Lumit® Cytokine Assay Automation for High-Throughput Screening

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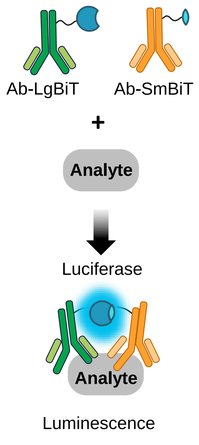
SLAS2024 Abstract # 1337-B

1. Introduction

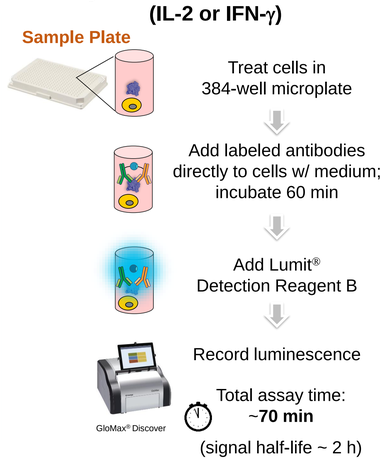
Lumit® technology enables a rapid, luminescent method for cytokine detection, ideal for high-throughput screening. These fully homogeneous Lumit® cytokine immunoassays eliminate the need for sample transfer or wash steps and are adaptable for automation in 384-well plate format. The assays offer a short turnaround time of approximately 70 minutes and utilize optimized volumes compatible with diverse liquid dispensing instruments. With a minimum detectable dose (MDD) under 10 pg/mL and a broad linear range extending over three logs of cytokine concentration, these assays significantly reduce the necessity for sample dilution. In this study, automated liquid handling was used to plate and treat human peripheral blood mononuclear cells (PBMCs) in 384-well format, as well as in the subsequent application of Lumit® cytokine immunoassays for assessment of IL-2 and IFN-γ release. Standard curves demonstrated excellent linearity and stable glow kinetics, with assay signal half-lives around 2 hours. Optimizing the number of PBMCs per well enabled us to achieve dose-response curves for IL-2 and IFN-γ release in response to Cell Stimulation Cocktail (CSC) treatment with signal-to-background (S/B) ratios exceeding 250-fold. Important for batch plate processing, we observed excellent S/B performance sustained for at least 2 hours post-signal generation. In summary, Lumit® cytokine immunoassays represent a streamlined, rapid, and effective methodology for cytokine detection. Their simplicity and adaptability for automation make them highly suitable for large-scale, high-throughput screening applications.

2. Assay Chemistry and Workflow

Lumit® assay chemistry



Cytokine assay workflow (IL-2 or IFN-γ)



3. Materials and Instrumentation Employed

Lumit® cytokine immunoassay sample and reagent volumes optimized for use with 384 well plates:

- 10 µl Human Peripheral Blood Mononuclear Cells (PBMCs)
  (For different cell numbers, 2-10 µl cells + medium to 10 µl total)
- 10 µl Cell Stimulation Cocktail (CSC) (Invitrogen™, Cat.#: 00-4970-93)
- 5 µl Lumit® immunoassay cytokine-specific antibody mixture
- 6 µl Lumit® Detection Reagent B

These volumes are suitable for dispensing with high precision on a variety of liquid handling instruments.

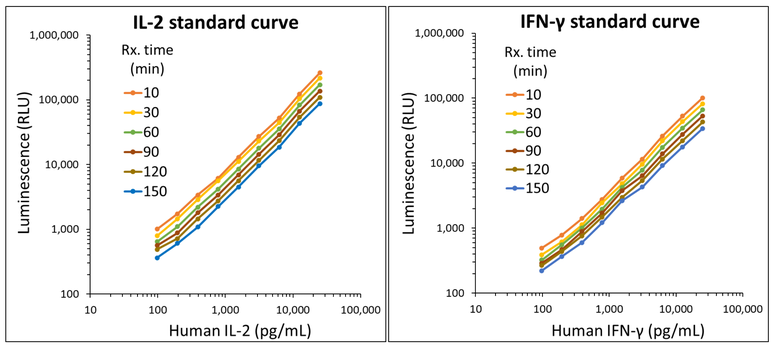
At Promega we used:

- Formulatrix Mantis for dispensing cells and Lumit® antibody mixtures
- Tecan Evo LiHa for Cell Stimulation Cocktail (CSC) serial dilutions
- MCA 96 tip head for 96- to 384-well plate transfer of CSC serial dilutions
- Thermo Fisher Combi_nL for Lumit® Detection Reagent B dispensing as well as for media backfill dispensings

For Luminescence signal acquisition, we used multi-functional microwell plate readers: Promega GloMax® Discover or Tecan Spark M20 Te-cool.

4. Recombinant Cytokine Standard Curves

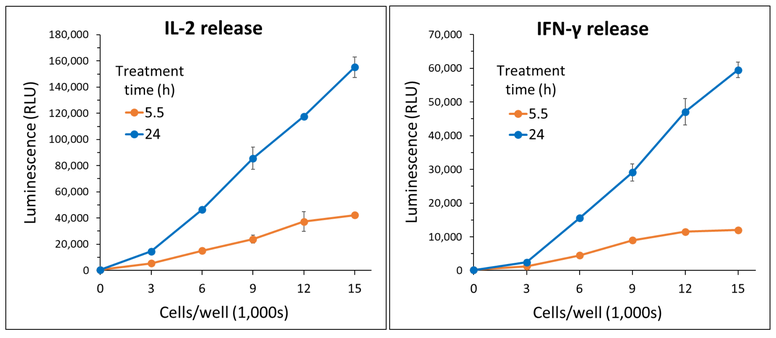
Cytokine standard curves assayed with Lumit® immunoassays.
Luminescence readings taken at various times after detection reagent addition.



- Lumit® cytokine immunoassay standard curves are linear over ~3 logs of cytokine concentration
- Assay signals exhibit glow kinetics ($T_{1/2}$ ~ 2 h)

5. Cytokine Release Observed Over Wide Range of Cell Number

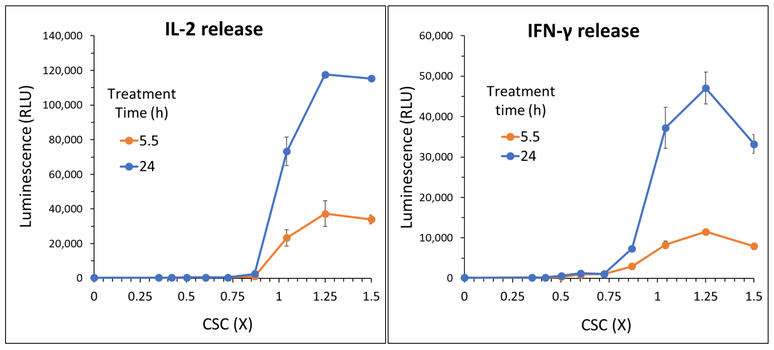
Max stimulated cytokine release (1.25X CSC) at short and longer treatment times was assayed at increasing numbers of PBMC dispensed per assay well.



- Assay signals increased with increasing cell number up to 15,000 cells/well.
- 12,000 cells/well was selected for subsequent assays to provide a large assay window and signals well within the linear ranges of the respective cytokine standard curves.

6. Dose Dependent Cytokine Release from PBMC

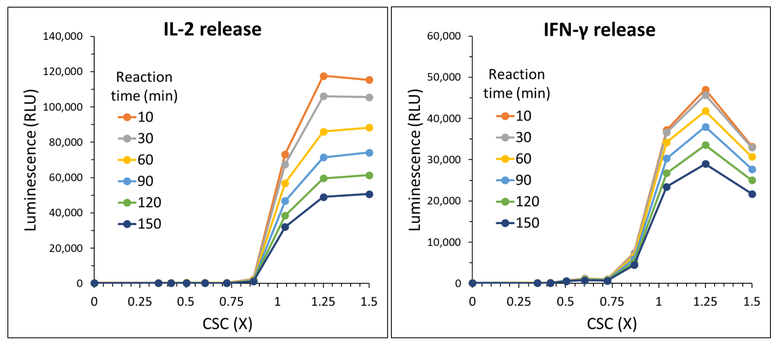
Human PBMC (12,000/well) dispensed in 384-well plates were treated with increasing concentrations of CSC followed by the application of Lumit® IL-2 and IFN-γ immunoassays.



- Dose-dependent stimulation of cytokine release was observed in response to CSC treatment at both short (5.5 h) and long (24 h) treatment times.
- While substantial signal was observed after short treatment time, 24 h treatment of PBMC with CSC provided a larger assay window.

7. Assay Signal Exhibits Glow Kinetics

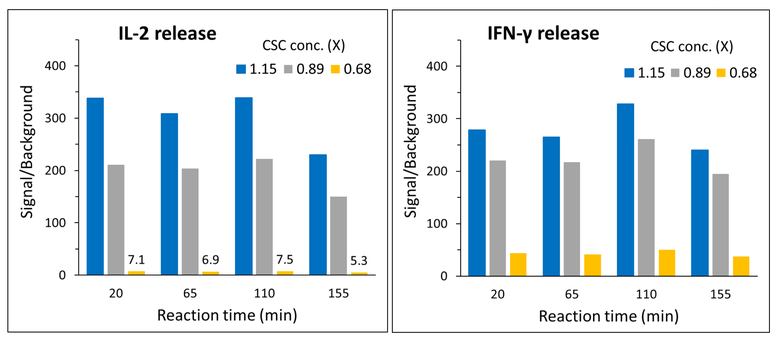
Human PBMC (12,000/well) treated with increasing concentrations of CSC for 24 h followed by the application of Lumit® IL-2 and IFN-γ immunoassays. Luminescence readings were obtained at increasing times (“reaction time”) after addition of detection reagent.



- Cell-based assay signals remain strong > 2 h, enabling batch plate processing.
- **Note**: Time-matched std curve enable accurate quantitation of cytokine release.

8. Strong Signal-to-Background Performance

Human PBMC (12,000/well) were treated with low, medium, and high concentrations of CSC for 24 h followed by cytokine assay. Luminescence readings were obtained at increasing times after addition of detection reagent.



- Maximum S/B values exceeded 250-fold for release of both cytokines.
- Both max and sub-max S/B values remained strong for > 2 h after detection reagent addition.

9. Conclusions

- Lumit® technology provides a fast (~ 70 min) and easy method for cytokine detection via luminescence detection.
- Lumit® cytokine immunoassay “add and read” protocol is very amenable to use of automation and 384-well plate format.
- Optimized Lumit® cytokine immunoassay volumes makes it easily compatible with diverse liquid dispensing instruments.
- Key assay performance characteristics:
  - excellent sensitivity (MDDs < 10 pg/mL)
  - broad linear ranges (≥ 3 logs of cytokine concentration)
  - very high signal/background ratios
  - assay signal glow kinetics ($T_{1/2}$ ~ 2 h)

The simple protocol and excellent performance of Lumit® cytokine immunoassays should make them very compatible with high-throughput screening applications.

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