Product Manual

LEADING LIGHT® Wnt Reporter Assay

For detection of canonical Wnt signaling in microplates
1x96 wells (with option for 1x384 wells)

Instruction Manual for:	Product No.
LEADING LIGHT® Wnt Reporter Assay Starter Kit	ENZ-61001-0001
LEADING LIGHT® Wnt Reporter Cell Line	ENZ-61002-0001
LEADING LIGHT® Wnt Reporter Cell Line Medium Pack	ENZ-60003-0001
LEADING LIGHT® Wnt Reporter Cell Line Assay Reagents	ENZ-60004-0001

NOTE: *This version contains a change to clarification on kit component for proteins included.*

For the latest product information, including support documentation, visit us online:
www.enzolifesciences.com

Technical Support (US): 800-942-0430
Technical Support (EU): +41 61 926 8989

USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research use only. Not for use in diagnostic procedures. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON-INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo and LEADING LIGHT® are trademarks of Enzo Life Sciences, Inc.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

TABLE OF CONTENTS

INTRODUCTION

Wnt ligands bind to Frizzled (Fz) and LRP5/6 receptors to trigger a signaling cascade that leads to stabilization of beta-catenin, which can enter into the nucleus to form a complex with T cell transcription factor (TCF/LEF) to activate Wnt target gene expression.[1] Canonical Wnt signaling is required for embryo-genesis and adult tissue maintenance and is involved in tumorigenesis and development of many human degenerative diseases.[2-6] Studies relating to Wnt signaling have advanced research in molecular embryology, stem cell biology, tumorigenesis, regenerative medicine, and rational drug discovery.[6,7]

The LEADING LIGHT® Wnt Reporter Assay is a cell-based luciferase activity test suitable for a 96-well or 384-well plate format. The system contains an engineered 3T3 mouse fibroblast cell line, which expresses the firefly luciferase reporter gene under the control of Wnt-responsive promoters (TCF/LEF). The luciferase activity from the reporter gene in this cell line can be up-regulated in a dose-dependent manner upon the addition of exogenous Wnt protein/Wnt agonist or down-regulated by a further addition of a Wnt antagonist to the cell culture medium. This system can be used to elucidate the functions/activities of different Wnt-related ligands such as Wnt, Dkk, etc. This system can also be used for screening small molecules and antibodies for their ability to act as Wnt inhibitors or Wnt agonists.

The assay described in this manual has been used successfully in different assay formats (including HTS applications) to identify several distinct categories of small molecule compounds that modulate the Wnt signaling pathway.[8,9]

The LEADING LIGHT® Wnt Reporter cell line included in this kit offers the following enhanced features in comparison with other commercially available cell lines:

- High sensitivity
- Stronger response to Wnt3a
- Excellent reproducibility
- High signal-to-noise ratio
- No lithium chloride required to enhance the signal
- Wnt pathway is intact, allowing investigation of the complete pathway

Wnt PATHWAY SCHEMATIC

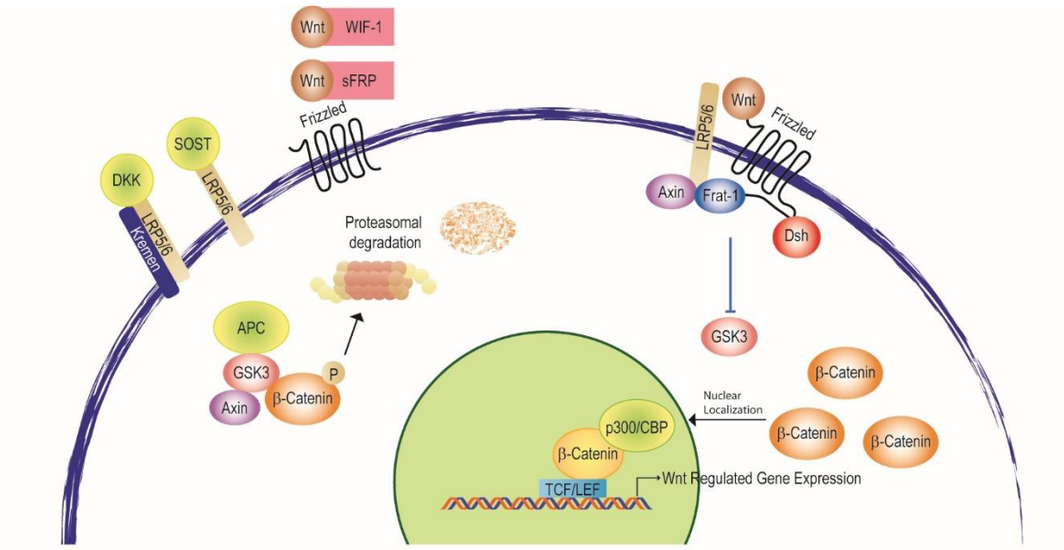

Figure 1: Schematic of the canonical Wnt signaling pathway and its components. Luciferase activity from the reporter gene in the LEADING LIGHT® Wnt reporter cell line can be up- or down-regulated in a dose-dependent manner upon the addition of exogenous Wnt protein/Wnt agonist or Wnt antagonist (Dkk) to the cell culture medium.

SAFETY WARNINGS AND PRECAUTIONS

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Handle with care

- Some components of this kit may contain hazardous substances. Reagents can be harmful if ingested or absorbed through the skin and may cause irritation to the eyes. Reagents should be treated as possible mutagens and should be handled with care and disposed of properly.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

STORAGE

Avoid freeze / thaw cycles

All reagents are shipped on dry ice. **Immediately upon receipt, store the vial of Wnt Reporter Cell Line in liquid nitrogen.** Alternatively, the Wnt Reporter Cell line may be cultured upon receipt. Store the remaining reagents at -80°C. When stored properly, these reagents are stable for 1 year upon receipt.

Avoid repeated freezing and thawing. Wnt3a Reagent is particularly susceptible to adverse reactions during Freeze Thaw. Please see the table below to determine proper storage conditions of Wnt-3a protein based on laboratory needs.

Wnt3a Stability Avoid Repeated Freezing and Thawing		
Form	**Temperature**	**Duration of Storage**
Lyophilized	-20ºC to -80ºC	1 year
Diluted	-20ºC to -80ºC	3 months
Diluted	2ºC to 8ºC	1 week

LEADING LIGHT® Wnt REPORTER ASSAY COMPONENTS

The reagents provided in the kit are sufficient for one 96-well or one 384-well microplate experiment.

Reagent	Quantity	Part No.	Included in Starter Pack
LEADING LIGHT® Wnt Reporter Cell line	1 mL 2 x 10^6 cells	ENZ-61002-0001	Yes
LEADING LIGHT® Wnt Reporter Cell Line Media Pack		ENZ-60003-0001	Yes
Growth Medium Concentrate	60 mL	80-2592	
Assay Medium Concentrate	4.5 mL	80-2582	
Freezing Medium	10 mL	80-2583	
LEADING LIGHT® Wnt Reporter Cell Line Assay Reagents		ENZ-60004-0001	Yes
Wnt-3a (mouse) protein	1 µg	61001-WNT-0001	
DKK-1 (mouse) protein	500 ng	61001-DKK1	
Luciferase Assay System	1 pack	80-2593	
2M Lithium Chloride (LiCl)	0.5 mL	WNT-LICL-0500	
1M HEPES	0.5 mL	80-2595	
96-well, Black, Clear Bottom, Tissue Culture Plate	1 each	80-2594	
Wnt-3a (mouse) protein*	1 µg	ENZ-60001-C001	Sold Separately
DKK-1 (mouse) protein	5 µg	ENZ-60002-C005	Sold Separately

***Any Wnt-3a (mouse) protein sold separately, or purchased from another vendor, will require optimization by the end user for desired results.**

ADDITIONAL MATERIALS REQUIRED

EQUIPMENT

- Microplate reader suitable for chemiluminescence detection
- Centrifuge
- Liquid nitrogen dewar and -80°C freezer
- Humidified 37°C/5% CO_2 incubator
- Multi-dispensing automated pipette, capable of dispensing 20 µL, 40 µL, 50 µL and 100 µL

CONSUMABLES

- Sterile vials and tubes for working solutions and cell manipulation
- 100mm tissue culture plates
- 384-well, clear bottom, black-walled tissue culture plates (optional)

MEDIA/REAGENTS

Media/Reagent	Recommended Source	Part #
DMEM (Dulbecco's modified Eagle medium, without phenol red, containing 4.5g/L glucose and 4mM L-glutamine)	Sigma	D1145*
	Cellgro	17-205-CV*
**L-glutamine must be added by the user*		
Phosphate Buffered Saline (PBS), sterile	Life Technologies	10010-049
Pen-Strep (Penicillin-Streptomycin)	Gibco	15140
0.05% Trypsin/EDTA	Gibco	25300

METHODS AND PROCEDURES

NOTE: *Unless otherwise stated, all media and solutions should be pre-warmed to 37°C for optimal performance before adding them to the cells.*

Wnt Reporter Cell Line Growth and Maintenance

Growth Medium Concentrate Dilution:

1. Add the contents of the Growth Medium Concentrate (60 mL) and 5 mL of Pen-Strep into 435 mL DMEM.
2. Mix well.
3. Store at 4°C. This solution must be used within one month.

Thawing of cells

1. Add 9 mL of 1X Growth Medium to a sterile 15 mL sterile conical tube.
2. Thaw the LEADING LIGHT® Wnt Reporter Cell Line in a 37°C water bath with gentle agitation.
3. Once thawed, add the cells to the tube containing Growth Medium.


Centrifuge

4. Centrifuge the cell suspension at 200 x *g* for 5 min.
5. Aspirate the supernatant and resuspend the cells in 10 mL Growth Medium.

Subculturing cells

NOTE: *It is recommended to passage the cells at least twice after thawing before using them in the luciferase assay. Cells should be passed every three days. Never allow the cells to exceed 60% confluency.*

1. Transfer the 10 mL of cells in Growth Medium after thawing to a 100mm tissue culture plate.
2. Allow the cells to grow to ~60% confluency in a humidified 37°C/5% CO_2 incubator.
3. Remove the growth medium and then add 1.5 mL of 0.05%Trypsin/EDTA to the plate. Incubate in a 37°C incubator until the cells become detached (usually requires 1-3 min). Add 10 mL Growth Medium to the plate.

4. Split the cells 1:4 or greater into 100mm tissue culture plates. Resuspend cells by adding an appropriate amount of Growth Medium to each plate to a final volume of 10-12 mL. It is recommended to seed 0.2 to 1 million cells per 100mm plate. Allow the cells to grow as before. Maintain cell confluency between 10-60%.

Freezing of cells


Centrifuge

1. Harvest the cells by removing the Growth Medium and adding 1.5 mL of 0.05% Trypsin/EDTA to the Plate. Incubate in a 37ºC incubator until the cells become detached (usually requires 1-3 min). Add 10 mL Growth Medium to the plate.
2. After detachment, count the cells. Centrifuge the cells down at 200 x *g* for 5 min, aspirate the supernatant and then resuspend the cell pellet at a concentration of 2 x 10^6 cells/mL in Freezing Medium held at 4°C.
3. Aliquot 1.0 mL of cell suspension per cryogenic vial. Store overnight at -80°C in an insulated container for slow cooling.
4. Place frozen vials of cells into a liquid nitrogen storage the next day for long term storage.

NOTE: *It is recommended to freeze the cells during early passages. The reporter cell line may lose its ability to respond to Wnt induction after reaching a high number of passages.*

Wnt Reporter Luciferase Assay

Quick Reference Guides – 96-well Assay

LUCIFERASE ASSAY FOR WNT AGONIST ACTIVITY 96-Well Assay			
	Test Agonist Wells	**Positive Control Wells**	**Background Control Wells**
Step 1 **96-well Plating Cells**	50 µL cells in Assay Medium (25,000-30,000 cells/well) *See Page 11 for preparation of medium*		
Step 2 **Incubation**	Incubate the plates overnight in humidified 37°C/5% CO_2 incubator.		
Step 3 **Adding Test Agonist**	100 µL Test Agonist in DMEM w/ 25mM HEPES	100 µL Wnt3a in DMEM w/ 25mM HEPES *plus* Test Agonist Solvent	100 µL DMEM w/25mM HEPES *plus* Test Agonist Solvent
Step 4 **Incubation**	Incubate the plates overnight at 37°C/5% CO_2 incubator.		
Step 5 **Preparation of Luciferase Assay System**	Luciferase Substrate (1 Bottle Lyophilized) + 1 Bottle Luciferase Assay Buffer		
Step 6 **Loading of Luciferase Substrate**	Invert plate and lightly blot to remove media – Add 100 µL per well of substrate mixture and incubate 10 min at room temperature.		
Step 7 **Detection**	Read chemiluminescence signal.		
Step 8 **Data Analysis**	See **Page 17**		

LUCIFERASE ASSAY FOR MODULATORS OF WNT ANTAGONIST 96-well Assay				
	Test Antagonist Wells	**Control Antagonist (DKK-1) Wells**	**Control Agonist Wells**	**Background Control Wells**
Step 1 **Plating Cells**	50µL cells in Assay Medium (25,000-30,000 cells/well) *See Page 11 for preparation of medium*			
Step 2 **Incubation**	Incubate the plates overnight in humidified 37°C/5% CO_2 incubator.			
Step 3 **Adding Test Agent**	100 µL Test Antagonist in DMEM w/25mM HEPES containing Wnt3a	100 µL Dkk-1 in DMEM w/25mM HEPES containing Wnt3a *plus* Test Antagonist Solvent	100 µL DMEM w/25mM HEPES containing Wnt3a *plus* Test Antagonist Solvent	100 µL DMEM w/25mM HEPES *plus* Test Antagonist Solvent
Step 4 **Incubation**	Incubate the plates overnight at 37°C/5% CO_2 incubator.			
Step 5 **Preparation of Luciferase Assay System**	Luciferase Substrate (1 Bottle Lyophilized) + 1 Bottle Luciferase Assay Buffer			
Step 6 **Loading of Luciferase Substrate**	Invert plate an lightly blot to remove media – Add 100 µL per well of substrate mixture and incubate 10 min at room temperature.			
Step 7 **Detection**	Read chemiluminescence signal.			
Step 8 **Data Analysis**	See **Page 17**			

LUCIFERASE ASSAY FOR WNT AGONIST ACTIVITY 384-Well Assay			
	Test Agonist Wells	**Positive Control Wells**	**Background Control Wells**
Step 1 **96-well Plating Cells**	25µL cells in Assay Medium (8,000-10,000 cells/well) *See Page 11 for preparation of medium*		
Step 2 **Incubation**	Incubate the plates overnight in humidified 37°C/5% CO_2 incubator.		
Step 3 **Adding Test Agonist**	25 µL Test Agonist in DMEM w/ 25mM HEPES	25 µL Wnt3a in DMEM w/ 25mM HEPES *plus* Test Agonist Solvent	25 µL DMEM w/25mM HEPES *plus* Test Agonist Solvent
Step 4 **Incubation**	Incubate the plates overnight at 37°C/5% CO_2 incubator.		
Step 5 **Preparation of Luciferase Assay System**	Luciferase Substrate (1 Bottle Lyophilized) + 1 Bottle Luciferase Assay Buffer		
Step 6 **Loading of Luciferase Substrate**	Invert plate and lightly blot to remove media – Add 20 µL per well of substrate mixture and incubate 10 min at room temperature.		
Step 7 **Detection**	Read chemiluminescence signal.		
Step 8 **Data Analysis**	See **Page 17**		

Quick Reference Guides – 384-well Assay

LUCIFERASE ASSAY FOR MODULATORS OF WNT ANTAGONIST 384-well Assay				
	Test Antagonist Wells	**Control Antagonist (DKK-1) Wells**	**Control Agonist Wells**	**Background Control Wells**
Step 1 **Plating Cells**	25 µL cells in Assay Medium (8,000-10,000 cells/well) *See Page 11 for preparation of medium*			
Step 2 **Incubation**	Incubate the plates overnight in humidified 37°C/5% CO_2 incubator.			
Step 3 **Adding Test Agent**	25 µL Test Antagonist in DMEM w/25mM HEPES containing Wnt3a	25 µL Dkk-1 in DMEM w/25mM HEPES containing Wnt3a *plus* Test Antagonist Solvent	25 µL DMEM w/25mM HEPES containing Wnt3a *plus* Test Antagonist Solvent	25 µL DMEM w/25mM HEPES *plus* Test Antagonist Solvent
Step 4 **Incubation**	Incubate the plates overnight at 37°C/5% CO_2 incubator.			
Step 5 **Preparation of Luciferase Assay System**	Luciferase Substrate (1 Bottle Lyophilized) + 1 Bottle Luciferase Assay Buffer			
Step 6 **Loading of Luciferase Substrate**	Invert plate an lightly blot to remove media – Add 20 µL per well of substrate mixture incubate 10 min at room temperature.			
Step 7 **Detection**	Read chemiluminescence signal.			
Step 8 **Data Analysis**	See **Page 17**			

***NOTE**: Certain solvents may affect assay performance .The effect of a test agent solvent (e.g. DMSO) in the assays described below should be assessed.*

The following instructions provide a detailed procedure for monitoring the Wnt/β-catenin signaling pathway by measuring luciferase activity.

Assay Medium Preparation

Add the contents of the Assay Medium Concentrate (4.5 mL) and 0.5 mL of Pen-Strep into 95 mL DMEM. Mix well. Store at 4°C. This solution must be used within one month.


Centrifuge

Assay Preparation

1. The day before the assay, remove the Growth Medium from plates, and then rinse the cells with 10 mL PBS. Aspirate the PBS and then add 1.5 mL 0.05%Trypsin/ EDTA to detach the cells. After the cells are detached, resuspend the cells in 10 mL of Assay Medium (as described above). Then transfer the cells into a sterile 15 mL conical tube. Spin cells down at 200 x g for 5 min.

   NOTE: *Only* ***Assay Medium*** *or* ***Assay Diluent*** *(not Growth Medium) should be used for manipulations with cells during assay. Failure to use appropriate media will result in poor assay performance.*

2. Discard the supernatant and then resuspend the cells in 10 mL PBS and spin cells down at 200 x g.
3. Resuspend cells in 10 mL PBS.
4. Count the cells. Centrifuge the cells at 200 x g for 5 min.
5. Carefully discard the supernatant, resuspend the cell pellet in an appropriate amount of pre-warmed Assay Medium to obtain a cell density of 5-6 x 10^5/mL.
6. Seed cells into a 96-well plate at 25-30 x 10^3 cells/50 µL/well or 384-well plate at 8-10 x 10^3 cells/25 µL/well. Incubate overnight in a humidified 37°C/5% CO_2 incubator.

   NOTE: *This cell density is necessary for assay sensitivity. Please follow the recommendation above.*

Assay Diluent Preparation

The following diluent should be used for all agonist, antagonist and control (i.e. Wnt3a, DKK1) dilutions for addition to 96-well or 384-well assay plates.

Prepare Assay Diluent by adding 1M HEPES to DMEM to a final concentration of 25 mM. To 10 mL DMEM add 0.250 mL 1M HEPES (supplied).

Preparation of Wnt3a Protein Stock Solution (61001-WNT-0001 and ENZ-60001-C001)

NOTE: *Follow storage recommendations for Wnt3a detailed on Page 4 and avoid freeze thaw cycles.*

Wnt3a protein is supplied lyophilized. Prepare 5 µg/mL stock solution of Wnt3a by adding 200 µL deionized Water into the vial of lyophilized Wnt3a. Unused stock solution of Wnt3a should be aliquoted and stored at -80°C for increased longevity.

Preparation of Wnt-3a Protein Working Solution (Positive Control)

Immediately prior to the assay, dilute only the required amount of Wnt3a protein. Do not store diluted Wnt3a protein since it will lose activity when stored for extended periods of time in the diluent.

Dilute Wnt3a protein stock solution (5 µg/mL) into Assay Diluent. The working concentration of Wnt3a protein should be adjusted for each particular experimental system, and if using Wnt3a protein purchased as a stand-alone item (sold separately). For use as control in the assays, add the same amount of solvent as contained in the test agents. The recommended working stock concentration range of Wnt3a protein is 150-500 ng/mL. Once added into wells containing cells with Assay Medium, these concentration equate to 100-333 ng/mL **in well.** See **Figure 2**.

Luciferase Assay for Wnt (or Wnt agonist) Activity

This assay can be used to screen compounds that directly regulate the Wnt signaling pathway.

1. Prepare the controls as follows:

   Wnt positive control - use Wnt3a protein in Assay Diluent that contains the same amount of solvent as in the test agonist. (See above for recommended Wnt3a dilution concentrations)

   Background Control - use Assay Diluent that contains the same amount of solvent as in the test agonist.

2. Prepare the test agonist as follows:

   Dilute the test agonist in Assay Diluent to appropriate concentrations to make a 1.5X solution.

3. Add 100 µL (96-well plate) or 25 µL (384-well plate) of each prepared test agonist and controls into plate wells that contain cells and Assay Medium. It is recommended to run the tests in triplicate.

4. Incubate the plates for at least 12 hr. The incubation time can

be extended up to 20 hr.

Immediately prior to beginning the assay, prepare the **Luciferase Assay System** as follows:

4.1 Allow the Luciferase Assay System components to equilibrate to room temperature.

4.2 Add the contents of the bottle of Luciferase Assay Buffer into the bottle of Luciferase Substrate and mix well.

NOTE: *Any remaining Luciferase Substrate Mixture can be aliquoted and stored at -80°C for future use. Avoid freeze thaw cycles of Luciferase Substrate Mixture.*

4.3 After incubation remove media from wells by gently inverting the plate and gently blotting on lint free absorbent towel.

4.4 Immediately add Luciferase Substrate Mixture into each well at 100 µL/well (96-well plate) or 20 µL/well (384-well plate).


Microplate

4.5 Allow plate to incubate for 10 min after adding the Luciferase Substrate Mixture and then read the signal in each well using a chemiluminescence microplate reader.

NOTE: *Luciferase Substrate signal starts to degrade ~ 25 min after addition to the plate. Reading the plate signal after this time period will lead to decrease in signal intensity and increase in variation.*

Preparation of Dkk-1 Protein (Wnt3a antagonist) Stock Solution

Dkk-1 protein is supplied lyophilized. Prepare 5µg/ mL stock solution of Dkk-1 by adding 100 µL deionized water into the vial of lyophilized Dkk-1. Unused stock solution of Dkk-1 should be aliquoted and stored at -80°C.

Luciferase Assay for Modulators of Wnt Antagonist

The test described below can be used to screen for modulators of Wnt antagonists. **Dkk-1** is one of the most well-known Wnt antagonists and is used here as an example for screening.

1. Prepare the controls as follows:

   For **Wnt antagonist control**, prepare Dkk-1 at 1.5X working solution by diluting the Dkk-1 protein stock solution (5 µg/mL) in Assay Diluent containing 150-500 ng/mL Wnt3a protein and the same amount of solvent as in the test antagonist. The working concentration of Wnt3a protein should be adjusted for each test system (a suggested starting point is 100-333ng/ mL final **in-well** concentration). The working concentration of Dkk-1 should be adjusted for each particular experimental system. The recommended working concentration range of Dkk-1 protein is 150-500 ng/mL with a final **in-well** concentration of 100-333 ng/mL (see Fig. 3). Discard any unused Dkk-1/Wnt3a diluted into Assay Diluent.

   For **Wnt positive control**, use Assay Diluent that contains Wnt3a protein and the same amount of solvent as in the test antagonist.

   For **background control**, use Assay Diluent that contains the same amount of solvent as in the test antagonist.

2. Prepare the test antagonist as follows:

   Dilute the test agents in Assay Diluent that contains only Wnt3a protein. Use the same Wnt3a protein concentration as that of the positive control (see above).

3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of each prepared test agent and controls into wells of the plate that contain cells and Assay Medium (see Page 10). It is recommended to run the tests in triplicate.

4. Incubate the plate for at least 12 hr. The incubation time can be extended up to 20 hr.

5. Immediately prior to beginning the assay, prepare the **Luciferase Substrate Mixture** as follows:

   5.1 Allow the Luciferase Assay System components to equilibrate to room temperature.

   5.2 Add the contents of the bottle of Luciferase Assay Buffer into the bottle of Luciferase Substrate and mix well.

NOTE: *Any remaining Luciferase Substrate Mixture can be aliquoted and stored at -80°C for future use. Avoid freeze thaw cycles of Luciferase Substrate Mixture.*

5.3 After incubation remove media from wells by gently inverting the plate and gently blotting on lint free absorbent towel.

5.4 Immediately add **Luciferase Substrate Mixture** into each well at 100 µL/well (96-well plate) or 20 µL/well (384-well plate).


Microplate

5.5 Allow plate to incubate for 10 mins after adding the Luciferase Substrate Mixture and then read the signal in each well using a chemiluminescence microplate reader.

NOTE: *Luciferase Substrate signal starts to degrade ~ 25 minutes after addition to the plate. Reading the plate signal after this time period will lead to decrease in signal intensity and increase in variation.*

Data Analysis

1. For the luciferase assay of Wnt (or Wnt agonist) activity, the readings for each point (concentration of activator or inhibitor) is calculated as the response ratio. The response ratio is calculated as the average chemiluminescence reading for that point divided by the average chemiuminescence reading of background wells only containing Assay Diluent with any test agent solvent. For Wnt3a activation, the final **in-well** concentration of Wnt3a should be between 1 and 400 ng/mL. The EC_{50} is the concentration of Wnt3a that gives half maximal stimulation of luciferase activity. Figure 2 shows a typical dose response of the LEADING LIGHT® Wnt reporter cells to Wnt3a protein under optimized conditions.

Activation of the Wnt Reporter Gene

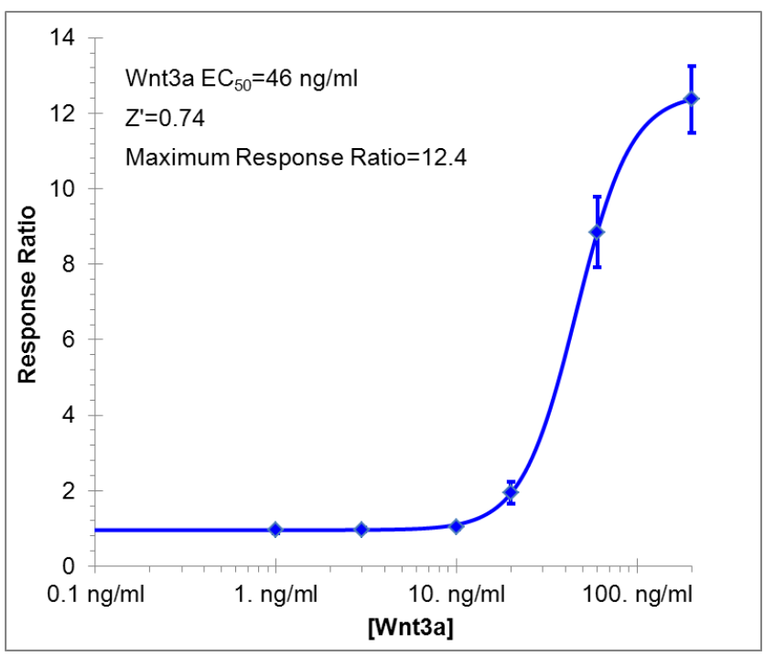

Figure 2: Dose Response of LEADING LIGHT® Wnt Reporter Cells to Wnt3a Protein. LEADING LIGHT® Wnt reporter cells were treated with indicated in-well concentrations of Wnt3a protein. The chemiluminescence in the Wnt3a-treated cells increased in a dose-dependent manner.

2. Figure 3 shows a typical dose response of LEADING LIGHT® Wnt reporter cells to inhibition by Dkk-1 protein. The readings are plotted as percent of Wnt induced activity remaining for concentration of the antagonist Dkk-1. This is calculated by dividing the response ratio of the cells induced with 200 ng/mL (**in-well)** Wnt3a in the presence of the given amount of antagonist by the response ratio of the cells treated with Wnt3a alone multiplied by 100. EC_{50} is the concentration of Dkk-1 that gives half maximal inhibition of Wnt3a signaling.

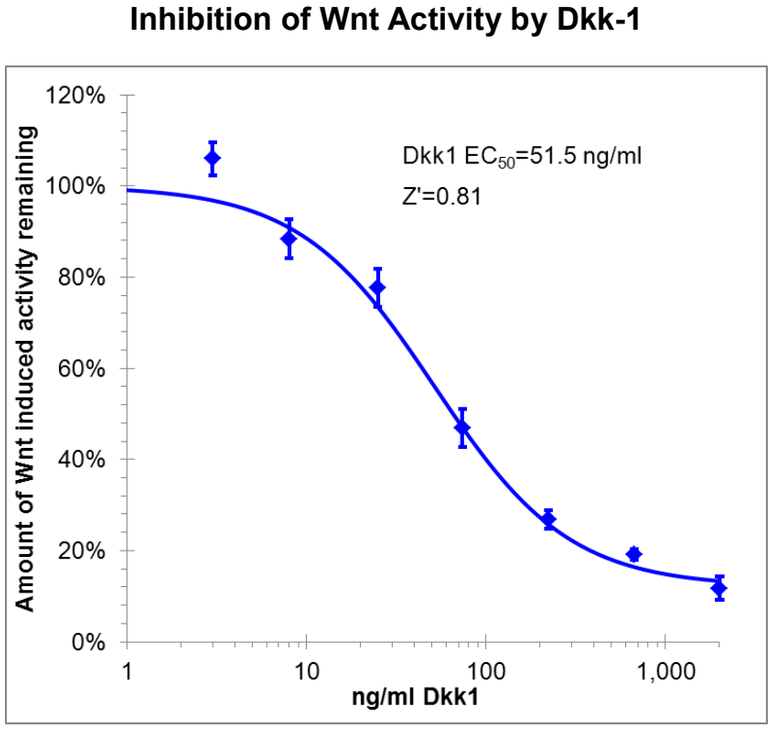

Figure 3: Dose Response of LEADING LIGHT® Wnt Reporter Cells to Dkk-1 Protein. LEADING LIGHT® Wnt reporter cells were treated with the indicated doses of Dkk-1 protein in the presence of 200ng/mL **(in-well)** Wnt3a protein. Dkk-1 inhibits Wnt3a-elevated luciferase levels in a dose dependent manner.

VALIDATING THE ABILITY OF THE CELL LINE TO RESPOND TO WNT ACTIVITY

Lithium chloride (LiCl) is an inhibitor of GSK-3 and can stabilize ß-catenin to induce Wnt target gene expression. In contrast with other commercial reporter cell lines, the reporter cell line in this system does not require LiCl to enhance the signal. LiCl is provided here only for cell line validation purposes. We recommend using this validation control in parallel with the tested reagents.

1. Dilute the 2M LiCl stock solution in Assay Diluent to 100 mM, 50mM, 25 mM and 12.5 mM concentrations.
2. Add 100 µL of each LiCl dilution into separate wells of a microtiter plate, with a like volume of cells. Each concentration should be performed in triplicate, and Assay Diluent alone should be used as a background control.
3. Run the luciferase assay as previously described. The results should be plotted as the response ratio (see Figure 4).

In Figure 4, the response ratio is plotted against the final concentration of LiCl used for induction. The EC_{50} is the concentration of LiCl that gives half maximal stimulation of luciferase activity and should be around 21.9 mM (**in-well**).

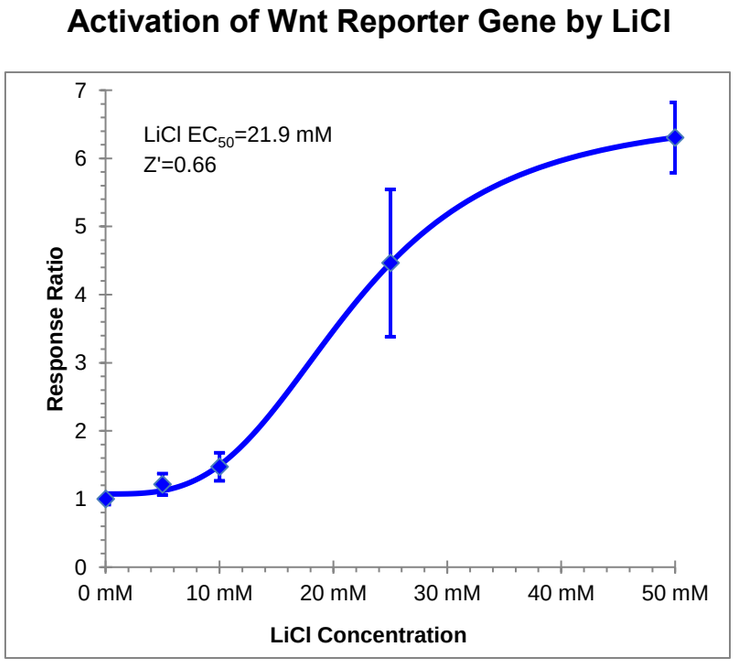

Figure 4: Dose Response of LEADING LIGHT® Wnt Reporter Cells to LiCl. LEADING LIGHT® Wnt reporter cells were treated with the indicated doses of LiCl. The chemiluminescence in the LiCl-treated cells increased in a dose-dependent fashion.

REFERENCES

1. He X, *et al.* (2004) LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: arrows point the way. *Development* 131: 1663-77

2. Molenaar M, *et al. (*1996*)* XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos. *Cell* 86*:* 391–399.

3. Korinek V., *et al. (*1997*)* Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma. *Science* 275*:* 1784–1787*.*

4. Morin P.J., *et al. (*1997*)* Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science* 275*:* 1787–1790

5. Clevers H. (2006) Wnt/β-catenin signaling in development and disease. *Cell* 127: 469–480

6. Logan C.Y., *et al.* (2004) The Wnt signaling pathway in development and disease *Annu Rev Cell Dev Biol* 20: 781-810.

7. Moon R.T., *et al.* (2004) Wnt and β-catenin signalling: diseases and therapies. *Nature Rev Genetics* 5: 689–699

8. Liu P., *et al.* (2005) Enhancement of bone formation by small molecule compounds that disrupt Dkk-LRP5/6 interaction. Proc American Society for Bone and Mineral Research Meeting, Nashville, TN, Abstract 1062

9. Jin R., *et al.* (2007) Small Organic Molecule Compound Protects Against Inflammatory Bone Loss in a Rat Model of Periodontitis. *Journal of Bone and Mineral Research* 22: S208

10. Ewan K., *et al.* (2010) A useful approach to identify novel small-molecule inhibitors of Wnt-dependent transcription *Cancer Res* 70: 5963-73.

TROUBLESHOOTING GUIDE

Problem	Potential Cause	Suggestion
Low response to Wnt induction	High passage cells are used in the assay.	Thaw cells with low passage from liquid nitrogen.
	Inappropriate medium has been used.	Use the medium provided in the kit and follow the instructions.
	Cells are not conditioned well due to incompletely replacing growth medium with assay medium.	Follow the instructions in the manual and make sure the growth medium is completely removed.
	Wnt3a was not diluted and stored properly.	Follow the instructions for storage and dilution of Wnt3a.
	Working concentration of Wnt3a was not set up properly.	Working concentration of Wnt3a should be adjusted for each particular experimental system.
	Cell density is too low in the assay plate.	Follow the recommended procedures.
Low response to Dkk-1 inhibition	Dkk-1 protein was not diluted and stored properly	Follow the instructions for storage and dilution of Dkk-1 protein.
	Working concentration of Dkk-1 protein was not set up properly.	Working concentration of Dkk-1 should be adjusted for each particular experimental system.
Cells detach from the plate.	Cell density is too high in the plate.	Follow the recommended procedures.
	Wrong medium was used in assay.	Use the media supplied in the kit, with DMEM containing 4.1 g/liter glucose and 4mM L-glutamine

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone: 631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:

www.enzolifesciences.com

Catalog Numbers: ENZ-61001-0001; ENZ-61002-0001;
ENZ-60003-0001; ENZ-60004-0001

Rev. 103019