Product Manual

Leading Light® Sclerostin-LRP Interaction Screening System

Catalog #: ENZ-61003

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Product Manual

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Please read entire booklet before proceeding with the assay.

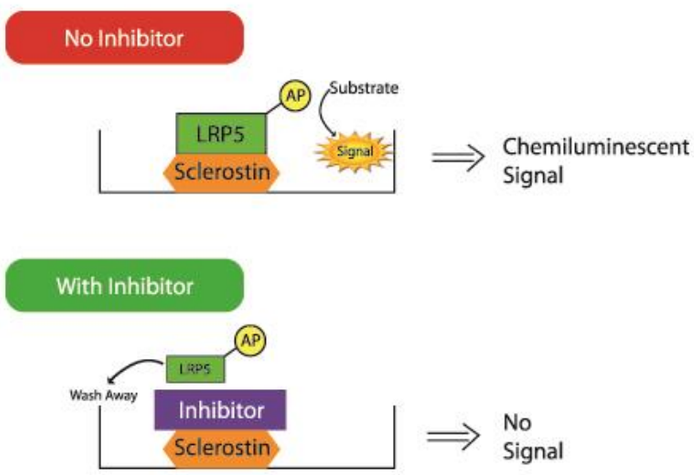
Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

Sclerostin is encoded by the SOST gene and serves as a negative regulator of bone formation [1, 2]. Wnt ligands bind to Frizzled (Fz) and LRP5/6 receptors to trigger the canonical Wnt signaling cascade [3], which has been reported to play an important role in bone development [4, 5]. Sclerostin can bind to LRP5/6 to inhibit the canonical Wnt signaling [6]. Antibodies against sclerostin can block sclerostin-mediated Wnt inhibition in cell based assays and increase bone mineral density in animal models [7]. Importantly clinical trial studies demonstrate that antibodies against sclerostin can also increase human bone mineral density [8]. Consequently, sclerostin is thought to be a superior therapeutic target for osteoporosis treatment over current implemented methods such as treatment with bisphosphonate derivatives.

The Leading Light® Sclerostin Screening System is 96-well plate assay based on sclerostin–LRP interaction. This system contains engineered LRP5-alkaline phosphatase (AP), containing domains 1 and 2 of LRP5, and baculovirus expressed human sclerostin immobilized in a multi-well plate. LRP5-alkaline phosphatase binds to sclerostin coated on the bottom of the well and is detected by the activity of alkaline phosphatase remaining after a washing step. This system can be used for drug screening to identify small molecule compounds, antibodies, DNA aptamers and peptides capable of modulating sclerostin-LRP interaction.



The Leading Light® Sclerostin–LRP Screening System represents a unique and novel tool for identifying Wnt pathway modulators with the following features:

- No requirement for cell line – functions as a biochemical assay
- No requirement for stable or transient transfection of a reporter gene
- Excellent reproducibility - Z'-factor 0.871 (96-well plates)

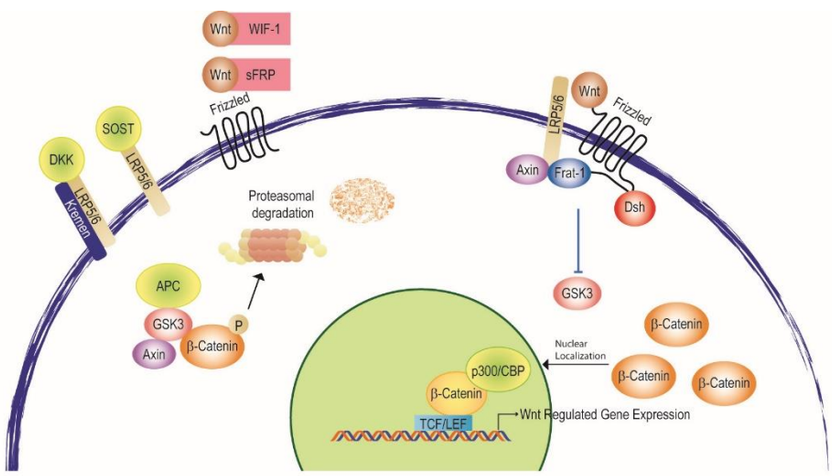


Figure 1: Schematic of the canonical Wnt signaling pathway and its components. This pathway causes an accumulation of β-catenin in the cytoplasm and its eventual translocation into the nucleus to act as a transcriptional coactivator of transcription factors that belong to the TCF/LEF family. Without Wnt signaling, β-catenin is ubiquitinylated and targeted for proteasomal degradation.

MATERIALS SUPPLIED

1. **Sclerostin Microtiter Plate, One Plate of 96 Wells**

   Catalog No. 80-2468

   A plate using break-apart strips coated with baculovirus expressed human sclerostin protein.

2. **LRP5-AP fusion protein concentrate, 2 x 600 µL**

   Catalog No. 80-2469

   Two vials of an 8Xsolution of LRP5-AP fusion protein containing domains 1 and 2 of LRP5.

3. **AP Concentrate (negative control), 300 µL**

   Catalog No. 80-2470

   A 10x solution of AP negative control.

4. **Assay Buffer 13 , 50 mL**

   Catalog No. 80-1500

   Tris buffered saline containing BSA and detergents

5. **Wash Buffer, 20X concentrate, 25 mL**

   Catalog No. 80-1286

   Tris buffered saline containing detergents.

6. **Acid Green 25 (Sclerostin binding inhibitor), 40 mg**
   Catalog No. 80-2473

7. **AP Substrate Reagent, 5 mL**

   Catalog No. 80-2474

   A substrate for chemiluminescent detection of alkaline phosphatase.

8. **Plate Sealer, 2 each**

Avoid freeze / thaw cycles

STORAGE

All reagents are shipped on Blue Ice. Upon receipt kit should be stored at -20ºC until kit is used. Upon first use of kit Assay Buffer 13, Wash Buffer 20x, and AP Substrate may be stored at 4ºC. Avoid repeated freezing and thawing.

OTHER MATERIALS NEEDED

1. Pure or well characterized sclerostin-LRP5 inhibitor targets of interest of known concentrations.
2. Deionized or distilled water.
3. Precision pipets for volumes between 5 µL and 1,000 µL,
4. Disposable polypropylene test tubes for dilution of samples and controls.
5. Repeater pipette for dispensing 50 µL.
6. Disposable beakers for diluting buffer concentrates.
7. Graduated cylinders.
8. Microplate shaker
9. Absorbent paper for blotting.
10. Microplate reader capable of chemiluminescence detection.

Handle with care

SAFETY WARNINGS & PRECAUTIONS

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

- 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. Dispose of unused materials in accordance with established safety procedures.

METHODS AND PROCEDURES

The following protocols provide the recommended procedures for screening Wnt pathway modulators by measuring Sclerostin–LRP5 interaction.

REAGENT PREPARATION

1X Wash Buffer

Add 475 mL distilled or deionized water and 25 mL of 20X Wash Buffer into a 500 mL bottle and mix well. Store at 4°C.

1X Assay Solution

Dilute 600 µL of the 8X LRP5-AP fusion protein concentrate with 4.2 mL of Assay Buffer 13. Two vials are provided.

Negative Control Solution

Dilute 300 µL AP Concentrate (negative control) with 2.7 mL of Assay Buffer 13.

Acid Green 25, Control Sclerostin Binding Inhibitor

Dissolve Acid Green 25 in 1.2 mL distilled water to make a 50mM stock solution. Allow 50 mM Acid Green 25 to sit at room temperature for approximately 20 minutes to ensure it is fully dissolved, vortex the solution. Dilute the 50 mM stock 1:25 with 1X Assay Solution to make a 2 mM working concentration. (Example, add 8 µL of 50mM Acid Green 25 to 192 µL 1X Assay Solution to make a 2mM solution of control inhibitor)

SCLEROSTIN-LRP BINDING ASSAY PROCEDURE

Assay Preparation

1. Prepare inhibitor candidates (e.g. compounds, antibodies, peptides, Acid Green 25, etc.) for testing by diluting each with 1X Assay Solution (from Reagent Preparation). Serial dilutions of test reagents are strongly recommended.
2. Remove plate from storage at -20ºC and allow it to come to room temperature before opening plate bag.
3. Add 50 µL of Negative Control Solution (from Reagent Preparation) into 3 wells.
4. Add 50 µL of 1X Assay Solution into 3 wells as a positive control.
5. Add 50 µL of 2mM Acid Green 25 (from Reagent Preparation) into 3 wells as an inhibition control.
6. Add 50 µL of the inhibitor candidates diluted in 1X Assay Solution into each well. It is recommended to test each candidate in triplicate per diluted concentration.
7. Seal the plate with the adhesive film to prevent evaporation.
8. Shake the sealed plate for 2 hours at room temperature on a horizontal shaker or orbital shaker.

   NOTE: A 1 hour incubation time is often sufficient to see 10-fold inhibition during testing. Users may lower the incubation time to 1 hour if inhibitor of interest shows a 10-fold or greater reduction after 1 hr.

Washing Procedure

1. Empty the contents of the wells and wash by adding a full well volume (~300 µL) of Wash Solution (from Reagent Preparation) to every well. Repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

   NOTE: When using Acid Green 25 control a slight green residue may be observed in the bottom of wells, this does not interfere with the chemiluminescent development or %CV of the control wells.

Development Procedure

1. Add 50 µL of AP Substrate Reagent (supplied) to each well.
2. Shake the plate at room temperature for 25 minutes on a horizontal shaker or orbital shaker.
3. Read luminescence intensity with a chemiluminescence microplate reader.
4. The intensity of the signal in the wells containing positive control (Assay Solution only) should be more than 10-fold higher than the intensity of the signal in the wells containing Negative Control (AP only).

DATA ANALYSIS

The intensity of the signal in the wells containing positive control (Assay Solution only) should be more than 10-fold higher than the intensity of the signal in the wells containing Negative Control (AP only). If the control binding inhibitor, Acid Green 25, is used, it should inhibit binding at a concentration of 1mM or higher.

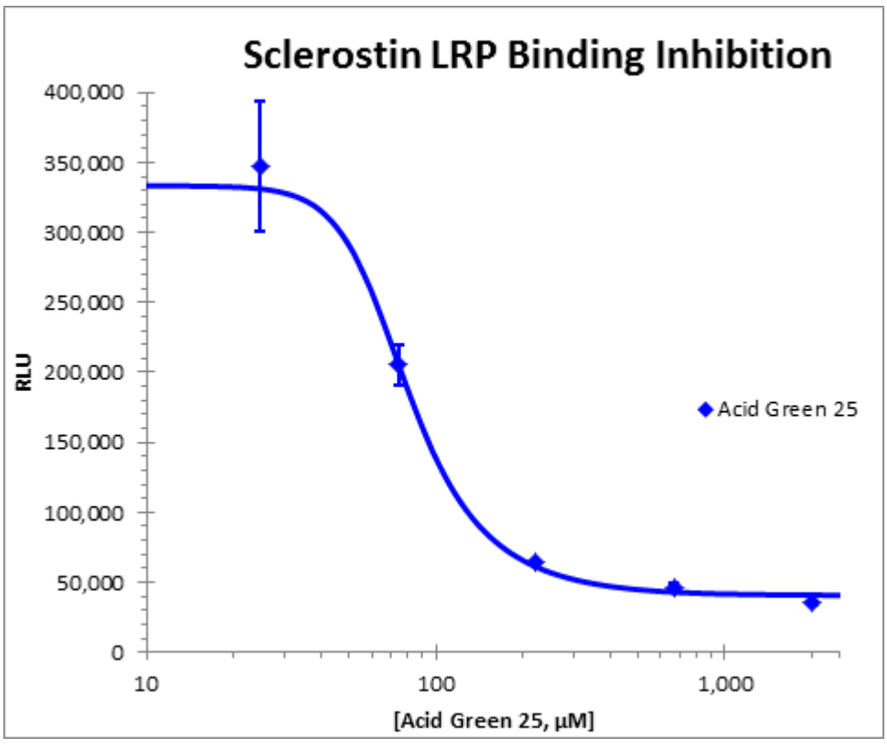


Figure 2: Inhibition of Sclerostin binding by Acid Green 25. The LRP5-AP was bound to Sclerostin in the presence of the indicated concentration of Acid Green 25 used to inhibit this interaction. Chemiluminescence was read using a BMG Labtech Fluostar Optima plate reader following the development steps.

REFERENCES

1. Balemans, W., et al., Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). Hum Mol Genet, 2001. 10(5): p. 537-43.
2. Brunkow, M.E., et al., Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cystine knot-containing protein. Am J Hum Genet, 2001. 68(3): p. 577-89.
3. He, X., et al., LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: arrows point the way. Development, 2004. 131(8): p. 1663-77.
4. Gong, Y., et al., LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell, 2001. 107(4): p. 513-23.
5. Boyden, L.M., et al., High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med, 2002. 346(20): p. 1513-21.
6. Li, X., et al., Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. J Biol Chem, 2005. 280(20): p. 19883-7.
7. Veverka, V., et al., Characterization of the structural features and interactions of sclerostin: molecular insight into a key regulator of Wnt-mediated bone formation. J Biol Chem, 2009. 284(16): p. 10890-900.
8. Padhi, D., et al., Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. J Bone Miner Res. 26(1): p. 19-26.

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