

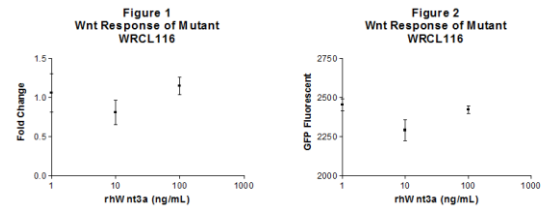
**Keep Frozen
Below – 80°C**

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 10⁴ cells/mL in complete McMoy's 5A medium incubate cell at 5% CO₂, 37°C incubator overnight, replace the complete medium with 198 μ L McMoy's 5A without serum, add 2 μ L of control buffer or Wnt3a (concentration range: 0.06 to 0.5 μ g/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.

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