MC3T3 Wnt TCF Reporter Cell Line-Active

A specialized producer of Wnt signaling products

Keep Frozen
Below – 80°C

Catalog Number
WRMC3T3A

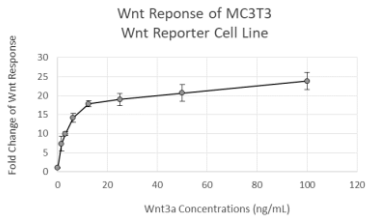
Source
Mouse bone/calvaria preosteoblast, MC3T3-E1 Subclone 4

Synonyms
Wnt reporter, TCF reporter, LEF reporter cell line

Background
The WNT gene family consists of structurally related genes that encode secreted signaling proteins, membrane bound receptors, and signaling transduction proteins. These proteins have been implicated in oncogenesis, adipogenesis, etc. and in several other developmental processes, including regulation of cell fate and patterning during embryogenesis. Activity of the Wnt signaling pathway leads to nuclear translocation of β-catenin and the formation of TCF transcription factor complex. The TCF complex interacts with Wnt gene transcriptional response elements and leads to the expression of Wnt-responsive genes.

Product Description
This Wnt reporter cell line is designed to monitor the activity of β-catenin-based Wnt signal transduction pathway. This mouse bone/calvaria preosteoblast cell line hosts CMV promoter, tandem repeats of the TCF transcriptional response element, luciferase gene, and GFP gene. GFP expressed constantly can serve as control of cell numbers. These cell lines are good models for studying in vitro osteoblast differentiation, particularly ECM signaling. They have behaviors similar to primary calvarial osteoblasts.

Activity
The luciferase activity from the Wnt reporter cell line increases 10-fold after 6-hour treatment with 5 ng/mL of recombinant mouse Wnt3a (Figure 1). The luciferase activity was normalized to endogenously expressed GFP.



Handling and Storage
The cell line may be shipped in dry ice or RT in either 25 cm^2 flask or 15 mL tube. If the cell line is shipped in dry ice, after receiving, store the cells at -80°C or in liquid nitrogen or culture under standard culture conditions.

Luciferase Assay

Using normal tissue culture-treated plate: Seed 0.5 mL of cells into each well of 24 or 48 wells plate at a density of 5 x 10^4 cells/mL in complete MEM Alpha Medium (Corning Ref: 10-022-CV plus 1 mM sodium pyruvate, 1500 mg/L sodium bicarbonate, Penicillin-Streptomycin (100 U/mL), and 10 % of fetal bovine serum), incubate cell at 5% CO_2, 37°C incubator overnight, replace complete MEM Alpha Medium with 198 µL MEM Alpha Medium without serum but with 0.1% BSA, add 2 µL of control buffer or Wnt3a (concentration range: 2 to 100 ng/mL), ,mix well and return plate into 5% CO_2, 37°C incubator and continue to incubate for 6 to 8 hours, suction out medium, lyse cells with 0.2 mL of cell lysis buffer (Promega, Cat: E1941), incubate for 10 min on rocking shaker at room temperature, transfer 50 µL of cell lysate from each well into the wells of a 96 well black plate and add 50 µL of lysis buffer into three wells of the same plate as fluorescent background, read fluorescent first, and then add 50 µL of Luciferase substrate (Promega, Cat: E2610) into each well, read Luciferase activity within 15 min. Fluorescent reading can serve as control of cell numbers.

Reference

Molenaar M. XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos. Cell. 1996; 86:391-9
Xing-Yao LI. A reporter gene system for screening inhibitors of Wnt signaling pathway. Nat. Prod. Bioprospect. 2013; 3: 24–28

Time Bioscience, LLC Address: 60 Prescott St. Worcester, MA 01605
Tel: 617-319-2278 Email: info@timebioscience.com