SW480 Wnt TCF Reporter Cell Line-Mutant

Catalog Number: AMS.WRSW480P

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.

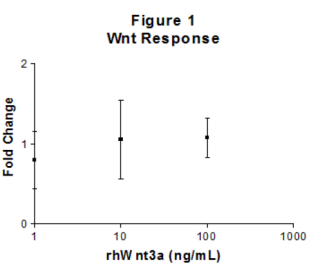
Most colorectal carcinomas harbor genetic alterations that result in stabilization accumulation of β-catenin. colorectal carcinoma cell line SW480 expresses a truncated form of adenomatous polyposis coli (APC) that is a key player in β-catenin destruction complex. The mutation results in accumulation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

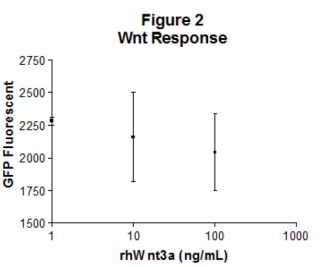
Product Description

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 RT in either 25cm2 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.

Luc Assay

Using normal tissue culture-treated plate: Seed 0.5 mL of cells into each well of 24 wells plate at a density of 20 x 10^4 cells/mL in complete Leibovitz's L-15 medium (ATCC Catalog No. 30-2008) incubate cells in 37°C incubator without CO_2 overnight. Replace complete medium with Wnt3a or inhibitors in Leibovitz's L-15

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without serum, return plate back into the incubator and continue to incubate for 16 hours, suction out medium, lyse cells with 0.2 mL of cell lysis buffer, incubate for 10 min on rocking shaker at room temperature, transfer 50 µL cell lysate from each well into the wells of a 96 well black plate, read fluorescent first, and then add 50 µL of Luciferase substrate into each well, read Luciferase activity within 30 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 Ll. 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

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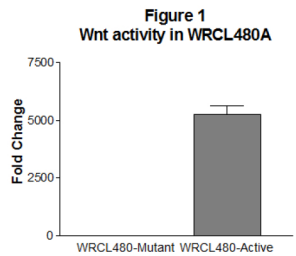
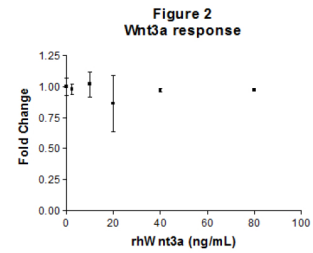
Product Description

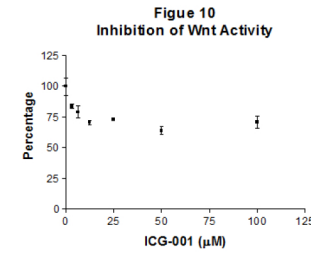
Wnt reporter cell line is designed to monitor the activity of β-catenin-based Wnt signal transduction pathway. This human colorectal carcinoma cell line hosts the TCF transcriptional response element, luciferase gene, and GFP gene. Since this carcinoma cell lines harbors a truncated form of adenomatous polyposis coli (APC) and an accumulation of β-catenin, the cells have high endogenous Wnt signaling and the Wnt signaling can not be stimulated further. GFP expressed constantly can serve as control of cell numbers.

Activity:

This Wnt reporter cell line expresses 5000-fold higher luciferase activity than the control cell line-WRCL480M (Fig. 1). RhWnt3a is no longer able to activate the Wnt signaling even at a concentration upto 80 ng/mL (Fig. 2). A fraction of endogenous Wnt signaling can be inhibited by ICG-001 with an IC_{50} of 3 µM (Figs. 3). The inhibition data were processed by setting the luciferase activity from SW480 Wnt reporter cell line-Mutant (Cat: WRSW480M) as 0 and without inhibitor as 100%.







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