HCT116 Wnt TCF Reporter Cell Line-Mutant

Keep Frozen
Below – 80°C

Catalog Number: WRHCT116M

Source: Human colorectal cancer cell line

Synonyms: Wnt reporter, TCF reporter, LEF reporter cell line

Background

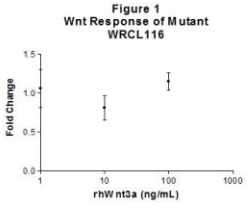
The WNT gene family consists of structurally related genes that encode secreted signaling proteins, membrane bound receptors, and signaling transduction proteins. These proteins have been implicated in oncogenesis, adipogenesis, etc. and in several other developmental processes, including regulation 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.

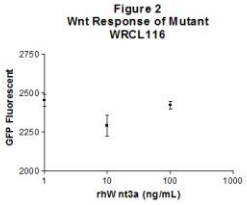
Product Description

This Wnt reporter cell line is a control cell line, designed to show background luciferase activity for HCT116 Wnt TCF Reporter Cell Line-Active (Cat: WRHCT116A). This human colorectal carcinoma cell line hosts CMV promoter, a mutant TCF transcriptional response element, luciferase gene, and GFP gene. GFP expressed constantly can serve as control of cell numbers

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. The cells should be cultured in complete McMoy's 5A medium (Corning Catalog No. 10-050-CV plus 100 U/mL of Penicillin-Streptomycin and 10 % of fetal bovine serum).

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 McMoy's 5A medium incubate cell at 5% CO_2, 37°C incubator overnight, replace the complete medium with 198 µL McMoy's 5A without serum, add 2 µL of control buffer or Wnt3a (concentration range: 0.06 to 0.5 µg/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
Sparks AB. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. Cancer Res. 1998; 58(6): 1130-4

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85