V 5.1

Rho Activator I
Shp-2 phosphatase mediated Rho activation
Cat. # CN01

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Background Information

The G-switch™ line of small G-protein tools has been developed with an emphasis on creating highly potent reagents that target endogenous Rho family proteins and pathways. In contrast to methods that rely on over-expression or knockdown of target proteins (e.g. DNA transfection of dominant negative or constitutively active Rho mutants, RNAi knockdown) , the G-switch™ reagents act rapidly on the endogenous target protein (in minutes to hours, depending on product), thereby optimizing the chance of generating a more physiologically relevant response. The G-switch™ product line includes reagents that directly and indirectly modulate Rho family signal transduction, thereby offering a wide range of mechanistic tools to study these critical cellular functions. See Cytoskeleton's web site for the latest G-switch™ information.

Rho Activator CN01 (calpeptin) activates RhoA, B and C in a variety of cell types (see Table 1). Rho activation is indirect via a mechanism involving inhibition of Shp-2 phosphatase (1). Inhibition of Shp-2 allows constitutive activation of Rho GEFs (1). CN01 also inhibits calpain-1 in a mechanism that is unrelated to Rho activation (2). This product is useful for studying upstream regulators of Rho and probing the mechanisms underlying Rho mediated events.

Material

Peptide sequence Z-Leu-Nle-CHO, mol wt 362.5. CAS number 117591-20-5. Supplied as lyophilized white solid, each vial contains 1 mg (10 units) of CN01. Purity ≥95% by HPLC. The material has been shown to be active in a biological assay for Rho activation (see below). One unit of CN01 is defined as the concentration, in units/ml, that is required to elicit a 2 fold activation of RhoA in Swiss 3T3 cells.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized powder can be stored desiccated at 4°C for 6 months. For reconstitution, briefly centrifuge to collect the product at the bottom of the tube and resuspend each vial in 50 µl of DMSO to give a stock concentration of 0.2 units/µl. Store the reconstituted product at -20°C for up to 6 months.

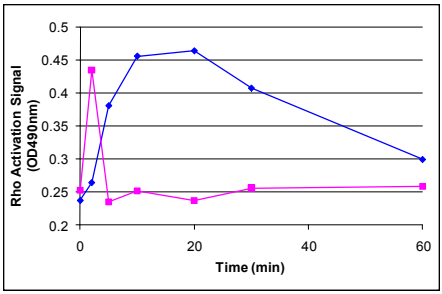
Biological Activity Assay

CN01 (1 unit/ml / equivalent to 5 µl/ml) was shown to induce a two fold RhoA activation (Fig. 1) and stress fiber induction (Fig. 2) in serum starved Swiss 3T3 cells after a 30 minute incubation at 37°C. Recommended conditions for Rho activation in several cell types are detailed in Table 1.

Activity Assay Method: Swiss 3T3 cell activation

1. Grow Swiss 3T3 cells at 37°C / 5% CO_2 to 30% confluency in two 10 cm^2 dishes containing 10 ml DMEM / 10% fetal bovine serum (FBS).
2. Serum starve cells by changing media to DMEM /1% FBS for 24h and then transferring to DMEM/0% FBS for 24h.
3. Briefly spin tube of CN01 to collect contents to the bottom of tube.
4. Reconstitute CN01 with 50 µl DMSO to give a stock solution of 0.2 units/µl.
5. Dilute contents of vial into 10 ml of serum free DMEM media to give a 1 unit/ml final CN01 working concentration (1 unit/ml is commonly used in many cell lines (see Table 1), however, optimal CN01 concentrations should be determined for any given cell line.
6. Aspirate serum free medium from both dishes of cultured cells and transfer CN01 containing media onto one dish.
7. The control dish should contain DMEM/1% DMSO and represents un-stimulated cells.
8. Incubate for 30 min at 37°C and 5-10% CO_2 (optimal CN01 incubation times should be determined for any given cell line, e.g. see Table 1). Assay Rho activity by G-LISA analysis (Cat # BK124; Fig.1) or cell morphology (Cat # BK005; Fig 2).

Figure 1 - CN01 Activation of RhoA in Swiss 3T3 cells



Legend: Cells were grown in DMEM plus 10% fetal bovine serum to 30% confluency, followed by 1% serum for 24h and 0% serum for 24h. CN01 was added at 1 unit/ml and LPA added at 1 µg/ml.Cells were harvested in G-LISA lysis buffer at each time point. RhoA activity was measured with the RhoA G-LISA Activation Assay (Cat. # BK124) and OD_{490nm}. LPA time course in magenta squares, and CN01 in blue diamonds. Note: The LPA signal is very short lived whereas the CN01 time course is broad.

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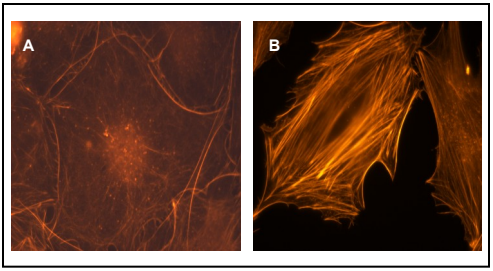
Figure 2 - CN01 Induction of stress fibers in Swiss 3T3 cells



Legend: Swiss 3T3 fibroblasts were plated on coverslips at 1000 cells / cm^2 and grown for three days in DMEM plus 10% fetal bovine serum at 37°C and 5% CO_2, Cells were then serum starved for 24h in DMEM/1% serum and 24h in DMEM/0% serum. Cultures were treated with 1 unit of CN01 per ml of medium for 30 min at 37°C. Cells were then fixed, stained with rhodamine-labeled phalloidin (Cat. # PHDR1 or BK005), and visualized by fluorescence microscopy. Images were taken at a magnification of 40x. The untreated control cells were treated with 5 ul DMSO per ml of medium. The cells treated with CN01 produced abundant stress fibers whereas control had less than 10% of CN01 levels of stress fibers (A and B respectively). Under similar conditions the activity of Rho increased by 2 fold as measured by the G-LISA® RhoA Activation Assay (Cat.# BK124; see Fig. 1).

Product Uses

- Activator for Rho pathway in many cell types (see Table 1).
- Study the effects of Rho activation on cell motility.
- Study the effects of Rho activation on the rearrangement of the actin cytoskeleton.
- Study of upstream regulators of Rho.
- Study the mechanisms underlying Rho mediated events.
- Investigate the effects of Rho activation with respect to cross talk with other signal transduction pathways.

Table 1 - CN01 Induction of Rho Activation in a variety of cell lines

Cell Type	Cell Line	Units/ml required for Rho Activation	Ref
Fibroblast	Swiss 3T3	1 unit/ml 30 min.	1
Fibroblast	REF-52	1 unit/ml 30 min.	1
Epithelial	MDCK	Not recommended.	3
Endothelial	HUVEC	1 unit/ml 30 min.	4

References

1. Schoenwaelder S.M. et al. 2000. The protein tyrosine phosphatase Shp-2 regulates RhoA activity. *Current Biol.* **10**, 1523-1526.
2. Schoenwaelder S.M. and Burridge K. 1999. Evidence for a calpeptin-sensitive protein-tyrosine phosphatase upstream of the small GTPase Rho. *J. Biol. Chem.* **274**, 14359-14367.
3. Gopalakrishnan S., et al. 2003. Differential regulation of junctional complex assembly in renal epithelial cell lines. *Am. J. Physiol. Cell Physiol.*, **285**, C102-C111.
4. Wittchen E.S. and Burridge K. 2008. Analysis of low molecular weight GTPase activity in endothelial cell cultures. *Meth. Enz.* **443**, 285-297.

Product Citations / Related Products

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