V 1.3

Rac/Cdc42 Activator II
EGF receptor mediated Rac/Cdc42 activation
Cat. # CN02

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.

Rac/Cdc42 Activator II, epidermal growth factor (EGF) acts through a tyrosine kinase receptor (EGFR) to rapidly stimulate actin reorganization at the cell membrane resulting in membrane ruffling. This primary morphological response, which occurs within 5-10 minutes in Swiss 3T3 cells, has been shown to be mediated through activation of Rac (1). Biochemical assays that quantitate the amount of active (GTP-bound) small G-proteins have demonstrated that both Rac and Cdc42 are rapidly activated (within 30 seconds–3 minutes) by EGF stimulation (2 and see Table 1). A later, secondary response to EGF treatment is the formation of actin stress fibers which is mediated through the activation of Rho (2). EGF is a useful tool in studying Rho signaling pathways, it should be noted, that EGF activates several other important signal transduction pathways including Ras/Raf/MAPK, JAK/STAT and PI3K/AKT and data should be interpreted accordingly.

Material

The Rac and Cdc42 Activator, epidermal growth factor (EGF) is a small polypeptide (approx. 6 KDa), isolated from murine submandibular glands. The product is negative for endotoxin contaminants and is >95% pure as determined by SDS-PAGE analysis, the CAS number is 62229-50-9. CN02 is supplied as a white lyophilized solid, each vial contains 10 units (1 µg) of EGF. One unit is defined as the concentration, in units/ml, that is required to elicit a 2 fold activation of Cdc42 in Swiss 3T3 cells (see biological assay section below).

The concentration of CN02 required for efficient activation of Rac and Cdc42 proteins can vary based on cell type (see Table 1) and should be determined by the end user. Typically the effective range is between 0.25 units/ml and 1.0 unit/ml for incubation in serum free medium. Incubation times between 0.5 to 10 min should be tested for each cell type. Recommended conditions for several cell types are detailed in Table 1.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized solid can be stored desiccated at 4°C for 12 months. For reconstitution, briefly centrifuge to collect the product at the bottom of the tube and resuspend each vial in 100 µl sterile phosphate buffered saline (PBS) pH 7.4 to yield a concentration of 0.1 unit/µl. After reconstitution, the product is in the following buffer; PBS, 5 mM PIPES, 0.5% sucrose, pH 7.4. Store the reconstituted product at –20°C for up to 6 months.

Biological Activity Assay

CN02 (1 unit/ml / equivalent to 10µl of CN02 stock/ml or 100ng/ml) was shown to induce a 2 fold activation of Cdc42 in serum starved Swiss 3T3 cells after a 3 minute incubation at 37°C (Figure 1) . Recommended conditions for Rac/Cdc42 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 18h and then transferring to DMEM/0% FBS for 24h.
3. Briefly spin tube of CN02 to collect contents to the bottom of tube.
4. Reconstitute CN02 with 100 µl ice cold sterile PBS.
5. Dilute contents of vial into 10 ml of serum free DMEM media to give a 1 unit/ml (100 ng/ml) final CN02 working concentration.
6. Aspirate serum free medium from both dishes of cultured cells and transfer CN02 containing media onto one dish.
7. The control dish should contain DMEM only and represents unstimulated cells.
8. Incubate for 3 minutes at 37°C and 5-10% CO_2 (optimal CN02 incubation times should be determined for any given cell line, e.g. see Figure 1 & Table 1). Assay Rac or Cdc42 activity by G-LISA analysis (Cat # BK128 [Rac] or Cat # BK127 [Cdc42] ; Figure 1) or cell morphology (Cat # BK005).

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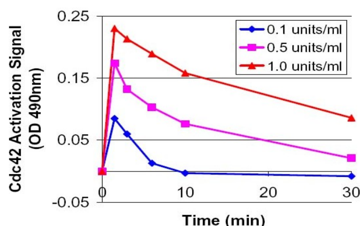
Table 1. Suggested Conditions for Cdc42/Rac activation in serum free medium

Cell Type	Cell Line	Units/ml required for >2X Rac Activation	Units/ml required for >2X Cdc42 Activation	Ref.
Glioblastoma	US7MG	0.5 units/ml for 5 min	Na	3
Epidermal	YAMC	1.0 unit/ml for 30 sec.	Na	4
Fibroblast	COS-7	Na	0.5 units/ml for 3-5 min	5
Epithelial	A431	0.25 units/ml for 2 min	0.25 units/ml for 2 min	6

Figure 1. **Time course of Cdc42 activation with CN02**



Legend: Swiss 3T3 cells were serum starved (SS) for 18 h at 1% serum and 24h with 0% serum and treated with CN02 (0.1, 0.5 and 1.0 units/ml for 1.5, 3.0, 6.0, 10 and 30 min). Cell lysates were subjected to the Cdc42 G-LISA® assay (Cat # BK127) and OD was read at 490 nm. The "controlled state" serum starved value (0.22) was subtracted from these samples prior to plotting. At 1.0 unit/ml (red line) Cdc42 activation was 2.0 fold higher than control, serum starved, sample at 3 min.

Product Uses

- Positive control for Rac and Cdc42 activation studies
- Study the effects of Rac and Cdc42 activation on cell motility
- Study the effects of Rac activation on the rearrangement of the actin cytoskeleton
- Investigate the effects of Rac and Cdc42 activation with respect to cross talk with other signal transduction pathways

References

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