



EphA2 Positive Control

Product Data Sheet

For Research Use Only, Not for use in diagnostic procedures

## Rho-Kinase II / ROK alpha Positive Control

(Human, a.a. 11-552, recombinant protein expressed in Sf21)

Cat# CY-E1160-1

Lot No.

For 200 Assays

(0.02 units /  $\mu\text{L}$  x 100  $\mu\text{L}$ )

### Product Description:

Human Rho-kinase II, residues 11-552, containing N-terminal His tag, expressed in recombinant baculovirus infected sf21 cells. Purified by using Ni-NTA agarose chromatography. The Rho-kinase II Positive control is designed to use for CycLex Rho-kinase Assay Kit (Cat# CY-1160). The Rho-kinase II Positive Control should be added to the well at 10 m units/well. For instance, diluted positive control 1:20, use 10  $\mu\text{L}$  for 1 assay. Unused Rho-kinase II Positive control should be stored at  $-70^{\circ}\text{C}$ .

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**Product Size:** Recombinant Rho-kinase II: 2 units/100  $\mu\text{L}$

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

**Source:** Human Rho-kinase II, residues 11-552, containing N-terminal GST tag and C-terminal His tag, expressed in sf21 cells.

**Molecular Weight:** Rho-kinase II Positive Control demonstrates a single 77 kDa band by SDS-PAGE analysis.

**Purity:** Rho-kinase II Positive Control is greater than 75% pure as determined by SDS-PAGE analysis.

**Substrates:** Rho-kinase II phosphorylates a number of substrates, including MBS/MYPT1, LIMK2, CPI-17 and MLC<sub>20</sub>

**Inhibitors:** Y-27632 and HA-1077.

**Unit Definition:** One unit is defined as the amount of kinase required to incorporate 1nmol of phosphate into the GST-MBS/MYPT1 (654-880), per minute at  $30^{\circ}\text{C}$ .

**Assay Conditions:** Assay activity of Rho-kinase II in a 50  $\mu\text{L}$  reaction containing 20 mM Hepes KOH (pH 7.5), 5 mM  $\text{MgCl}_2$ , 1 mM DTT, 100  $\mu\text{M}$  [ $\gamma$   $^{32}\text{P}$ ] ATP (1  $\mu\text{Ci}$ ), and 4  $\mu\text{g}$  of GST-MBS/MYPT1 fusion protein. Start the reaction by adding 10 $\mu\text{L}$  of the enzyme, diluted 50-fold in a buffer containing 20 mM Hepes KOH (pH 7.5), 1 mM DTT, 0.03 % Brij35. Incubate for 30 minutes at  $30^{\circ}\text{C}$ . Terminate the reaction by adding 600  $\mu\text{L}$  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  $-70^{\circ}\text{C}$  from date of shipment. For maximum recovery



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of product, centrifuge the original vial after thawing and prior to removing the cap. Aliquot enzyme to avoid repeated freezing and thawing.

#### **Related Products:**

- \* Rho-kinase Assay Kit: Cat# CY-1160
- \* DMPK Positive control: Cat# CY-E1160-2
- \* Anti-phospho-MBS/MYPT1-Thr697 monoclonal antibody (AF-20): Cat# CY-M1011

#### **References:**

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2. Fujisawa, K., Fujita, A., Ishizaki, T., Saito, Y. and Narumiya, S. *J. Biol. Chem.* 271: 23022-23028, 1996.
3. Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K, Iwamatsu A, Kaibuchi K. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase) *Science*. 1996 Jul 12;273(5272):245-8.
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5. Nagumo H, Sasaki Y, Ono Y, Okamoto H, Seto M, Takuwa Y. Rho kinase inhibitor HA-1077 prevents Rho-mediated myosin phosphatase inhibition in smooth muscle cells. *Am. J. Physiol. Cell Physiol.* 2000 Jan;278(1):C57-65.
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