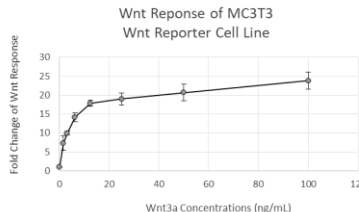


MC3T3 Wnt TCF Reporter Cell Line-Active

**Keep Frozen
Below – 80°C**

Catalog Number	WRMC3T3A
Source	Mouse bone/calvaria preosteoblast, MC3T3-E1 Subclone 4
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 bone/calvaria preosteoblast cell line hosts CMV promoter, tandem repeats of the TCF transcriptional response element, luciferase gene, and GFP gene. GFP expressed constantly can serve as control of cell numbers. These cell lines are good models for studying in vitro osteoblast differentiation, particularly ECM signaling. They have behaviors similar to primary calvarial osteoblasts.
Activity	<p>The luciferase activity from the Wnt reporter cell line increases after 6-hour treatment with 5 ng/mL of recombinant mouse (Figure 1). The luciferase activity was normalized to endogenously expressed GFP.</p> <div style="display: flex; align-items: center; justify-content: space-between;"> <div style="flex: 1;">  <p style="text-align: center;">Wnt Response of MC3T3 Wnt Reporter Cell Line</p> </div> <div style="flex: 0 0 100px; text-align: right;"> <p>10-fold Wnt3a</p> </div> </div>
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 MEM Alpha Medium (Corning Ref: 10-022-CV plus Penicillin-Streptomycin (100 U/mL), and 10 % of fetal bovine serum).
Luciferase Assay	Using normal tissue culture-treated plate: Seed 0.5 mL of cells into each well of 24 or 48 wells plate at a density of 5 x 10 ⁴ cells/mL in complete MEM Alpha Medium, incubate cell at 5% CO ₂ , 37°C incubator overnight, replace complete MEM Alpha Medium with 198 μ L MEM Alpha Medium without serum but with 0.1% BSA, add 2 μ L of control buffer or Wnt3a (concentration range: 2 to 100 ng/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 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	<p>Molenaar M. XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos. Cell. 1996; 86:391-9</p> <p>Xing-Yao Li. A reporter gene system for screening inhibitors of Wnt signaling pathway. Nat. Prod. Bioprospect. 2013; 3: 24–28</p>