Enzo Life Sciences

APPLICATION NOTE

Detecting Leading Light® Sclerostin-LRP Screening System using the CLARIOstar® and LVF-Monochromator

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Leading Light® Sclerostin-LRP Interaction Screening System (ENZ-61003)

- Enzo's Leading Light Sclerostin-LRP Screening System is a novel tool to identify Wnt-pathway modulators
- The CLARIOstar can detect this assay using the LVF-monochromator
- Excellent Z' values are indicative of the robust assay and sensitive detection

Introduction

Bone remodeling is the process where bone is continually removed and replaced. Disruption of this process can lead to osteoporosis, characterized by low bone mineral density or bone thickening characteristic of sclerosterosis. Bone remodeling involves the actions of two cell types found in bone, osteoblasts (bone resorption) and osteoclasts (bone deposition). The actions of these two cell types is coordinated by a third type of bone cell called an osteocyte. Sclerostin has now been identified as a molecule expressed by osteocytes that is able to modulate the Wnt-signaling pathway which is important in regulation of bone formation[1].

Wnt exerts its effect on bone formation by binding to the LRP 5/6 –Frizzled receptor on osteoblasts[2]. This leads to stabilization of intracellular β-catenin and regulation of transcription that promotes bone formation. By binding to the LRP 5/6 receptor sclerostin antagonizes Wnt-signaling and inhibits bone formation[1]. Therefore treatments which block the sclerostin LRP 5/6 interaction could serve as treatments for osteoporosis.

Assay Principle

The Leading Light® Sclerostin-LRP Interaction Screening System uses LRP5 engineered such that it is linked to alkaline phosphatase (AP). Sclerostin is coated on 96-well plates and bound LRP5-AP is detected by the activity remaining after a washing step (Figure 1). In this way a variety of different compounds can be screened for their ability to disrupt the sclerostin / LRP5 interaction. Since this is a biochemical assay, there is no requirement for a cell line or the stable or transient transfection of a reporter gene.

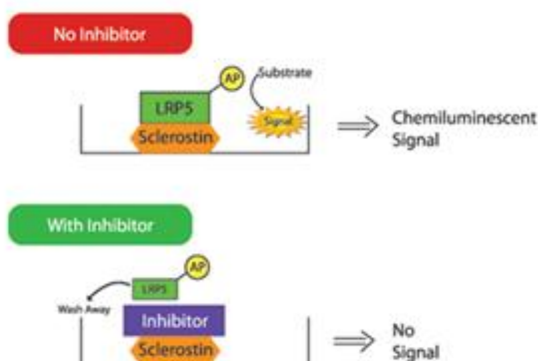

Figure 1. Sclerostin-LRP System Assay Principle
In the absence of binding inhibitors LRP5-AP binds to the Sclerostin with which the 96-well plate is coated. Therefore a chemiluminescent signal is produced upon activation of the enzyme. In contrast, the presence of inhibitors leads to decreased LRP5-AP binding which is washed away. As a result no enzyme activity is detected.

Materials and Methods

- Leading Light® Sclerostin-LRP Interaction Screening System kit (ENZ-61003)
- CLARIOstar microplate reader from BMG LABTECH (Figure 2)

Figure 2. The CLARIOstar microplate reader (**BMG LABTECH**) features an LVF Monochromator for wavelength selection in luminescence and fluorescence experiments.

Reagents supplied in the Sclerostin-LRP Screening kit were prepared according to the procedures described in the product manual. All plate shaking steps were employed by the CLARIOstar orbital shaking feature.
To determine appropriate monochromator settings spectral scanning was performed using the following CLARIOstar settings:

Measurement Method	Luminescence
Reading Mode	Spectral scan
Emission wavelength	500 – 600 nm
Emission bandwidth	20 nm
Gain	3600
Focal height	11.0

The plate was then read in the CLARIOstar with the following setting.

Measurement Method	Luminescence
Reading Mode	Endpoint
Measurement interval	1 second
Emission wavelength	550.0 nm
Emission bandwidth	various
Gain	3600
Focal height	11.0

Results:

The CLARIOstar spectral scan feature allows you to obtain an image of the luminescence emission of your lumiphore at resolution of 1 nm (Figure 3).

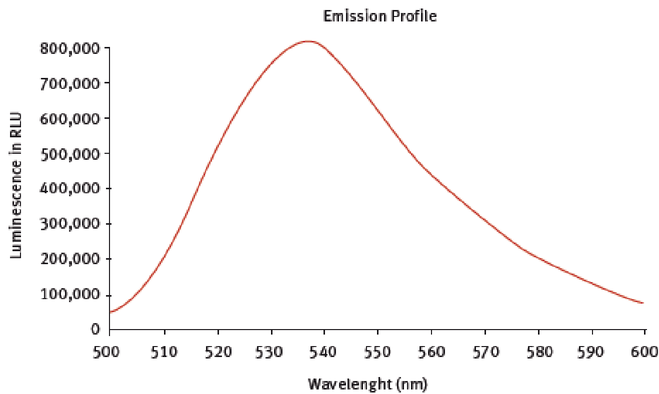

Figure 3. The emission spectrum for alkaline phosphatase substrate in the Leading Light® Sclerostin-LRP Screening Assay (Enzo Life Sciences).

Based on the broad emission spectrum in this experiment we believed that capturing the entire emission spectrum from 500 nm to 600 nm provided the best results. The LVF Monochromator in the CLARIOstar makes detecting bandwidths up to 100 nm possible. Using the monochromator setting of 550-100, we were able to collect light from this entire spectral range and obtain excellent results in the assessment of the inhibitory capacity of Acid Green 25 on the interaction between sclerostin and LRP5 (Figure 4).

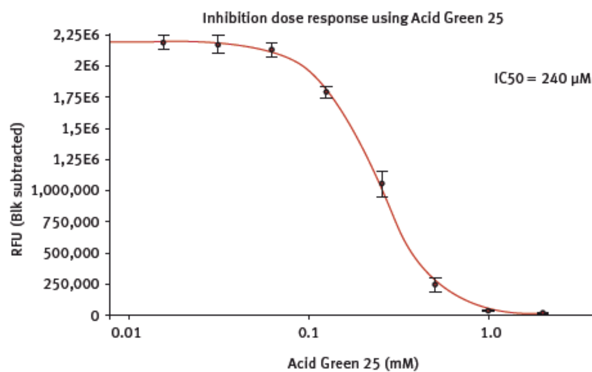

Figure 4. Acid Green 25 Inhibition Data
RLU plotted vs. Acid Green 25 Concentration was analyzed in MARS and found to conform to a 4-parameter fit-curve with an R^2 = 0.9998. From this an IC_{50} of 240 μM was calculated for Acid Green 25.

Table 1 provides a comparison of various monochromator setting to assess the effect of decreasing bandwidth on assay performance parameters. As we would expect, decreasing the bandwidth leads to a decrease in RLU which correlates with a slight decrease in assay window. The assay window is the ratio of positive control and negative control. The effect on Z', however, is negligible.

Bandwidth (500-)	100 nm	90 nm	80 nm
Avg. PC*	2,216,000	2,022,000	2,004,000
Assay window	274.9	254.4	248.4
Z'	0.972	0.968	0.968

Avg. PC* = average luminescence value of positive control

Conclusions

The CLARIOstar LVF Monochromator exhibits robust luminescent detection sensitivity for Enzo's Leading Light® Sclerostin-LRP Interaction Screening System. This assay system and instrumentation proves to be an excellent platform to assess modulators of sclerostin-LRP interactions.

References

1) M. Semënov *et al* (2005) **J. Biol. Chem.** 280: 26770
2) M. Kato *et al* (2002) **J. Cell Biol.** 157: 303

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