HEK293 Wnt TCF Reporter Cell Line-Active

Catalog Number	WRHEK293A-HEW
Clone Number	2A9E8E8E
Source	Human embryonic kidney 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 human embryonic kidney 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.
Activity	The luciferase activity from the Wnt reporter cell line increases 2 fold after 6- to 8-hour treatment with 10 ng/mL of recombinant mouse Wnt3a (Fig. 1) or 8 fold when 500 ng/mL of recombinant human Wnt3a was used (Fig. 2).

Figure 1 rmWnt3a Response

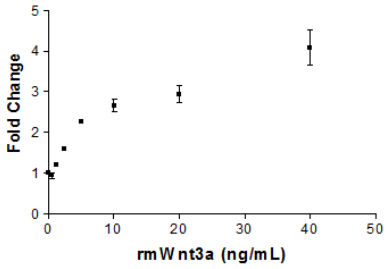
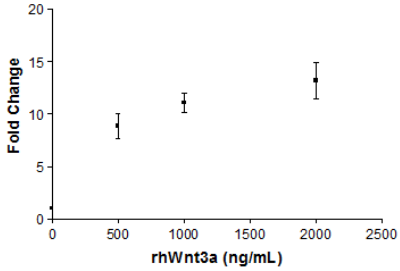


Figure 2 rhWnt3a Dose Response



Both response to Wnt3a and endogenous Wnt signaling can be inhibited by ICG-001 with an IC_{50} of 3 μM (Figs. 3 and 5). Endogenous GFP expression from this Wnt reporter cell line is shown in Figure 4 and 6. The inhibition data were processed by setting the luciferase activity from HEK293 Wnt reporter cell line-Mutant (Catalog: WRHEK293M) as 0 and without inhibitor but with Wnt3a as 100%.

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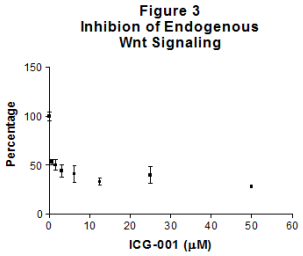

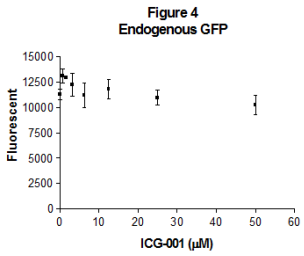

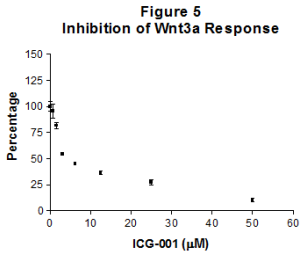

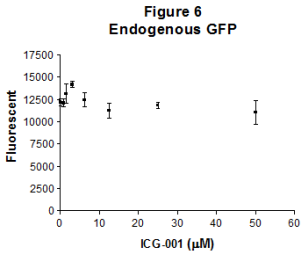

Handling and Storage

The cell line may be shipped in dry ice or RT in either 25cm^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.

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 10 x 10^4 cells/mL in complete EMEM medium (Corning Ref: 10-010-CV plus 1 mM sodium pyruvate and 1500 mg/L sodium bicarbonate), incubate cell at 5% CO_2, 37°C incubator overnight, replace complete EMEM with 198 µL EMEM without serum, add 2 µL of control buffer or Wnt3a (concentration range: 0.06 to 1 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, incubate for 5 to 10 min on rocking shaker at room temperature, transfer 50 µL cell lysate from each well into the wells of a 96 well black plate, read fluorescent first, and then add 50 µL of Luciferase substrate into each well, read Luciferase activity within 30 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