

HCT116 Wnt TCF Reporter Cell Line-Active

**Keep Frozen
Below – 80°C**

Catalog Number: WRHCT116A
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 of β -catenin. colorectal carcinoma cell line HCT116 harbors both wild-type and mutated (deletion of codon 45) β -catenin genes.

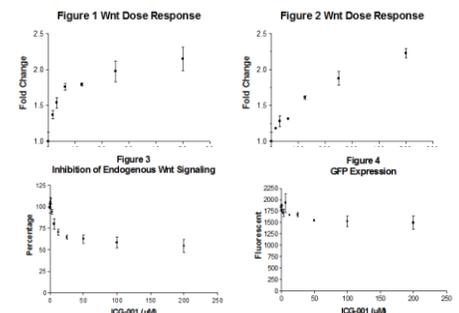
Product Description

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 both wild-type and mutated β -catenin genes, the cells have high endogenous Wnt signaling but can be stimulated further. GFP expressed constantly can serve as control of cell numbers.

Activity:

The luciferase activity from the Wnt reporter cell line increases 2 fold after 6- to 8-hour treatment with 50 ng/mL of recombinant mouse Wnt3a (Fig. 1) or 2 fold when 500 ng/mL of recombinant human Wnt3a was used (Fig. 2).

Half of endogenous Wnt signaling can be inhibited by ICG-001 with an IC_{50} of 3 μ M (Figs. 3). Endogenous GFP expression from this Wnt reporter cell line is shown in Figure 4. The inhibition data were processed by setting the luciferase activity from HCT Wnt reporter cell line-Mutant (Catalog: WRHCT116M) as 0 and without inhibitor as 100%.



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 the cells 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 20×10^4 cells/mL in complete McMoy's 5A medium (Corning Catalog No. 10-050-CV plus 2200 mg/L sodium bicarbonate) incubate cell at 5% CO₂, 37°C incubator overnight, replace complete medium with 198 μ L McMoy's 5A without serum, add 2 μ L of control buffer or Wnt3a (concentration range: 5 to 100 ng/mL), mix well and return plate into 5% CO₂, 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 μ L of cell lysate from each well into the wells of a 96 well black plate and add 50 μ L of lysis buffer into three wells of the same plate as fluorescent background, read fluorescent first, and then add 50 μ L 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