

Certificate of Analysis

PI3 Kinase (p120y)

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-558, 14-558-K, 14-558M

Parent Lot # 2298365

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 full-length human PI3 kinase (p120y) expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 92.4% by SDS-PAGE and Coomassie blue staining. MW = 130kDa.

Specific Activity (Parent lot# 2298365): 34U/mg, where one unit of PI3 Kinase (p120y) activity is defined as 1nmol phosphatidylinositol 3,4,5-trisphosphate formed per minute at 22°C with a final ATP concentration of 100µM.

Formulation: 1.481mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 10% glycerol, 0.1mM EGTA, 2mM DTT.

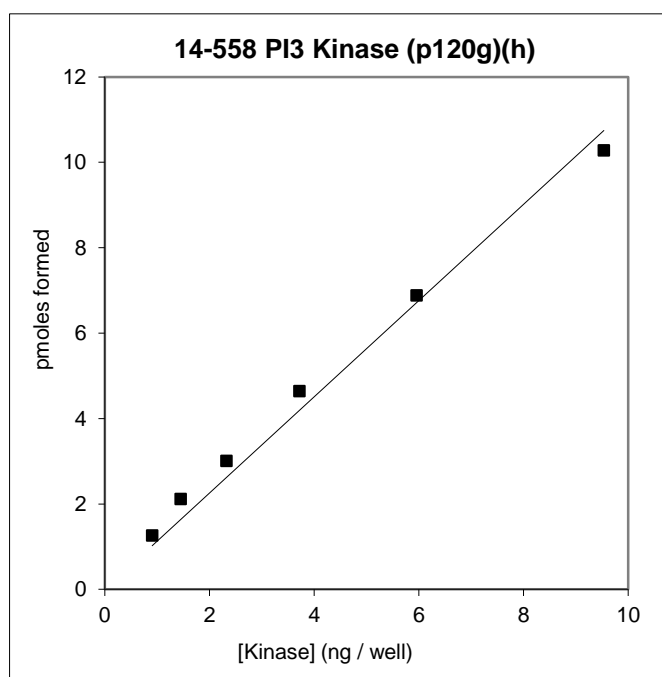
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

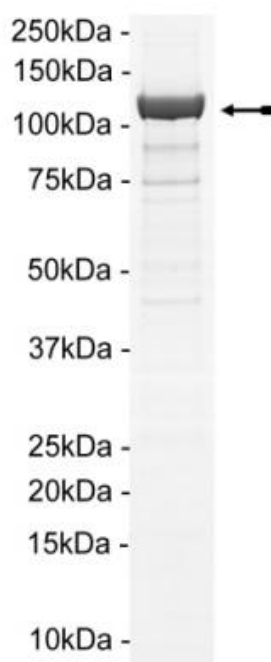
Quality Control Testing

Kinase Assay: 0.9–9.5ng of this enzyme phosphorylated 10µM phosphatidylinositol 4, 5-bisphosphate in the assay referenced on page two.



MS Tryptic Fingerprint: Confirmed product identity as PI3 Kinase (p120y) with the translated sequence listed on page two.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active PI3 kinase (p120y).



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Eurofins PI3 Kinase Homogeneous Time-resolved Fluorescence (HTRF) Class I Reagent Kits

The following Eurofins kits are suitable for use with this enzyme:

Cat. No	Kit Description
33-016	PI3 Kinase 4-Step Assay Reagent 1-Plate Kit
33-017	PI3 Kinase 4-Step Assay Reagent 5-Plate Kit
33-036	PI3 Kinase 4-Step Assay Reagent Kit (10000 wells)
33-037	PI3 Kinase 4-Step Assay Reagent Kit (50000 wells)
33-040	PI3 Kinase 3-Step Assay Reagent Kit (384 wells)
33-041	PI3 Kinase 3-Step Assay Reagent Kit (1920 wells)
33-047	PI3 Kinase 3-Step Assay Reagent Kit (10000 wells)

Kits 33-016, 33-017, 33-036 and 33-037 provide reagents and assay details for the Eurofins standard 4-step HTRF assay. This assay format is suitable for the majority of small and medium throughput screening work. The 3-step HTRF assay (kits 33-040, 33-041, 33-047) was introduced to reduce the number of assay steps to aid high throughput screening. Items 33-040 and 33-041 are intended as introductory kits for 3-step procedure work up. Please contact us for any further information regarding different kit formats (discoveryservices@eurofins.com).

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P13 Kinase (p120y) Sequence Information

<u>Protein</u>	human P13 Kinase (p120y)
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M34 of the recombinant protein is equivalent to M1 of human P13 Kinase (p120y)
<u>Accession number</u>	GenBank AF327656. The recombinant protein contains the amino acid substitution R459Q with respect to this accession number. This conflict is reported in GenBank XM_027912. The residue coordinates in the native sequence are given.

Recombinant P13 Kinase (p120y) amino acid sequence:

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1 MSYHHHHHHH DYDIPTTENL YFQAMGSGI RPCMELENYK QPVVLRDNC RRRRRMKPRS
61 AAASLSSMEL IPIEFLVPTS QRKCKSPETA LLHVAGHGNV EQMKAQVWLR ALETVAADF
121 YHRLGPHHFL LLYQKKGQWY EIYDKYQVVQ TLDCLRYWKA THRSPGQIHL VQRHPPSEES
181 QAFQRQLTAL IGYDVTDVSN VHDDELEFTR RGLVTPRMAE VASRDPKLYA MHPWVTSKPL
241 PEYLWKKIAN NCIFIVIHRS TTSQTIKVSP DDTPGAILQS FFTKMAKKKS LMDIPESQSE
301 QDFVLRVCGR DEYLVGETPI KNFQWVRHCL KNGEEIHVVL DTPDPALDE VRKEEWPLVD
361 DCTGVTGYHE QLTIHGKDHE SVFTVSLWDC DRKFRVKIRG IDIPVLPRNT DLTVFVEANI
421 QHGQQVLCQR RTSPKPFTEE VLWNVWLEFS IKIKDLPKGA LLNLQIYCGK APALSSKASA
481 ESPSESCKGK VQLLYVNNLL LIDHRFLLRR GEYVLHMWQI SGKGEDQGSF NADKLTSATN
541 PDKENSMSIS ILLDNYCHPI ALPKHQPTPD PEGDRVRAEM PNQLRKQLEA IIATDPLNPL
601 TAEDKELLWH FRYESLKHPK AYPKLFSSVK WGQQEIVAKT YQLLARREVV DQSALDVGLT
661 MQLLDCNFSN ENVRAIAVQK LESLEDDVVL HYLLQLVQAV KFEPYHDSAL ARFLLKRGRLR
721 NKRIGHFLFW FLRSEIAQSR HYQRFQAVIL EAYLRGCGTA MLHDFDQQVQ VIEMLQKVTL
781 DIKLSAEKY DVSSQVISQL KQKLENLQNS QLPESFRVPY DPGLKAGALA IEKCKVMASK
841 KKPLWLEFKC ADPTALSNET IGIIFKHGDD LRQDMLILQI LRIMESIWET ESLDCLLPY
901 GCISTGDKIG MIEIVKDATT IAKIQQSTVG NTGAFKDEVL NHWLKEKSPT EEKFQAAVER
961 FVYSCAGYCV ATFVLGIGDR HNDNIMITET GNLFHIDFGH ILGNYKSFLG INKERVPFVL
1021 TPDFLFVMGT SGKKTSPHFQ KFQDICVKAY LALRHHTNLL IILFSMMLMT GMPQLTSKED
1081 IEYIRDALT V GKNEEDAKKY FLDQIEVCRD KGWTVQFNWF LHLVLGIKQG EKHSA

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Recombinant P13 Kinase (p120y) nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatggg atccggaatt cgcccttgca tggagctgga gaactataaa
121 cagcccgtgg tgcctgagaga ggacaactgc cgaagcgcc ggaggatgaa gccgcgcagt
181 gctgcggcca gcctgtcctc catggagctc atccccatcg agttcgtgct gccaccacgc
241 cagcgaat gcaagagccc cgaaacggcg ctgctgcacg tggccggcca cggcaactg
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361 taccaccggc tgggaccgca tcacttcctc ctgctctatc agaagaagg gcagtggtac
421 gagatctacg acaagtacca ggtggtgcag actctggact gcctgcgcta ctggaaggcc
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541 caagccttc agcggcagct cacggcctg attggctatg acgtcactga cgtcagcaac
601 gtgcacgacg atgagctgga gttcacgcgc cgtggcttgg tgaccccgcg catggcggag
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1081 gactgcacgg gactcaccgg ctaccatgag cagcttacca tccacggcaa ggaccacgag

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1141 agtgtgttca ccgtgtccct gtgggactgc gaccgcaagt tcagggtcaa gatcagaggc
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3361 ctacatcttg ttcttggcat caaacaagga gagaacatt cagcctaa

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