

Detecting Sclerostin-LRP5/6 interaction using the CLARIOstar® and LVF Monochromator™

Carl Peters¹ and Courtney Noah²
¹BMG LABTECH ² Enzo Life Sciences

- 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 sclerostosis. 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.

Wnt exerts its effect on bone formation by binding to the LRP 5/6 – Frizzled receptor on osteoblasts. 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. Therefore treatments which block the sclerostin LRP 5/6 interaction could serve as treatments for osteoporosis. In this application note we show that the CLARIOstar microplate reader from BMG LABTECH can be used to screen for Sclerostin-LRP5/6 binding inhibitors.

Assay Principle

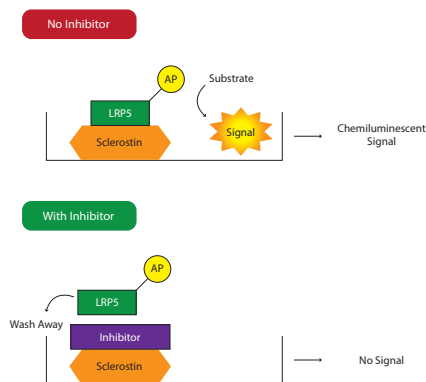


Fig. 1: Sclerostin-LRP System Assay Principle In the absence of binding inhibitors LRP5-AP binds to the Sclerostin with which the 96-well plate is coated. Therefore a chemiluminescent signal is produced upon activation of the enzyme. In contrast, the presence of inhibitors leads to decreased LRP5-AP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

The Leading Light™ Sclerostin-LRP Interaction Screening System uses LRP5 engineered such that it is linked to the enzyme 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.

Materials & Methods

- CLARIOstar microplate reader from BMG LABTECH
- Leading Light™ Sclerostin-LRP Interaction Screening System kit (ENZ-61003) from Enzo® Life Sciences
- white 96-well microplate, Costar

Reagents and microplate supplied in the Sclerostin-LRP Screening kit were prepared according to the procedures described in the product manual. The kit contains a positive control (LRP5-AP fusion protein), a negative control (AP concentrate only) and an inhibition control (Acid Green 25). All plate shaking steps employed the CLARIOstar orbital shaking feature. Upon finishing assay preparation and washing steps the alkaline phosphatase substrate was added.

After a 25 minute incubation the chemiluminescence signal was measured in the CLARIOstar.

To determine appropriate monochromator settings an emission scan was performed using the following instrument 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 adjustment.

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



Results & Discussion

The CLARIOstar spectral scan feature allows you to obtain an image of the luminescence emission of your lumiphore at resolution of 1 nm [Figure 2].

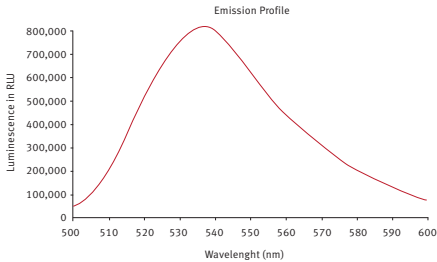


Fig. 2: Emission spectrum for the reaction of alkaline phosphatase with its substrate in the Leading Light® Sclerostin-LRP Screening Assay.

Based on the broad emission spectrum in this experiment we believed that capturing the entire emission spectrum from 500 nm to 600 nm will give 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. Excellent results in the assessment of the inhibitory capacity of Acid Green 25 on the interaction between sclerostin and LRP5 were obtained (Figure 3).

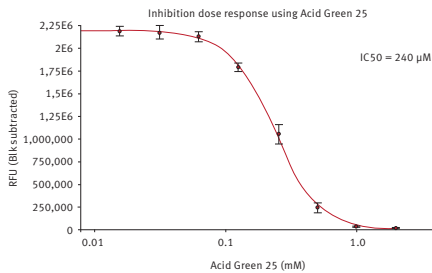


Fig. 3: Acid Green 25 inhibition data, plotted in a 4-parameter fit using the MARS Data Analysis software [R2 = 0.9998]. From the inhibition response curve an IC50 value of 240 µM was calculated.

Table 1 provides a comparison of various monochromator settings to assess the effect of decreasing bandwidth on assay performance parameters.

Bandwidth (550-)	100 nm	90 nm	80 nm
Avg. PC*	2,216,000	2,022,000	2,004,000
Assay window	275	254	248
Z'	0.972	0.968	0.968

Avg. PC* = average luminescence value of positive control

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.

Conclusion

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.



CLARIOstar®