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Certificate of Analysis

DRAK1, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-668, 14-668-K, 14-668M
Parent Lot # D8MN024U

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, full length, human DRAK1, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 82.5% by SDS-PAGE and Coomassie blue staining. MW = 50.4kDa.

Specific Activity (Parent lot# D8MN024U): 89U/mg, where one unit of DRAK1 activity is defined as 1nmol phosphate incorporated into 250µM (KKLNRTLSFAEPG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.05mg/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.

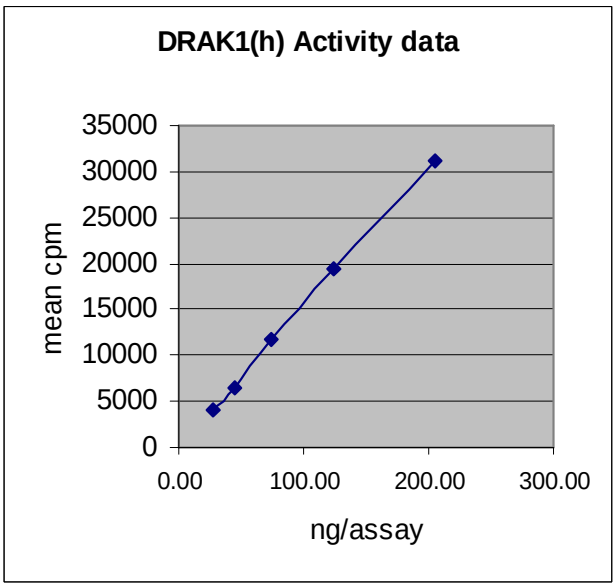
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

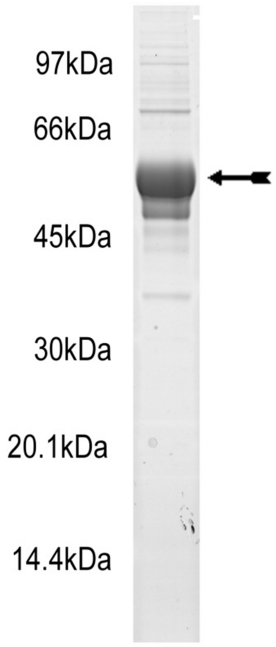
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 26.9–205ng of this lot of enzyme phosphorylated 250µM (KKLNRTLSFAEPG) 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 DRAK1 with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of DRAK1, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **(KKLNRTLSFAEPG):** Use at a final concentration of 250µM. Make a 2.5mM stock. Use 2.5µl of stock solution per assay point.
3. **DRAK1, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 26.9–205ng 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 **(KKLNRTLSFAEPG)**.
3. Add **2.5µl (26.9–205ng) DRAK1, 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 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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DRAK1 Sequence Information

Protein Human DRAK1

Tags *N*-terminal 6His

Native sequence M31 of the recombinant protein is equivalent to M1 of human DRAK1

Accession number GenBank NM_004760

Recombinant DRAK1 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MIPLEKPGSG GSSPGATSGS GRAGRGLSGP
 61 CRPPPPPQAR GLLTEIRAVV RTEPFQDGYS LCPGRELGRG KFAVVRKCIK KDSGKEFAAK
121 FMRKRRKGQD CRMEIIHEIA VLELAQDNPW VINLHEVYET ASEMILVLEY AAGGEIFDQC
181 VADREEAFKE KDVQRLMRQI LEGVHFLHTR DVVHLDLKPQ NILLTSESPL GDIKIVDFGL
241 SRILKNSEEL REIMGTPEYV APEILSYDPI SMATDMWSIG VLTYVMLTGI SPFLGNDKQE
301 TFLNISQMNL SYSEEEFDVL SESAVDFIRT LLVKKPEDRA TAEECLKHPW LTQSSIQEPS
361 FRMEKALEEA NALQEGHSVP EINSDTDKSE TEESIVTEEL IVVTSYTLGQ CRQSEKEKME
421 QKAISKRFKF EEPLLQEIPG EFIY
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Recombinant DRAK1 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc atgatccctt tggagaagcc aggcagcggc
 121 ggctcctccc caggcgccac ctcaggctcg ggccgggcag gccggggtct gagcgggccg
 181 tgccggccgc cgccgccgcc ccaggcccgc gggctgctga cagagatacg cgccgtggtg
 241 cgcaccgagc ccttccagga cggctacagc ctgtgcccgg gccgggagct gggcaggggg
 301 aaatttgcag tggtgagaaa atgtataaag aaagattctg ggaaagaatt tgctgcaaag
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1261 caaaaggcca tttccaaacg atttaaattt gaggaacctt tgctacaaga aattccagga
1321 gaatttatct actga
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Reviewed and approved by site quality representative.