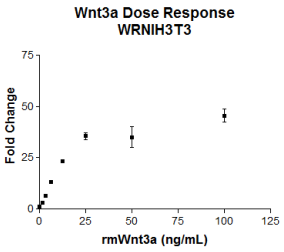
NIH3T3 Wnt Reporter Cell Line

Keep Frozen
Below – 80°C

Catalog Number	WRNIH3T3A
Source	Mouse fibroblast 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.
Product Description	This Wnt reporter cell line is designed to monitor the activity of β-catenin-based Wnt signal transduction pathway. This mouse fibroblast cell line hosts CMV promoter, tandem repeats of the TCF transcriptional response element, and luciferase gene.
Activity	The luciferase activity from the Wnt reporter cell line increases 25-fold after 8 hours treatment with 10 ng/mL of recombinant mouse Wnt3a.



Handling and Storage	The cell line may be shipped in dry ice or RT in either 25 cm^2 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. 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 plate: Seed 0.5 mL of cells into each well of 24 wells plate (tissue culture-treated) at a density of 10 x 10^4 cells/mL in complete DMEM medium, incubate cell at 5% CO_2, 37°C incubator overnight, replace the complete DMEM with 198 µL DMEM plus 0.1 % BSA but without serum, add 2 µL of control buffer or Wnt3a (concentration range: 1 to 100 ng/mL), return plate into 5% CO_2, 37°C incubator and 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.
Mycoplasma Detection	Negative. Detection Kit: Mycoplasma Detection Kit (PCR) from Southern biotech
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

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