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Certificate of Analysis

KDR, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-630, 14-630-K, 14-630M
Parent Lot # WAB0366

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 KDR amino acids 790–end, expressed by baculovirus in Sf21insect cells. Purified using Ni^{2+}/NTA agarose. Purity 85% by SDS-PAGE and Coomassie blue staining. MW = 67.9kDa.

Specific Activity (Parent lot# WAB0366): 397U/mg, where one unit of KDR, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.75mg/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.

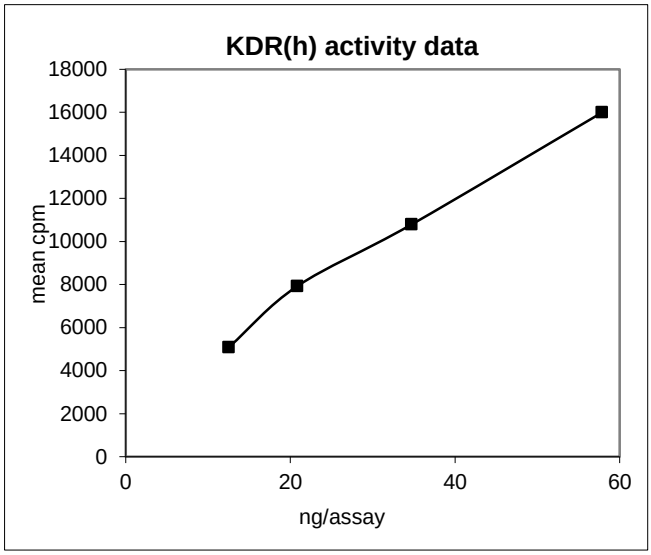
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

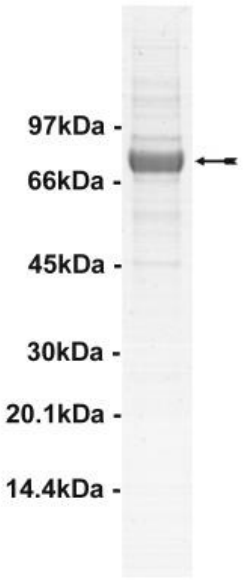
Quality Control Testing

Kinase Assay: 12–58ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein (MBP) 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 KDR 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 KDR, active



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Use 2.5µl of stock per assay point.
3. **KDR, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 12–58ng 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 **myelin basic protein (MBP)**.
3. Add **2.5µl (12–58ng) KDR, 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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KDR Sequence Information

Protein	Human KDR
Tags	*N*-terminal 6His
Native sequence	K37 of the recombinant protein is equivalent to K790 of human KDR
Accession number	GenBank NM_002253

Recombinant KDR amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRLKRAN GGELKTGYLS IVMDPDELPL
 61 DEHCERLPYD ASKWEFPRDR LKLGKPLGRG AFGQVIEADA FGIDKTATCR TVAVKMLKEG
121 ATHSEHRALM SELKILIHIG HHLNVVNLLG ACTKPGGPLM VIVEFCKFGN LSTYLRSKRN
181 EFVPYKTKGA RFRQGKDYVG AIPVDLKRRL DSITSSQSSA SSGFVEEKSL SDVEEEEAPE
241 DLYKDFLTLE HLICYSFQVA KGMEFLASRK CIHRDLAARN ILLSEKNVVK ICDFGLARDI
301 YKDPDYVRKG DARLPLKWMA PETIFDRVYT IQSDVWSFGV LLWEIFSLGA SPYPGVKIDE
361 EFCRRLKEGT RMRAPDYTTP EMYQTMLDCW HGEPSQRPTF SELVEHLGNL LQANAQQDGK
421 DYIVLPISET LSMEEDSGLS LPTSPVSCME EEEVCDPKFH YDNTAGISQY LQNSKRKSRP
481 VSVKTFEDIP LEEPEVKVIP DDNQTDSGMV LASEELKTLE DRTKLSPSFG GMVPSKSRES
541 VASEGSNQTS GYQSGYHSDD TDTTVYSSEE AELLKLIEIG VQTGSTAQIL QPDSGTTLSS
601 PPV
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Recombinant KDR nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacttaa gcgggccaat
 121 ggaggggaac tgaagacagg ttacttgtcc atcgtcatgg atccagatga actcccattg
 181 gatgaacatt gtgaacgact gccttatgat gccagcaaat gggaattccc cagagaccgg
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1741 gtgcaaaccg gtagcacagc ccagattctc cagcctgact cggggaccac actgagctct
1801 cctcctgttt aa
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Reviewed and approved by site quality representative.