SW480 Wnt TCF Reporter Cell Line-Mutant

Keep Frozen Below – 80°C

Catalog Number: WRSW480M

Source: Human colorectal cancer cell line

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.

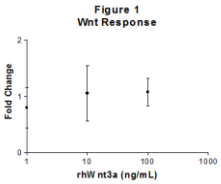
Most colorectal carcinomas harbor genetic alterations that result in stabilization accumulation of β-catenin. colorectal carcinoma cell line SW480 expresses a truncated form of adenomatous polyposis coli (APC) that is a key player in β-catenin destruction complex. The mutation results in accumulation of β-catenin and expression of the oncogenes regulated by canonical Wnt signaling

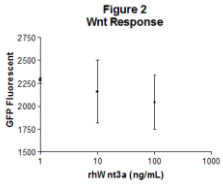
Product Description

Wnt reporter cell line is designed to monitor the activity of β-catenin-based Wnt signal transduction pathway. This human colorectal carcinoma cell line hosts CMV promoter, a mutant TCF transcriptional response element, luciferase gene, and GFP gene.

Activity:

This Wnt reporter cell line expresses low luciferase. The luciferase activity does not increase dramatically in response to Wnt3a stimulation at 100 ng/mL (Fig. 1). Endogenous GFP expression from this Wnt reporter cell line is shown in Figure 2.





Handling and Storage

The cell line may be shipped in dry ice or room temperature in either 25 cm^2 flask or 15 mL tube. If the cell line is shipped in dry ice, after receiving, store vials at -80°C or in Liquid Nitrogen or culture under standard culture conditions. The cells should be cultured in complete DMEM medium (Corning Catalog: 15-013-CV plus 2 mM L-glutamine, 10 % FBS, and 100 units/ml penicillin-streptomycin).

Luc Assay

Using normal tissue culture-treated plate: Seed 0.5 mL of cells into each well of 24 wells plate (0.25 mL for wells of 48 wells plate) at a density of 20 x 10^4 cells/mL in complete DMEM medium, incubate cell at 5% CO^2, 37°C incubator overnight. On second day, replace complete DMEM with 198 µL of DMEM without serum, add inhibitors into the culture, return plate back into the incubator and continue to incubate for 16 hours or overnight, 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.

Mycoplasma Detection

Negative. Detection Kit: Mycoplasma Detection Kit (PCR) from Southern biotech

Reference

Xing-Yao LI. A reporter gene system for screening inhibitors of Wnt signaling pathway. Nat. Prod. Bioprospect. 2013; 3: 24–28

Sparks AB. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. Cancer Res. 1998; 58(6): 1130-4

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