



p38 alpha Positive Control

Product Data Sheet

For Research Use Only, Not for use in diagnostic procedures

## p38 alpha Positive Control (Human, full length, recombinant enzyme expressed in *E. coli*)

Cat# CY-E1177

Lot No. 26F06

For 200 assays

(10 m units/ $\mu$ L  $\times$  200 $\mu$ L)

**Product Description:** Full length human p38 alpha, containing a *N*-terminal GST tag, expressed in *E. coli*. Purified by GSH agarose chromatography. The p38 alpha Positive Control is designed to use for CycLex p38 Kinase Assay /Inhibitor Screening Kit [Cat# CY-1177]. The p38 alpha Positive Control should be added to the well at 10 m units/well. For instance, diluted positive control 1:10, use 10  $\mu$ L for 1 assay. Unused the p38 alpha Positive Control should be stored at -70°C.

**Product Size:** Full length human p38 alpha: 2 units/200  $\mu$ L

**Formulation:** The p38 alpha Positive Control is supplied frozen in a buffer containing 20mM HEPES-KOH (pH 7.5), 8mM 2-Mercaptoethanol, 50mM NaCl and 50% glycerol.

**Source:** Recombinant Full length human p38 alpha containing *N*-terminal GST tag, expressed in *E. coli*.

**Molecular Weight:** Human p38 alpha demonstrates a single 64 kDa band by SDS-PAGE analysis.

**Purity:** Human p38 alpha is greater than 70% pure as determined by SDS-PAGE analysis.

**Substrates:** Human p38 alpha reported to phosphorylate endogenous substrate , ATF2.

**Inhibitors:** SB 202190, SB 220025 and SB 203580 are known as effective p38 inhibitor.

**Unit Definitions:** One unit is defined as the amount of kinase required to incorporate 1 nmol of phosphate into the ATF2, per minute at 30°C.

**Assay Conditions:** Assay activity of Human p38 alpha in a 50  $\mu$ L reaction containing 20 mM Hepes KOH (pH 7.5), 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM DTT, 100  $\mu$ M [ $\gamma$ -<sup>32</sup>P] ATP (1  $\mu$ Ci), and 4  $\mu$ g of GST-ATF2 fusion protein. Start the reaction by adding 10 $\mu$ L of the enzyme, diluted 50-fold in a buffer containing 20 mM Hepes KOH (pH 7.5), 1 mM DTT. Incubate for 30 minutes at 30°C. Terminate the reaction by adding 600  $\mu$ L of cold 10 % TCA solution containing 0.2 % sodium pyrophosphate and stand on ice for 15 min. Filtrate acid insoluble material through GFC filters (Whatman Inc.), wash 4 times with 1 % TCA and rinse filters with ethanol. Dry filters and count in a liquid scintillation counter.

**Storage and Stability:** Stable for 12 months at -70°C from date of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Aliquot enzyme to avoid repeated freezing and thawing.

### Related Products:

\* CycLex p38 Kinase Assay/Inhibitor Screening Kit: Cat# CY-1177



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**General References:**

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8. Enslin H, Ringeaud J, Davis RJ. Selective activation of p38 mitogen-activated protein (MAP) kinase isoforms by the MAP kinase kinases MKK3 and MKK6. *J Biol Chem.* 1998;273: 1741-1748
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**PRODUCED BY**

**CycLex Co., Ltd.**  
**1063-103 Terasawaoka**  
**Ina, Nagano 396-0002**  
**Japan**  
**Fax: +81-265-76-7618**  
**e-mail: [info@cyclex.co.jp](mailto:info@cyclex.co.jp)**  
**URL: <http://www.cyclex.co.jp>**

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