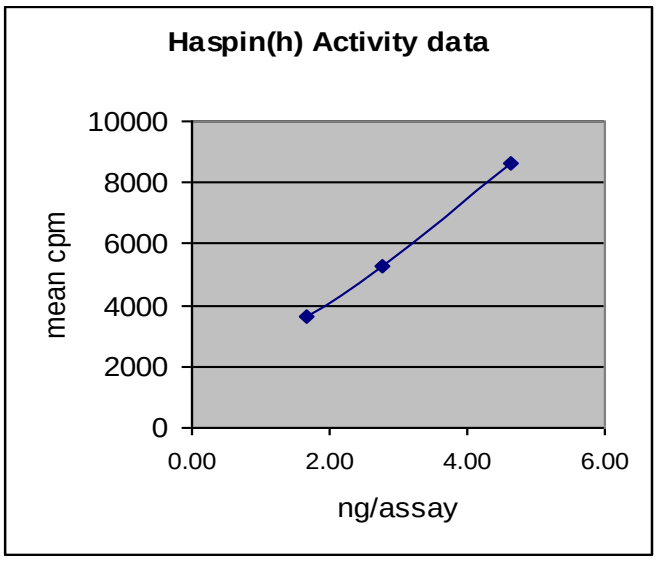
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

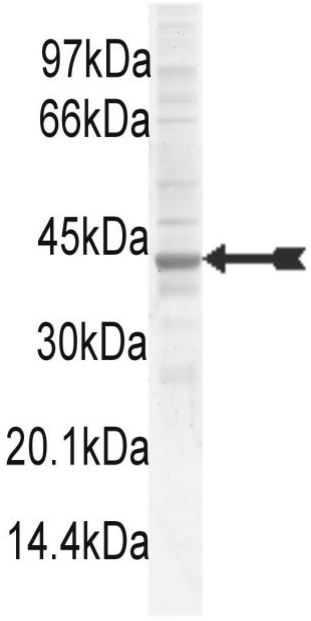
Quality Control Testing

Kinase Assay: 1.7–4.6ng of this lot of enzyme phosphorylated 500µM (RARTLSFAEPG) 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 Haspin 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 Haspin, active.



Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(RARTLSFAEPG):** Use at a final assay concentration of 500 µM. Prepare a 5mM stock and add 2.5µl of stock solution per assay point.
3. **Haspin, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 150mM NaCl, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.7–4.6ng per assay point.
4. **[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 **(RARTLSFAEPG)**.
3. Add **2.5µl (1.7–4.6ng) Haspin, 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 50mM 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.

eurofins | Discovery Services

Certificate of Analysis

Haspin Sequence Information

Protein human Haspin

Tags *N*-terminal 6His

Native sequence G31 of recombinant sequence = G471 of native human Haspin

Accession number GenBank NM_031965

Recombinant Haspin amino acid sequence:

```
   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF GPVPFSHCLP TEKLQRCEKI GEGVFGEVFQ
  61 TIADHTPVAI KIIAIEGPDL VNGSHQKTFE EILPEIIISK ELSLLSGEVC NRTEGFIGLN
 121 SVHCVQGSYP PLLLKAWDHY NSTKGSANDR PDFFKDDQLF IVLEFEFGGI DLEQMRTKLS
 181 SLATAKSILH QLTASLAVAE ASLRFEHRDL HWGNVLLKKT SLKKLHYTLN GKSSTIPSCG
 241 LQVSIIDYTL SRLERDGIVV FCDVSMDEDL FTGDGDYQFD IYRLMKKENN NRWGEYHPYS
 301 NVLWLHYLTD KMLKQMTFKT KCNTPAMKQI KRKIQEFHRT MLNFSSATDL LCQHSLFK
```

Recombinant Haspin nucleotide sequence:

```
   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc ggtcctgtcc cctttagcca ttgccttccc
 121 acagaaaaac tgcaacgctg tgagaagatt ggggaagggg tgtttggcga agtgtttcaa
 181 acaattgctg atcacacacc cgtagccata aaaatcattg ctattgaagg accagattta
 241 gtcaatggat cccatcagaa aacctttgag gaaatcctgc cagagatcat catctccaaa
 301 gagttgagcc tcttatccgg tgaagtgtgc aaccgcacag aaggctttat cgggctgaac
 361 tcagtgcact gtgtccaggg atcttaccct cccttgctcc tcaaagcctg ggatcactat
 421 aattcaacca aaggctctgc aaatgaccgg cctgattttt ttaaagacga ccagctcttc
 481 attgtgctgg aatttgagtt tggagggatt gacttagagc aaatgcgaac caagttgtct
 541 tccttggcta ctgcaaagag cattctacac cagctcacag cctccctcgc agtggcagag
 601 gcatcactgc gctttgagca ccgagactta cactgggggа acgtgctctt aaagaaaacc
 661 agcctcaaaa aactccacta caccctcaat gggaagagca gcactatccc cagctgtggg
 721 ttgcaagtga gcatcattga ctacaccctg tcgcgcttgg aacgggatgg gattgtggtt
 781 ttctgtgacg tttccatgga tgaggacctg tttaccggtg acggtgacta ccagtttgac
 841 atctacaggc tcatgaagaa ggagaataac aaccgctggg gtgaatatca ccctttatagt
 901 aatgtgctct ggttacatta cctgacagac aagatgctga aacaaatgac cttcaagact
 961 aaatgtaaca ctcctgccat gaagcaaatt aagagaaaaa tccaggagtt ccacaggaca
1021 atgctgaact tcagctctgc cactgacttg ctctgccagc acagtctgtt taagtaa
```

Reviewed and approved by site quality representative.