

Certificate of Analysis

YopH, active

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

Item # 14-590, 14-590-K, 14-590M

Parent Lot # D7DN012U

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, *Yersinia* YopH, full length, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 77.4kDa.

Specific Activity (Parent lot# D7DN012U): 27700U/mg, where one unit of YopH 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: 3.31mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 10% glycerol;, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

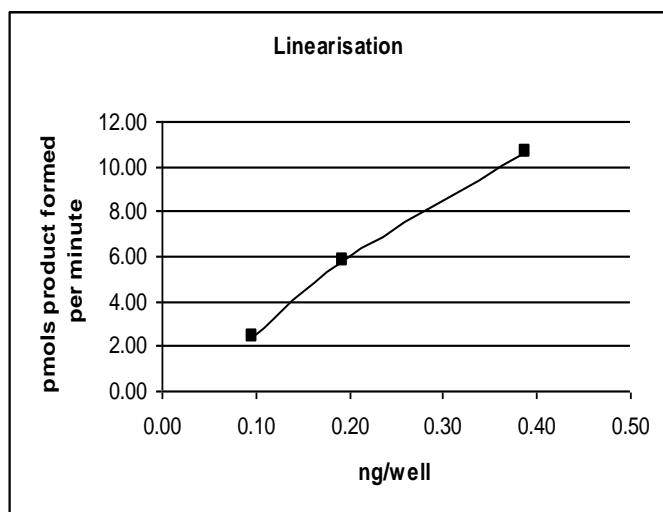
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

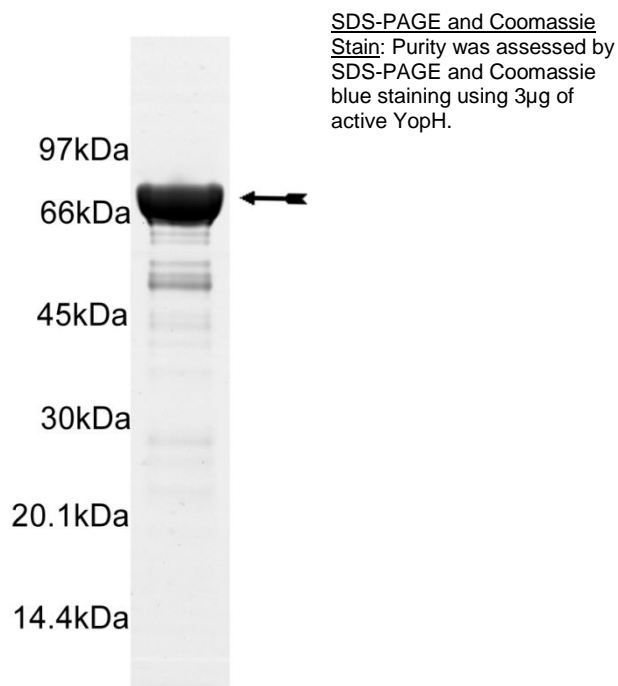
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Phosphatase Assay: 0.1–0.4ng 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 YopH with the translated sequence listed on page three.



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

Assay Procedure:

1. Dilute YopH in reaction buffer and use **0.1–0.4ng** in **15 μ l** per assay point.
2. Add 10 μ l DiFMUP 500 μ M stock solution (200 μ M final assay concentration.)
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5 μ 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.

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YopH Sequence Information

<u>Protein</u>	<i>Yersinia</i> YopH
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M228 of the recombinant protein is equivalent to M1 of <i>Yersinia</i> YopH
<u>Accession number</u>	GenBank Y00551. The recombinant protein contains the amino acid substitution R211A with reference to Y00551. This mutation is reported in GenBank M30457.

Recombinant YopH amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGDHPPKSD  LVPRGSHMNL  SLSDLHRQVS
241 RLVQQESGDC  TGKLRGNVAA  NKETTFQGLT  IASGARESEK  VFAQTVLSHV  ANVVLTQEDT
301 AKLLQSTVKH  NLNNYDLRSV  GNGNSVLVSL  RSDQMTLQDA  KVLLEAALRQ  ESGARGHVSS
361 HSHSALHAPG  TPVREGLRSH  LDPRTPLPP  RERPHTSGHH  GAGEARATAP  STVSPYGPEA
421 RAELSSRLTT  LRNTLAPATN  DPLYLQACGG  EKLNRFRDIQ  CCRQTAVRAD  LNANYIQVGN
481 TRTIACQYPL  QSQLESHFRM  LAENRTPVLA  VLASSEIAN  QRFGMPDYFR  QSGTYGSITV
541 ESKMTQQVGL  GDGIMADMYT  LTIREAQKT  ISVPVVHVGN  WPDQTAVSSE  VTKALASLVD
601 QTAETKRNM  Y  ESKGSSAVGD  DSKLRPVIHC  RAGVGRTAQL  IGAMCMNDSR  NSQLSVEDMV
661 SQMRVQRNGI  MVQKDEQLDV  LIKLAEGQGR  PLLNS

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Recombinant YopH nucleotide sequence:

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1  atgtccccta  tactaggtta  ttgaaaaatt  aagggccttg  tgcaaccac  tgcacttctt
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1501 ctggcagaaa  accgaacgcc  agtgttggt  gttttagcgt  ccagttctga  gatagccaat

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1741 tggcccgatc agaccgcagt cagctctgaa gttaccaagg cactcgcttc actggtagat
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1981 agccaaatgc gagtacaaag aaatggtatt atggtacaaa aagatgagca acttgatggt
2041 ctgattaagt tggctgaagg acaagggcga ccattattaa atagctaa
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