

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 Ni²⁺/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 (KKLNRTLSEFAEPG) 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.

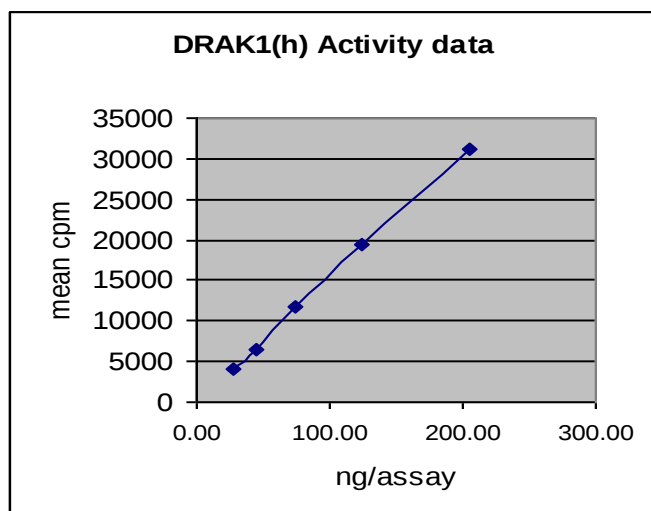
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

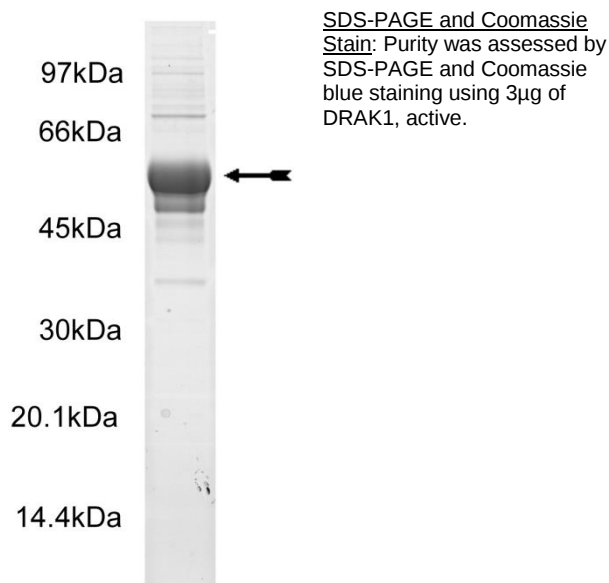
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 26.9–205ng of this lot of enzyme phosphorylated 250µM (KKLNRTLSEFAEPG) 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.



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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 -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 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 dH₂O.
5. Add 10µl of diluted [γ -³³P]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

<u>Protein</u>	Human DRAK1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of human DRAK1
<u>Accession number</u>	GenBank NM_004760

Recombinant DRAK1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MIPLEKPGSG GSSPGATSGS GRAGRGLSGP
61 CRPPPPPPQAR GLLTEIRAVV RTEPFQDGYS LCPGRELGRG KFAVVRKCIK KDSGKEFAAK
121 FMRKRRKGQD CRMEIIHEIA VLELAQDNPW VINLHEVYET ASEMILVLEY AAGGEIFDQC
181 VADREEAFKE KDVQRLMRQI LEGVHFLHTR DVVHLDLKPQ NILLTSESPL GDIKIVDFGL
241 SRILKNSEEL REIMGTPPEYV APEILSYDPI SMATDMWSIG VLTYVMLTGI SPFLGNDKQE
301 TFLNISQMNL SYSEEEFDVL SESAVDFIRT LLVKKPEDRA TAEELKHPW LTQSSIQEPS
361 FRMEKALEEA NALQEGHSVP EINSDDTKSE 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 ggtcctccc caggcgccac ctcaggctcg ggcggggcag gccggggtct gagcgggccc
181 tgccggccgc cgccgccgc ccaggccgc gggctgctga cagagatacg cgccgtggtg
241 cgcaccgagc ccttcaggga cggctacagc ctgtgcccg gccgggagct gggcaggggg
301 aaatttgcag tgggtgagaaa atgtataaag aaagattctg ggaaagaatt tgctgcaaag
361 ttcattgagaa aaagaagaaa aggccaagat tgtcggatgg aaataattca tgagattgct
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1261 caaaaggcca tttccaaacg atttaaatth gaggaacctt tgctacaaga aattccagga
1321 gaatttatct actga

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