Monitoring interactions between protein kinases and small molecule inhibitors

Lumit™ Protein Interaction Immunoassay

The Lumit™ Protein Interaction Immunoassay using proteins tags is a homogenous bioluminescent assay to measure protein-protein or protein-small molecule interactions. It combines immunodetection and NanoLuc Binary Technology (NanoBiT®). In Lumit™ Protein Interaction Immunoassays using protein tags, NanoBiT® subunits (SmBiT and LgBiT) are conjugated to two antibodies against common protein tags. When anti-Tag-LgBiT and anti-Tag-SmBiT are added to an interacting protein pair with the corresponding tags, LgBiT and SmBiT are brought into close proximity to form an active luciferase enzyme that generates luminescence in the presence of substrate (Fig 1A). Lumit™ Protein Interaction Immunoassays have simple, no-wash format (Fig 1B), require small sample volumes, and can be run in a high-throughput format.

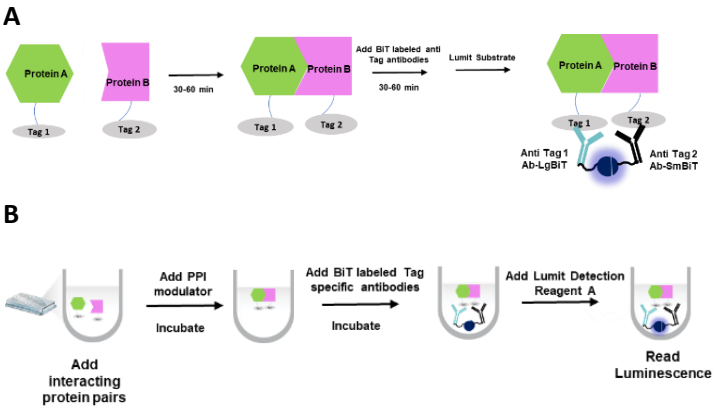

Figure 1. Schematic of Lumit™ Protein Interaction Immunoassay. A. *When LgBiT and SmBiT are brought into proximity by the interaction of Protein A and Protein B, an active luciferase generates luminescence in the presence of substrate.* ***B.*** *Overview of the simple no-wash protocol.*

Small molecule kinase inhibitors

Protein kinases are ubiquitously expressed proteins that are categorized by the amino acid they phosphorylate: serine, threonine, or tyrosine. All three types of protein kinases modulate numerous cellular signaling pathways. Because their function is often to initiate pathways of growth, survival and differentiation, kinases that are constitutively active or overexpressed lead to disease states such as cancer. Thus, kinase inhibitors represent a popular class for anticancer agents. Lumit Protein Interaction Immunoassays using protein tags offer a simple bioluminescent method for screening of kinase inhibitors as shown in Fig 2.

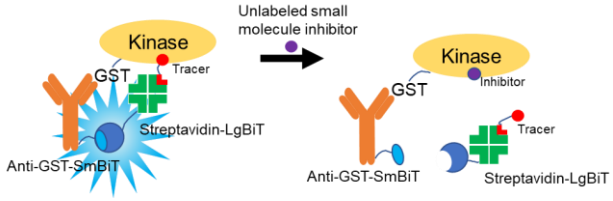

Figure 2. Lumit™ kinase-small molecule inhibition immunoassay. *In this assay, small molecule kinase inhibitors compete with Ibrutinib-Biotin tracer for binding, resulting in a decrease in luminescence signal when measured using BiT-labeled anti-Tag antibody and Streptavidin.*

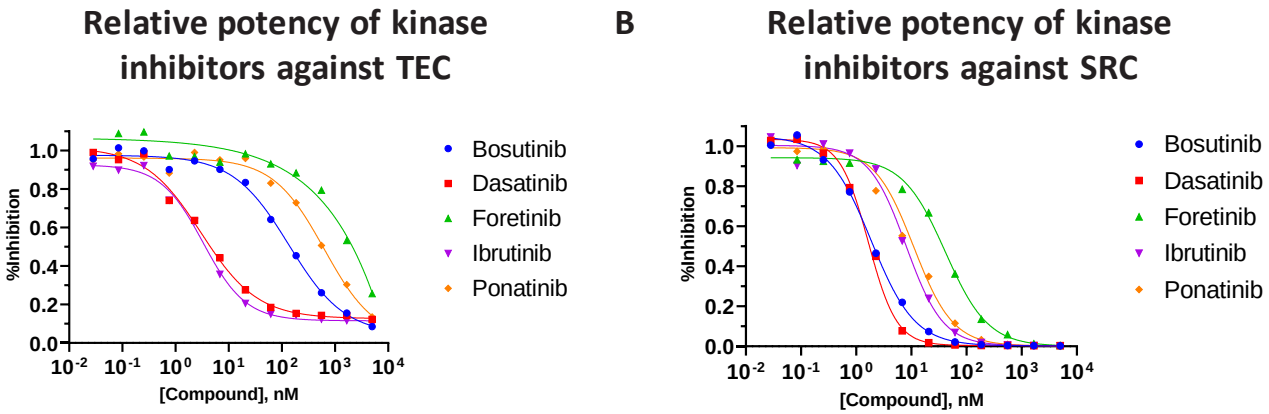

Figure 3. Comparing relative IC_{50} values of small molecule kinase inhibitors. *Unlabeled small molecule drugs compete with tracer (ibrutinib-biotin) for (A) TEC-GST or (B) SRC-GST binding. Graphs convey relative IC_{50} values of small molecule inhibitors for each. Concentrations are expressed as final concentration in each well.*

Lumit™ immunoassay for Kinase-Small Molecule Interaction

1. In Kinase Buffer, make a master mix of 25nM ibrutinib-biotin with serial dilutions of unlabeled small molecule inhibitor.
2. Add 10nM of kinase to a master mix and add 20µl of mixture to wells.
3. Incubate with shaking for 60 minutes.
4. Add 20µl mixture of anti-GST-LgBiT (0.38µg/ml) and Streptavidin-SmBiT (0.18µg/ml) diluted in 1x Lumit™ Immunoassay Dilution Buffer A.
5. Incubate with shaking for 30 minutes.
6. Add 10µl of Lumit™ Detection Substrate A diluted 1:50 in Lumit™ Immunoassay Dilution Buffer A to each well.
7. Incubate with shaking for 2 minutes.
8. Read luminescence.

Materials Needed

Item	Supplier	Cat. #
Bosutinib	Selleck Chemicals	S1014
Dasatinib	LC Laboratories	D-3307
Foretinib	Selleck Chemicals	S1111
Ibrutinib	Selleck Chemicals	S2680
Ponatinib	Selleck Chemicals	S1490
Ibrutinib-biotin	MedChemExpress	HY-100342

Ordering Information

Item	Cat. #
TEC Kinase Enzyme System	VA7306
SRC Kinase Enzyme System	V2921
Lumit™ Streptavidin-LgBiT and -SmBiT	CS332215
Lumit™ anti-GST-LgBiT and -SmBiT	CS332212
Lumit™ Immunoassay Detection Reagent A	VB2010