

Certificate of Analysis

VHR, active

(Recombinant enzyme expressed in *E. coli* cells)

Item # 14-594, 14-594-K, 14-594M

Parent Lot # LB194p158

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 GST-tagged, recombinant, human VHR, full length, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 98% by SDS-PAGE and Coomassie blue staining. MW = 47.8kDa.

Specific Activity (Parent lot# LB194p158): 506U/mg, where one unit of VHR, active activity is defined as the release of 1nmol of phosphate per minute from the phosphorylated substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) at room temperature.

Formulation: 2.33mg/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.

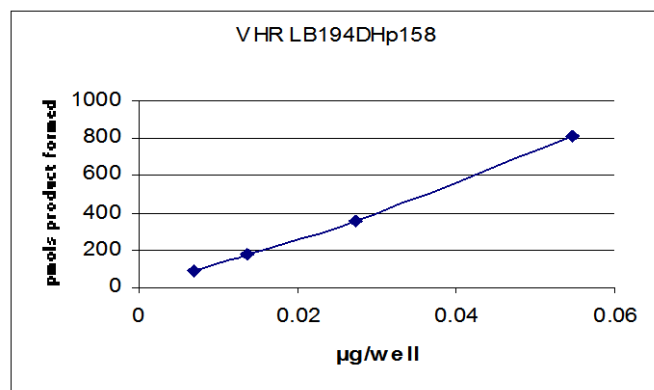
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

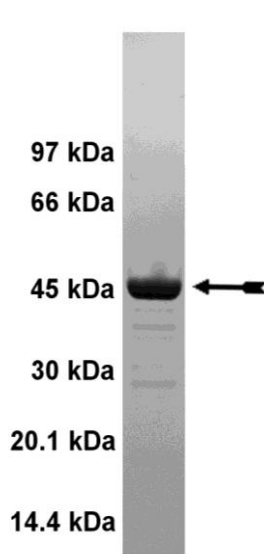
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Phosphatase Assay: 5–60ng of this lot of enzyme dephosphorylated 200µM DiFMUP in the assay described on page two. Assay background was subtracted from the actual Fluorescence Intensity (FI) to yield the results shown below. Quantification of FI was against a 6,8-difluoro-7-hydroxy-4-methylcoumarin (DiFMU) standard curve.



MS Tryptic Fingerprint: Confirmed identity as VHR 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 VHR, active.

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Phosphatase Assay Protocol

Stock Solutions:

1. **Reaction Buffer:** 32mM Hepes pH7.2, 50mM NaCl, 2.5mM EDTA, 0.17mM DTT, 0.83 (v/v)% glycerol, 0.017 (w/v)% BSA, 0.002% Brij-35.
2. 500µM DiFMUP (Molecular Probes Catalogue # D6567) in water.
3. 100mM sodium orthovanadate.
4. 500µM DiFMU (Molecular Probes Catalogue # D6566) in water for the calibration curve.

Assay Procedure:

1. Dilute **VHR** in reaction buffer and use 5–60ng in 15µl per assay point.
2. Add 10µl DiFMUP 500µM stock solution (200µM final assay concentration).
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5µl of 100mM sodium orthovanadate.
5. Read FI using an appropriate reader (Excitation 340nm; Emission 450nm).
6. Subtract the zero enzyme values from each FI reading and calculate the enzyme activity by conversion to nmoles product formed using a DiFMU standard calibration curve.

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VHR Sequence Information

Protein	VHR
Tags	N-terminal GST
Native sequence	M238 of the recombinant protein is equivalent to M1 of human VHR
Accession number	GenBank NM_004090

Recombinant VHR amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSPGIS  GGGGGILMSG
241 SFELSVQDLN  DLLSDGSGCY  SLPSQPCNEV  TPRIYVGNAS  VAQDIPKLQK  LGITHVLNAA
301 EGRSEFMHVT  NANFYKDSGI  TYLGIKANDT  QEFNLSAYFE  RAADFIDQAL  AQKNGRVLVH
361 CREGYSRSPT  LVIAYLMMRQ  KMDVKSALSI  VRQNREIGPN  DGFLAQLCQL  NDRLAKEGKL
421 KP

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Recombinant VHR nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121 tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatattgat
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421 acataattta  atgggtgatca  tgtaacccat  cctgacttca  tgttgatatga  cgctcttgat
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1261 aaaccctag

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