

## 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.

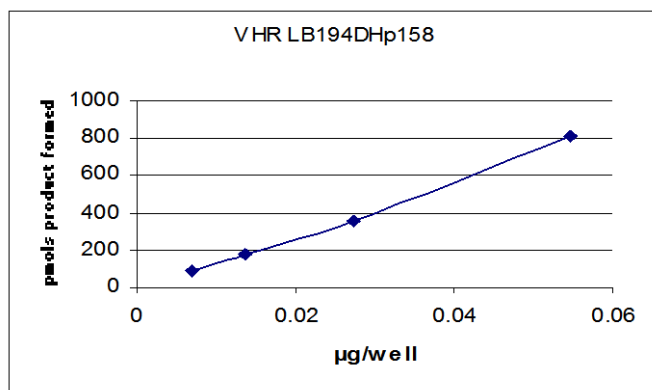
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

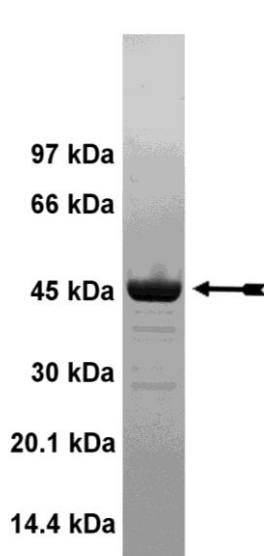
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### 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.

## Certificate of Analysis

### 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. 500 $\mu$ M DiFMUP (Molecular Probes Catalogue # D6567) in water.
3. 100mM sodium orthovanadate.
4. 500 $\mu$ M DiFMU (Molecular Probes Catalogue # D6566) in water for the calibration curve.

#### Assay Procedure:

1. Dilute **VHR** in reaction buffer and use 5–60ng in 15 $\mu$ l per assay point.
2. Add 10 $\mu$ l DiFMUP 500 $\mu$ M stock solution (200 $\mu$ M final assay concentration).
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5 $\mu$ l 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.

## Certificate of Analysis

### VHR Sequence Information

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

#### Recombinant VHR amino acid sequence:

```

1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGDHPPKSD LVPRGSPGIS GGGGILMSG
241 SFELSVQDLN DLLSDGSGCY SLPSQPCNEV TPRIYVGNAS VAQDIPKLQK LGITHVLNAA
301 EGRSEFMHVNT NANFYKDSGI TYLGIKANDT QEFNLSAYFE RAADFIDQAL AQKNGRVLVH
361 CREGYSRSPT LVIAYLMMRQ KMDVKSALSI VRQNREIGPN DGFLAQLCQL NDRLAKEGKL
421 KP
    
```

#### Recombinant VHR nucleotide sequence:

```

1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccacac tcgacttctt
61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatacggtt atatagctga caagcacaac
241 atgttggttg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcgggtttg
301 gatattagat acgggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttctg aagatcgttt atgtcataaa
421 acataatttaa atgggtgatca tgtaaccacat cctgacttca tgttgatga cgctcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggttccgc gtggatcccc gggaaatttcc ggtggtgggtg gtggaattct aatgtcgggc
721 tcgttcgagc tctcgggtgca ggatctcaac gacctgctct cggacggcag cggctgctac
781 agcctcccga gccagccctg caacgaggtc accccgcgga tctacgtggg caacgcgtct
841 gtggctcagg acatccccaa gctgcagaaa ctaggcatca cccatgtgct gaacgcggct
901 gagggcaggt ccttcatgca cgtcaacacc aatgccaaact tctacaagga ctccggcatc
961 acatacctgg gcatcaaggc caacgacaca caggagtcca acctcagcgc ttactttgaa
1021 agggctgccg acttcattga ccaggctttg gctcaaaaaga atggccgggt gctcgtccac
1081 tgccgggaag gttatagccg ctccccaacg ctagtattcg cctacctcat gatgcggcag
1141 aagatggacg tcaagtctgc cctgagcatc gtgaggcaga accgtgagat cggccccaac
1201 gatggcttcc tggcccagct ctgcccagtc aatgacagac tagccaagga ggggaagttg
1261 aaaccctag
    
```

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.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.