



EphA2 Positive Control

Product Data Sheet

For Research Use Only, Not for use in diagnostic procedures

EphA2 Positive Control

(Human, recombinant protein expressed in Sf9)
Cat# CY-E1092

Lot No.
For 100 Assays
(1 unit / μL x 100 μL)

Product Description:

Catalytic domain of human EphA2, corresponding to 613-871 a.a. containing a *N*-terminal GST tag and a C-terminal His tag, expressed in recombinant Baculovirus-infected sf9 cells. Purified by sequentially using GSH agarose and Ni-NTA agarose chromatography. The EphA2 Positive control is designed to use for CycLex EphA2 Kinase Assay/Inhibitor Screening Kit (Cat# CY-1092). The EphA2 Positive Control should be added to the well at 1 unit/well. For instance, diluted positive control 1:10, use 10 μL for 1 assay. Unused EphA2 Positive Control should be stored at -70°C .

Product Size: Recombinant EphA2: 100 units/100 μL

Formulation: The EphA2 Positive Control is supplied frozen in a buffer containing 20mM Hepes-KOH (pH 7.5), 1 % BSA, 1mM EDTA, 2 mM DTT, 50mM NaCl, 0.03 % Brij35 and 50% glycerol.

Source: Human EphA2 containing N-terminal GST-tag and C-terminal His tag, expressed in sf9 cells.

Molecular Weight: EphA2 Positive Control demonstrates a single 62 kDa bands by SDS-PAGE analysis.

Purity: EphA2 Positive Control is greater than 85 % pure as determined by SDS-PAGE analysis.

Substrates: EphA2 phosphorylates poly[Glu, Tyr] 4:1 as a exogenous substrate.

Inhibitors: Specific EphA2 inhibitor has not been discovered yet.

Unit Definition: One unit is defined as the amount of kinase required to incorporate 1 nmol of phosphate into the EphA2 (autophosphorylation) under oligomerized/activated condition per 60 minute at 30°C .

Assay Conditions: Assay activity of EphA2 in a 50 μL reaction containing 20 mM Hepes KOH (pH 7.5), 4 mM MgCl_2 , 2 mM MnCl_2 , 1 mM DTT, 50 μM [γ - ^{32}P] ATP (1 μCi), and 4 μg of CycLex-“Tyrosine kinase-binding module”. Start the reaction by adding 10 μL of the enzyme, diluted 10-fold in a buffer containing 20 mM Hepes KOH (pH 7.5), 1 mM DTT, 0.03 % Brij35. Incubate for 60 minutes at 30°C . Terminate the reaction by adding 600 μL 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.



EphA2 Positive Control
Product Data Sheet

For Research Use Only, Not for use in diagnostic procedures

Related Products:

* CycLex EphA2 Kinase Assay/Inhibitor Screening Kit: Cat# CY-1092

References:

1. Holder N., Klein R. Eph receptors and ephrins: effectors of morphogenesis. *Development (Camb.)*, **126**: 2033-2044, 1999.
2. Dodelet V. C., Pasquale E. B. Eph receptors and ephrin ligands: embryogenesis to tumorigenesis. *Oncogene*, **19**: 5614-5619, 2000
3. Easty D. J., Herlyn M., Bennett D. C. Abnormal protein tyrosine kinase gene expression during melanoma progression and metastasis. *Int. J. Cancer*, **60**: 129-136, 1995.
4. Nemoto T., Ohashi K., Akashi T., Johnson J., Hirokawa K. Overexpression of protein tyrosine kinases in human esophageal cancer. *Pathobiology*, **65**: 195-203, 1997.
5. Rosenberg I. M., Goke M., Kanai M., Reinecker H. C., Podolsky D. K. Epithelial cell kinase-B61: an autocrine loop modulating intestinal epithelial migration and barrier function. *Am. J. Physiol.*, **273**: G824-G832, 1997.
6. Zelinski D., Zantek N., Stewart J., Irizarry A., Kinch M. EphA2 overexpression causes tumorigenesis of mammary epithelial cells. *Cancer Res.*, **61**: 2301-2306, 2001.
7. Walker-Daniels J., Coffman K., Azimi M., Rhim J. S., Bostwick D. G., Snyder P., Kerns B. J., Waters D. J., Kinch M. S. Overexpression of the EphA2 tyrosine kinase in prostate cancer. *Prostate*, **41**: 275-280, 1999.
8. Kinch M. S., Moore M. B., Harpole D. H., Jr. Predictive value of the EphA2 receptor tyrosine kinase in lung cancer recurrence and survival. *Clin. Cancer Res.*, **9**: 613-618, 2003.
9. Miyazaki T., Kato H., Fukuchi M., Nakajima M., Kuwano H. EphA2 overexpression correlates with poor prognosis in esophageal squamous cell carcinoma. *Int. J. Cancer*, **103**: 657-663, 2003.
10. Miao H., Burnett E., Kinch M., Simon E., Wang B. Activation of EphA2 kinase suppresses integrin function and causes focal-adhesion-kinase dephosphorylation. *Nat. Cell Biol.*, **2**: 62-69, 2000.
11. Dohn M., Jiang J., Chen X. Receptor tyrosine kinase EphA2 is regulated by p53-family proteins and induces apoptosis. *Oncogene*, **20**: 6503-6515, 2001.
12. Hess A. R., Seftor E. A., Gardner L. M., Carles-Kinch K., Schneider G. B., Seftor R. E., Kinch M. S., Hendrix M. J. Molecular regulation of tumor cell vasculogenic mimicry by tyrosine phosphorylation: role of epithelial cell kinase (Eck/EphA2). *Cancer Res.*, **61**: 3250-3255, 2001.

PRODUCED BY

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

CycLex/CircuLex products are supplied for research use only. CycLex/CircuLex products and components thereof may not be resold, modified for resale, or used to manufacture commercial products without prior written approval from CycLex Co., Ltd.. To inquire about licensing for such commercial use, please contact us via email.