

Certificate of Analysis

DDR1, activated

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-942, 14-942-K, 14-942M

Parent Lot # D13NP016N

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 DDR1 amino acids 492-end expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Activated *in vitro* by auto-phosphorylation. Purity 77% by SDS-PAGE and Coomassie blue staining. MW = 50kDa.

Specific Activity (Parent lot# D13NP016N): 396U/mg, where one unit of DDR1, activated activity is defined as 1nmol phosphate incorporated into 250µM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.264mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 20mM β-glycerophosphate, 0.25mM sodium orthovanadate, 10mM NaF, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

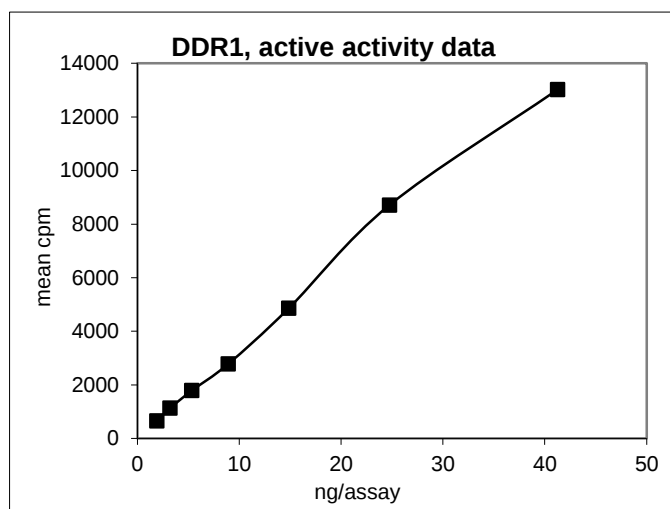
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

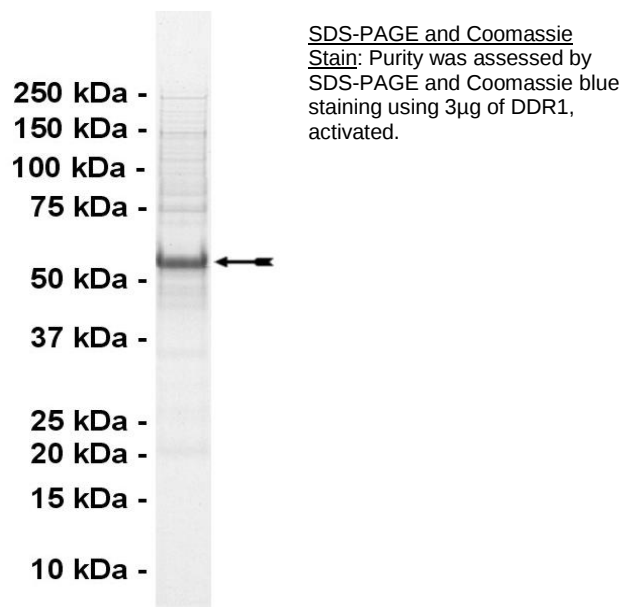
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2–41ng of this lot of enzyme phosphorylated 250µM (KKKSPGEYVNIEFG) 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 DDR1 with the translated sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKKSPGEYVNIEFG):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **DDR1, activated:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2–41ng per assay point.
4. **[γ-³³P]ATP:** 2.5 x MgAc/[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 (KKKSPGEYVNIEFG).
3. Add **2.5µl (2–41ng) DDR1, activated.**
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.

Certificate of Analysis

DDR1 Sequence Information

Protein	human DDR1
Tags	N-terminal 6His
Native sequence	N31 of the recombinant protein is equivalent to N492 of human DDR1
Accession number	GenBank U48705 The recombinant protein contains the amino acid substitution V833L with reference to GenBank U48705. V833L is reported in GenBank NM_001954, NM_013993 and NM_013994.

Recombinant DDR1 amino acid sequence:

```

1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF NPPHSAPCVP NGSALLLSNP AYRLLLATYA
61 RPPRGPGPPT PAWAKPTNTQ AYSGDYMEPE KPGAPLLPPP PQNSVPHYAE ADIVTLQGV
121 GGNTYAVPAL PPGAVGDGPP RVDFPRSRRLR FKEKLGEGQF GEVHLCEVDS PQDLVSLDFP
181 LNVKRGHPLL VAVKILRPDA TKNARNDFLK EVKIMSRLKD PNIIRLLGVC VQDDPLCMIT
241 DYMENGLDNQ FLSAHQLEDK AAEGAPGDGQ AAQGPTISYP MLLHVAAQIA SGMRYLATLN
301 FVHRDLATRN CLVGENFTIK IADFGMSRNL YAGDYRVQVG RAVLPIRWMA WECILMGKFT
361 TASDVWAFGV TLWEVLMLCR AQPFGQLTDE QVIENAGEFF RDQGRQVYLS RPPACPQGLY
421 ELMRLCWSRE SEQRPPFSQL HRFLAEDALN TV

```

Recombinant DDR1 nucleotide sequence:

```

1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgcatgga tccggaattc aatccgcccc actccgctcc ctgtgtcccc
121 aatggctctg cgttgctgct ctccaatcca gcctaccgcc tccttctggc cacttacgcc
181 cgtccccctc gaggcccggg cccccccaca cccgcctggg ccaaaccac caacaccag
241 gcctacagtg gggactatat ggagcctgag aagccaggcg ccccgcttct gccccacct
301 cccagaaca gcgtcccca ttatgccgag gctgacattg ttacctgca gggcgctacc
361 gggggcaaca cctatgctgt gcctgcactg ccccgagggg cagtcgggga tgggcccccc
421 agagtggatt tccctcgatc tcgactccgc ttcaaggaga agcttggcga gggccagttt
481 ggggagggtgc acctgtgtga ggtcgacagc cctcaagatc tggttagtct tgatttcccc
541 cttaatgtgc gtaagggaac ccctttgctg gtagctgtca agatcttacg gccagatgcc
601 accaagaatg ccaggaatga tttcctgaaa gaggtgaaga tcatgtcgag gctcaaggac
661 ccaaacaatc ttcggctgct gggcggtgtg gtgcaggacg accccctctg catgattact
721 gactacatgg agaagggcga cctcaaccag ttcctcagtg cccaccagct ggaggacaag
781 gcagccgagg gggcccctgg ggacgggcag gctgcgcagg ggcccaccat cagctacca
841 atgctgctgc atgtggcagc ccagatcgcc tccggcatgc gctatctggc cacactcaac
901 tttgtacatc gggacctggc cacgcggaac tgcctagttg gggaaaattt caccatcaaa
961 atcgagact ttggcatgag ccggaacctc tatgctgggg actattaccg tgtgcagggc
1021 cgggcagtg cgtccatccg ctggatggcc tgggagtgca tcctcatggg gaagttcacg
1081 actgcgagtg acgtgtgggc ctttgggtgt accctgtggg aggtgctgat gctctgtagg
1141 gcccagccct ttgggcagct caccgacgag caggtcatcg agaacgcggg ggagttcttc
1201 cgggaccagg gccggcaggt gtacctgtcc cggccgcctg cctgcccgca gggcctatat
1261 gagctgatgc ttcgggtgct gagccgggag tctgagcagc gaccaccctt ttcccagctg
1321 catcggttcc tggcagagga tgcactcaac acggtgtga

```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.