

Certificate of Analysis

DDR2, active

(Recombinant enzyme expressed Sf21 insect cells)

Item # 14-579, 14-579-K, 14-579M

Parent Lot # 1597492

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 DDR2 amino acids 467–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 57.3% by SDS-PAGE and Coomassie blue staining. MW = 48kDa.

Specific Activity (Parent lot# 1597492): 33U/mg, where one unit of DDR2, active activity is defined as 1nmol phosphate incorporated into 250µM (KKSREGDYMTMQIG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.595mg/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.

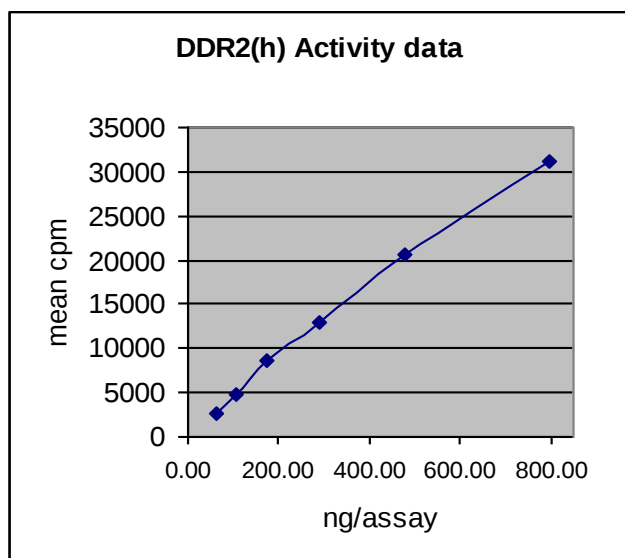
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

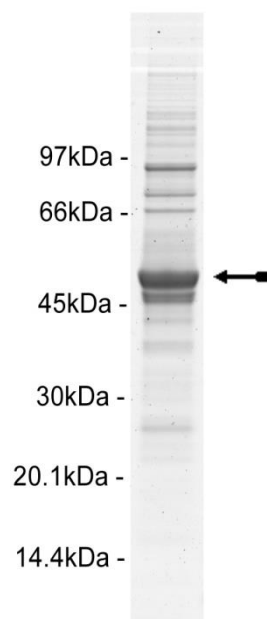
Quality Control Testing

Kinase Assay: 63–798ng of this lot of enzyme phosphorylated 250µM (KKSREGDYMTMQIG) 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 DDR2 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 DDR2, active.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **MnCl₂:** Use at a final assay concentration of 10mM. Prepare a 200mM stock in dH₂O and add 1.25µl of stock per assay point.
3. **(KKS₂RGDYMTMQIG):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
4. **DDR2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 63–798ng per assay point.
5. **[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 **(KKS₂RGDYMTMQIG)**.
3. Add 1.25µl of MnCl₂.
4. Add **2.5µl (63–798ng) DDR2, active**
5. Add 3.75µl of dH₂O.
6. Add 10µl of diluted [γ-³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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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DDR2 Sequence Information

<u>Protein</u>	human DDR2
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	S33 of recombinant sequence is equivalent to S467 of native human DDR2
<u>Accession number</u>	GenBank NM_006182. The recombinant protein contains the amino acid substitution S642A (native coordinates) with respect to GenBank NM_006182. This conflict is reported in GenBank accession nos; NM_022563, BC052998, BQ678490, BU168475 and BQ682363.

Recombinant DDR2 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMSLRL GSSNSTYDRI FPLRPDYQEP SRLIRKLPEF
61 APGEEESGCS GVVKPVQPSG PEGVPHYAEA DIVNLQGVGT GNTYSVPAVT MDLLSGKDVA
121 VEEFPRKLLT FKEKLGEGQF GEVHLCEVEG MEKFKDKDFA LDVSANQPVL VAVKMLRADA
181 NKNARNDFLK EIKIMSRLKD PNIIHLLAVC ITDDPLCMIT EYMENGDLNQ FLSRHEPPNS
241 SSSDVRTVSY TNLKFMATQI ASGMKYLSSL NFVHRDLATR NCLVGKNYTI KIADFGMSRN
301 LYSGDYYRIQ GRAVLPIRWM SWESILLGKF TTASDVWAFG VTLWETFTFC QEQPYSQLSD
361 EQVIENTGEF FRDQGRQTYL PQPAICPDSV YKLMLSCWRR DTKNRPSFQE IHLILLQQGD
421 E

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Recombinant DDR2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgtc ccttcgtctc ggatcctcca actcgactta cgatcgcatc
121 tttcccttc gccctgacta ccaggagcca tccaggctga tacgaaaact cccagaattt
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241 cctgaggggg tgccccacta tgcaaggctg gacatagtga acctccaagg agtgacagga
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1201 gatacgaaga accgtccctc attccaagaa atccaccttc tgctccttca acaaggcgac
1261 gagtag

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