

Leading Light[®] Sclerostin-LRP Interaction Screening System
ASSAY LAYOUT SHEET
for use with Enzo Life Sciences' Catalog No. ENZ-61003

• **ASSAY PROTOCOL FLOW CHART: Vol. Added**

Well ID:	Blank	(+) LRP5-AP (1x)	Acid Green 25 (2mM)	(-) AP Control (1x)	Samples
Mix all reagents gently	---	---	---	---	---
Assay Buffer 13	50 μ L	---	---	---	---
LRP5-AP in Assay Buffer 13	---	50 μ L	---	---	---
LRP5-AP w/Acid Green 25 in Assay Buffer 13	---	---	50 μ L	---	---
LRP5-AP w/ Sample Inhibitor in Assay Buffer 13	---	---	---	---	50 μ L
(-) Control AP in Assay Buffer 13	---	---	---	50 μ L	---
Tap plate gently to mix	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Incub. 2 hour @ RT, sealed, shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x ~400 μ L (Full Well Volume) TBST Wash Buffer	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
AP Substrate Reagent	50 μ L	50 μ L	50 μ L	50 μ L	50 μ L
Incub. 25 min. @ RT, sealed, shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Read luminescence intensity	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

Leading Light® Sclerostin-LRP Interaction PLATE LAYOUT:

A1 (+) LRP5-AP	A2 (-) AP Control	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 (+) LRP5-AP	B2 Blank	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1 (+) LRP5-AP	C2 Blank	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 Acid Green 25	D2 Blank	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 Acid Green 25	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 Acid Green 25	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1 (-) AP Control	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 (-) AP Control	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Kit Lot No. _____ Exp. Date _____ Date _____ Tech. _____

1st Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____

2nd Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____