Rapid and Sensitive Determination of Cytokine Release from Cells without Sample Transfer

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1. Introduction

We have utilized NanoLum® Binary Technology (NanoBIT™) to develop a rapid, homogeneous assay method (≤ 70 min completion time) to measure cytokines released from cells in culture without the need for sample transfer and requiring only a standard, plate-reading luminometer for signal acquisition. In this Lumi™ immunoassay approach, separate primary antibodies to a specific cytokine are individually labeled with either the small, 11-aa amino acid subunit of NanoBIT™ luciferase (SmBiT) or its 17.6 kDa complementary subunit (LgBiT). When these labeled antibodies conjugate on the target cytokine, the SmBiT and LgBiT subunits reconstitute a bright luciferase that produces bioluminescence proportional to analyte levels when the substrate furimazine is present. In this manner, immunoassays were developed for several human cytokines, including IL-2, IL-6, IFN-γ, and TNF-α. These assays share excellent sensitivities (LLLODs < 10 pg/mL) and broad linear ranges (≥ 2 logs of cytokine concentration), significantly mitigating the need for sample dilution. Direct addition of assay reagents to 96-well, white plates containing human PBMC treated with various stimuli revealed wide-ranging, time- and dose-dependent release of TNF-α and IL-6. Similar direct application to activated T cells mixed with target Raj B cells demonstrated IL-2 and IFN-γ release in response to the bispecific T-cell engager Blincyto® (EC50 of ~0.2 ng/mL). Further evaluation demonstrated Lumi™ cytokine immunoassays are scalable to 384-well format for HTS purposes, delivering excellent statistical performance (response CVs < 10%, Z’ values > 0.66). The implementation of this novel detection chemistry will enable rapid, "add-and-read" assays for cytokine detection for both low- and high-throughput screening applications.

2. Assay Principle for Lumi™ Cytokine Immunoassays

Recombinant NanoBIT™ luciferase subunits (SmBiT and LgBiT)
NanoBIT™-labeled 1st mAbs (mAb1-SmBiT and mAb2-LgBiT)
Sample (analyte) + NanoBIT™-labeled 2nd mAbs + furimazine substrate

• Very low-affinity interaction
• Negligible association
• Analyte-dependent mAb colocalization
• Facilitated reconstitution of NanoBIT™ luciferase
• Luminous assay signal proportional to analyte levels

3. Workflow for Direct Application to Cell Culture Wells

Plate and treat cells in microplate format
Add 5x mix of labeled mAbs directly to treated cell wells
Add Lumi Detection Reagent (with furimazine)
Record luminescence
Total assay time: ~70 min

6. Rapid Detection of Cytokine Released from PBMC

Lumi™ Immunoassay

• Optional sample transfer
• No sample dilution
• No wash steps
• 70 min for assay completion

Standard ELISA

• Sample transfer required
• Sample dilution required (15-fold)
• Multiple wash steps (8 total)
• > 5 h for assay completion

4. Excellent Sensitivity and Dynamic Range for Lumi™ Cytokine Immunoassays

A. Human cytokine standards dispensed in white, 96-well assay plates
B. Labeled mAbs and furimazine substrate added sequentially to assay wells
C. Luminescence signal recorded on a plate-reading luminometer (GloMax Discover)

Lower limits of detection (LLD) consistently < 10 pg/mL
Linear range of assays exceed three logs of cytokine concentrations

5. Dynamic Range Mitigates Need for Sample Dilution

A. Recorded plate data
B. Calculated cytokine levels

Human PBMC (100,000/well) treated with CSC, cytokine, and LgBiT reconstitutes bright luciferase
Assay reagents added directly to treated cell wells in 96-well format
Data interpolation using standard curve fit with 4-parameter logistic fit
Assay sensitivity and broad linear range delivers signal (A) proportional to released cytokine levels (B) over a very wide range of biological response

7. Cytokine Release Assessed in Mixed Culture Model Treated with Bispecific T Cell Engager

A. Cell model components
B. IL-2 release
C. IFN-γ release

• Assay reagents added directly to mixed cell culture model in 96-well format (A)
• Data interpolation using standard curve fit with 4-parameter logistic fit enables interpolation of IL-2 (B) and IFN-γ (C) levels produced in response to Blincyto® treatment
• Lumi Immunoassays enable simple and rapid determination of cytokine release associated with cytokytic activity of a biologic agent in a complex culture model

8. Assay Format Compatible with HTS Applications: Lumi IL-2 (Human) Immunoassay

A. Representative 384-well assay plate
B. 96-well plate
C. 384-well plate

• Human PBMC treated with CSC for 24 h
• IL-2 assay reagents added directly to 96 wells
• Scalable from 96-well (A) to 384-well (B) formats
• Z’ determinations in 384-well plates (C) indicates HTS compatibility of Lumi cytokine immunoassays

9. Summary and Conclusions

• Lumi™ cytokine immunoassays utilize NanoBIT™-labeled primary antibodies
• Binding of mAb1-SmBiT and mAb2-LgBiT to cytokine reconstitutes bright luciferase
• Homogeneous assay enables simplified detection of cytokines released from cell culture without the need for reagent addition to treated cell wells
• no transfer or wash steps required
• minimal protocol steps
• short execution times (~70 min)
• readings on standard plate-reading luminometer
• Excellent sensitivity (LLLOD < 10 pg/mL) and broad linear range (≥ 3 logs of cytokine concentration) mitigates need for sample dilution
• 384-well performance indicates assay amenable to HTS application

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