Monitoring ternary complex formation using PROTACs

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 NanoLuc luciferase generates luminescence in the presence of substrate.* ***B.*** *Overview of the simple no-wash protocol.*

PROTACs

PROTACs (PROteolysis TArgeting Chimeras) are heterobifunctional small molecules that bring a target protein into proximity of an E3 ubiquitin ligase for degradation. A PROTAC consists of two ligands connected by a linker. One ligand binds an E3 ligase (often Cereblon or VHL) to engage the cells' ubiquitin system, and the other binds a target protein of interest. When the PROTAC engages with the target and E3, the E3 ligase recruits enzymes that lead to ubiquitination and ultimately proteasomal degradation of the target protein.

PROTACs hold significant therapeutic promise as an alternative to traditional inhibitors. Unlike small molecule drugs, PROTACs are recycled within the cell which enables sustained degradation, and the PROTAC binding site is not limited to the target active site which creates an opportunity to target new sites on previously "undruggable" proteins. Lumit Protein Interaction Immunoassays using protein tags offers a simple bioluminescent method for screening of PROTACs as shown in Fig 2.

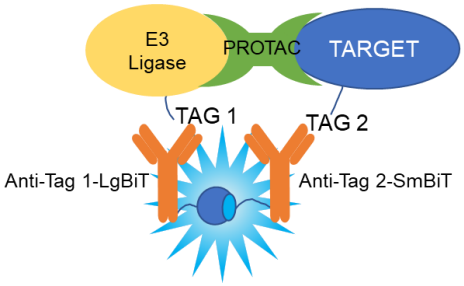

Figure 2. Lumit™ PROTAC Immunoassay*. PROTACs bring E3 ligase and target proteins into close proximity, which can be detected using BiT-labeled anti-Tag antibodies.*

A PROTAC driven VHL complex/BRD3(BD2) ternary complex formation

B Titration of ternary complex inhibitors

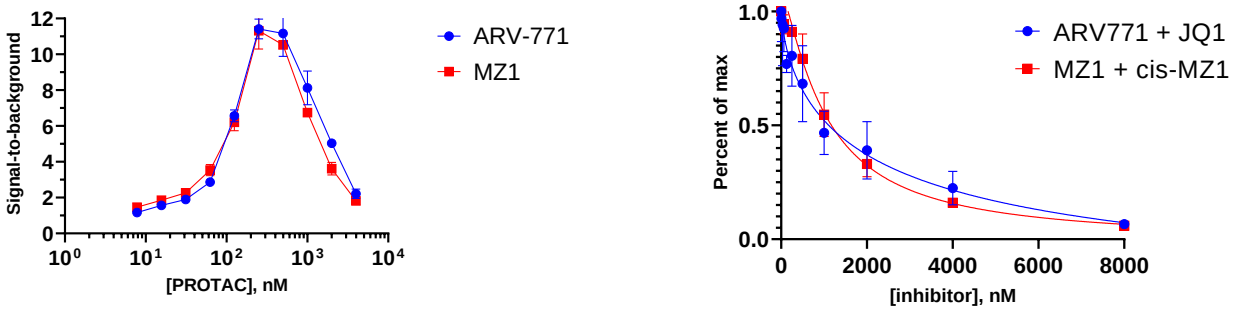

Figure 3. Measuring ternary complex formation with different PROTACs *(A) PROTACs ARV-771 and MZ1 induce VHL complex and BRD3(BD2) ternary complex formation. Until around 250nM, as the concentration of PROTAC increases the luminescent signal goes up, indicating increasing amounts of ternary complex. At the peak luminescent signal, the PROTAC has saturated E3 and/or target. Higher PROTAC concentrations result in a decreasing luminescent signal or a "hook effect," caused by the formation of non-productive binary complexes. (B) Increasing concentrations of either JQ1 or cis-MZ1 were added to PROTAC/protein mixture to compete with productive ternary complexes. Concentrations are expressed as final concentration in each well.*

Lumit™ PROTAC-mediated VHL complex/BRD3(BD2) Immunoassay

1. Mix 100nM BRD3(BD2) and 100nM VHL complex with (A) titrations of ARV-771 or MZ1 or (B) 250nM PROTAC and titrations of target protein warhead (for ARV-771: JQ1, for MZ1: cis MZ1). Add 20µl of mixture to wells.
2. Incubate with shaking for 1 hour.
3. Add 20µl of anti-FLAG-SmBiT (1.5µg/ml) and anti-GST-LgBiT (1.5µg/ml) diluted in 1x Lumit™ Immunoassay Dilution Buffer A.
4. Incubate with shaking for 30 minutes.
5. Add 10µl of Lumit™ Detection Substrate A diluted 1:50 in Lumit™ Immunoassay Dilution Buffer A to each well.
6. Incubate with shaking for 2 minutes
7. Read luminescence.

Materials Needed

Item	Supplier	Cat. #
VHL Complex	BPSBioscience	100373
BRD3(BD2)	BPSBioscience	31033
ARV-771	MedChemExpress	1949837-12-0
MZ1	Tocris	6154
JQ1	Tocris	4499
cis-MZ1	Tocris	6155

Ordering Information

Item	Cat. #
Lumit™ anti-FLAG-LgBiT and -SmBiT	CS332213
Lumit™ anti-GST-LgBiT and -SmBiT	CS332212
Lumit™ Immunoassay Detection Reagent A	VB2010