Lumit™ Immunoassays: Bioluminescent, Sensitive, and Homogeneous Analyte Detection Using Labeled Antibodies

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1. Introduction

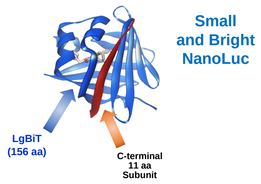
NanoLuc® Binary Technology (NanoBiT®), a two-part complementation system based on NanoLuc luciferase, is a proven technology for analyzing proteins at a cellular level. NanoBiT is comprised of an 11-amino acid subunit (low-affinity SmBiT or high-affinity HiBiT) that binds to its cognate large subunit partner (LgBiT) to form a bright luciferase that produces light when furimazine is added. We are building NanoBiT proximity immunoassays where complementary antibodies (or other affinity reagents) are labeled with NanoBiT subunits such that binding to analyte brings SmBiT and LgBiT into proximity, thereby producing signal proportional to analyte levels. This homogeneous detection chemistry has several advantages, including simple, add-and-read protocols, no requirement for sample transfer, no washes, and a broad linear dynamic range mitigating the need for sample dilutions. Moreover, time to assay completion is <30 to ≤ 90 minutes, depending on the specific assay. In development are assays for detection of cytokines (e.g., IL-1β), metabolic targets (e.g., Insulin), FcRn binding, cellular pathway analyses (total and phospho-protein levels), as well as labeling kits to build your own Lumit immunoassays.

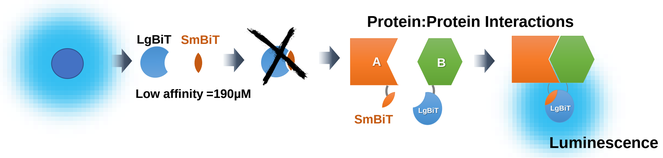
2. NanoLuc Binary Technology (NanoBiT)

The small NanoLuc luciferase (19kDa) was divided into two subunits and individually optimized for **assisted** complementation

Two subunits:

- Large BiT (LgBiT; 17.6kDa) and
- Small BiT (SmBiT; 11 amino acid peptide)

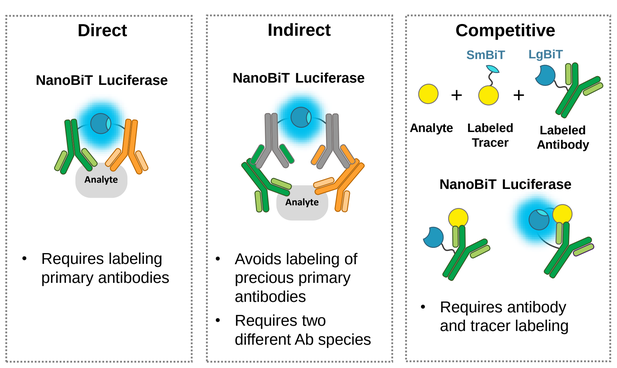




Dixon, A.S., *et al.* (2016) NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells. *ACS Chem. Biol.* **11**, 400-8.

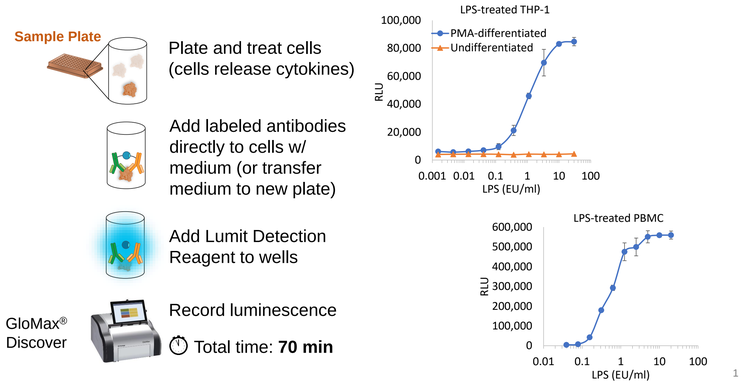
3. Lumit™ Immunoassay Configurations

Bioluminescent immunodetection platform based on NanoBiT technology



4. Lumit Cytokine Immunoassays

Protocol for Detecting Released IL-1β from Cells



Additional cytokine assays in development include, but are not limited to, **IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α, and VEGF**

5. Lumit Immunoassay Cellular System

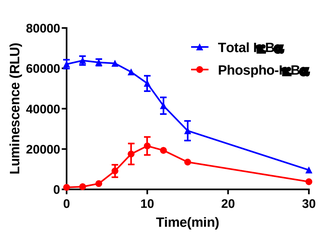
The assay is fast and homogeneous, with an easy "Add and Read" format

Hwang, B., Engel, L., Goueli, S.A. *et al.* A homogeneous bioluminescent immunoassay to probe cellular signaling pathway regulation. *Commun Biol* 3, 8 (2020) doi:10.1038/s42003-019-0723-9

6. NF-κB Signaling Pathway

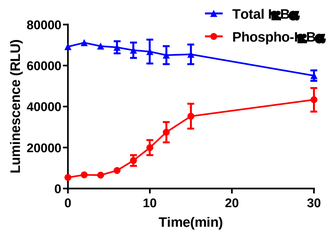
Activation of NF-κB Pathway with TNFα treatment in MCF-7 cells

Detection of Total and Phospho IκBα upon TNF treatment



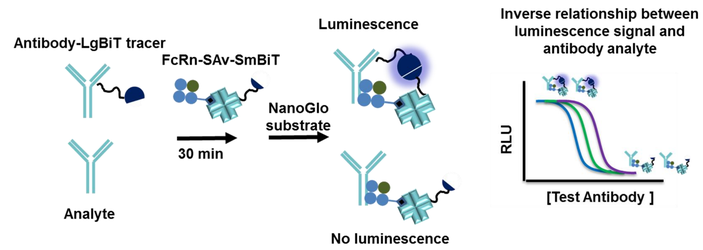
Lumit IκB detection reveals the predicted biology of NF-κB signaling pathway upon TNF treatment: **IκBα phosphorylation** (pS32) immediately followed by its **fast degradation.**

Detection of Total and Phospho IκBα upon TNF treatment in the presence of MG132



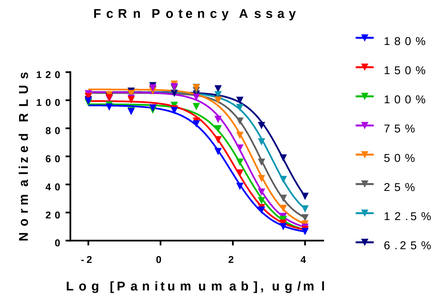
Detection of the predicted response of NF-κB pathway to the proteasome inhibitor treatment: **decrease** in IκBα degradation and **accumulation** of phosphorylated IκBα.

7. Lumit FcRn Immunoassay



- Lumit FcRn Immunoassay is solution based; minimizes artifacts introduced by immobilization
- Assay is homogeneous (add-and-read) and requires no washing
- Luminescence based detection provides wide dynamic range and a large assay window
- Assays are quick (30min) and require low sample volume (10-20µl)
- Use of 96/384 well white plates enables flexible throughput and automation capabilities

8. Measurement of Relative Antibody Potency



	180%	150%	100%	75%	50%	25%	12.5%	6.25%
IC50	86.66	113.9	200.4	231.1	369.9	604.7	1203	2553

Dose response curves for Panitumumab-FcRn binding corresponding to 180%, 150%, 100%, 75%, 50%, 25%, 12.5% and 6.25% of the nominal concentration, plotted versus nominal (100%) concentration values

9. Conclusions

Bioluminescent immunodetection platform based on NanoBiT technology

- No-wash assay can be performed directly on cells
- Fast, 30- to 90-minutes total time
- Scalable for high throughput use
- Large dynamic range reduces sample dilutions required
- Uses standard plate-reading luminometer
- Platform will include ready-to-use kits and reagents; user can create novel detection assays
- Custom antibody labeling and immunoassay development available; Contact: CAS@promega.com

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