Interleukin 2 Pathway Detection using the T Cell Activation Bioassay (IL2), Bio-Glo™ Luciferase Assay System and GloMax® Discover

Promega Corporation

Materials Required

- Bio-Glo™ Luciferase Assay System (Cat.# G7940)
- T Cell Activation Bioassay (IL2) (Cat.# J1651, J1655)
- GloMax® Discover System (Cat.# GM3000)
- White, 96-well assay plates (Corning Cat.# 3917)
- Blinatumomab Bispecific Antibody (Blincyto™; Amgen)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocol: *GloMax Discover System Technical Manual* #TM397 available at: **www.promega.com/protocols/**

Immune response signaling pathways are common targets for immunotherapy drug development. Several cytokine signaling targets play important roles in the immune system and the onset of autoimmune diseases. One such target is Interleukin 2 (IL2), which directly affects T cell differentiation and regulation.

The T Cell Activation Bioassay (IL2) with thaw-and-use cells provides a plate-based, homogeneous bioluminescent method for measuring IL2 pathway expression. Cells activating the IL2 pathway can easily be detected, and activation measured, using this bioassay with the GloMax® Discover System.

This Application Note describes a protocol to measure IL2 pathway expression using the T Cell Activation Bioassay (IL2) thaw-and-use cells with Blinatumomab Bispecific Antibody and the GloMax® Discover. The assay protocol integrated on the GloMax® Discover provides extended dynamic range and superior detection sensitivity. We also present results showing the sensitivity and reliability of the GloMax® Discover System compared to other plate readers.

T Cell Activation Bioassay (IL2) Protocol

For detailed instructions and protocol notes for various assay volumes and plate formats, see the *T Cell Activation Bioassay (IL2) Technical Manual* #TM492.

1. Plate 25µl of CD19-expressing Raji cells into white 96-well assay plates.
2. Add 25µl Blinatumomab (anti-CD3/CD19 bispecific Ab) diluted as described in the *T Cell Activation Bioassay (IL2) Technical Manual,* #TM492.
3. Add 25µl thaw-and-use TCR/CD3 Effector Cells (IL-2).
4. Incubate for 6 hours at 37°C, 5% CO_2 in a humidified incubator.
5. Equilibrate the assay plate and Bio-Glo™ Luciferase Assay Reagent to room temperature.
6. Add 75µl of Bio-Glo™ Luciferase Assay Reagent to the assay plate and incubate for 10 minutes.
7. Measure luminescence on the GloMax® Discover using the Bio-Glo protocol.

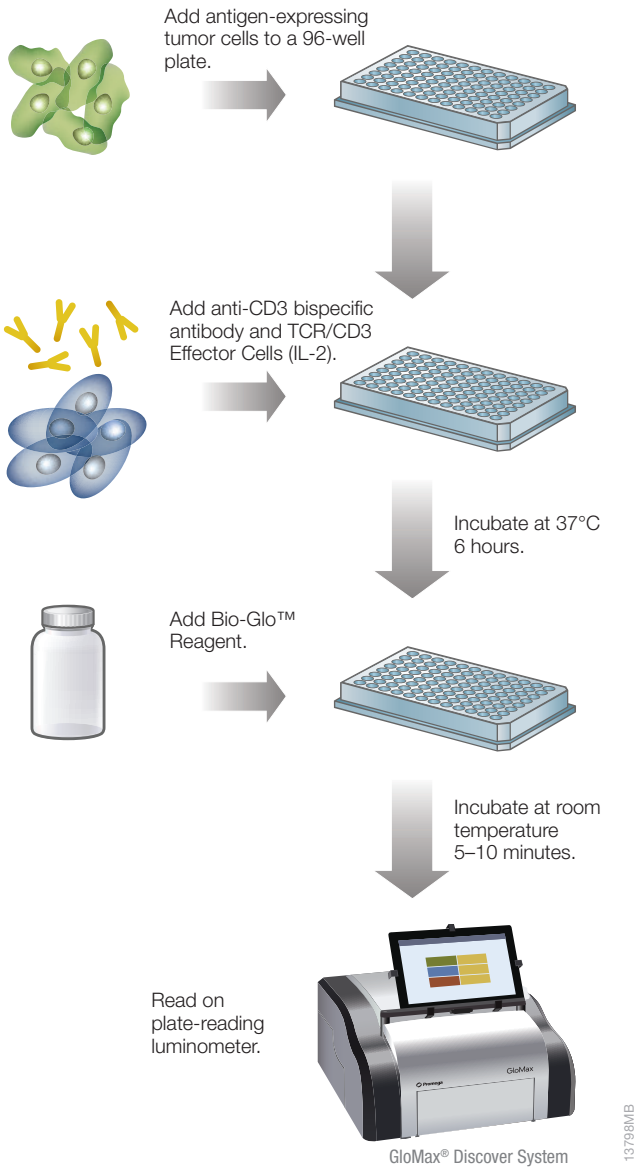

Figure 1. T Cell Activation (IL2) Bioassay Protocol.

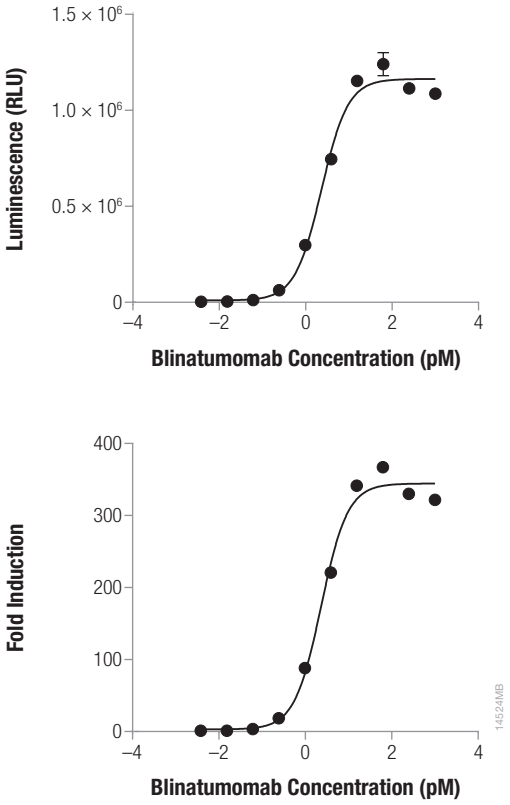

Figure 2. Thaw-and-Use T Cell Activation (IL2) Jurkat cells in Blinatumomab (CD3/CD19 Bispecific Antibody) Assay. Luminescence was determined using the GloMax® Discover System. Four-parameter logistic curve analysis was performed with GraphPad Prism® software. The calculated EC_{50} was 2.328pM. Similar data has been produced in 384- well plate assays (see *T Cell Activation Bioassay (IL2) Technical Manual* #TM492).

Plate Reader Comparison

Senstivity: Assay performance can be greatly affected by the plate reader used. We tested assay sensitivity using firefly luciferase and the Bio-Glo™ Luciferase Assay System, and compared performance of the GloMax® Discover and GloMax® Navigator instruments with that of several other plate readers.

Serial dilutions of QuantiLum® Recombinant Luciferase (Cat.# E1701) in 1X Passive Lysis Buffer (Cat.# E1941) with 1mg/ml BSA (Cat.# W3841) were used to assess instrument sensitivity. For each instrument, 100µl of each dilution was added to a 96-well white opaque plate in triplicate. All test plates were prepared at the same time using the same reagents and then frozen. For each instrument, three plates were thawed and assayed using the Bio-Glo™ Luciferase Assay System. Test plates and Bio-Glo™ Luciferase Assay Reagent were brought to room temperature and 100µl of Bio-Glo™ Luciferase Assay Reagent added to each well. After brief mixing, the plates were incubated inside the instrument for 10 minutes, and then luminescence was measured (Figure 3).

The limit of detection (LOD) was determined for each instrument based on a concentration of 4.1×10^{-18} moles of luciferase, which was within the linear range of the detection limit for all instruments. The following industry-recognized formula was applied to determine the instrument LOD:

LOD = [(Mean RLU at 4.1×10^{-18}) — (Mean RLU of blank well)] × [(3 × Standard Deviation of blank well)]

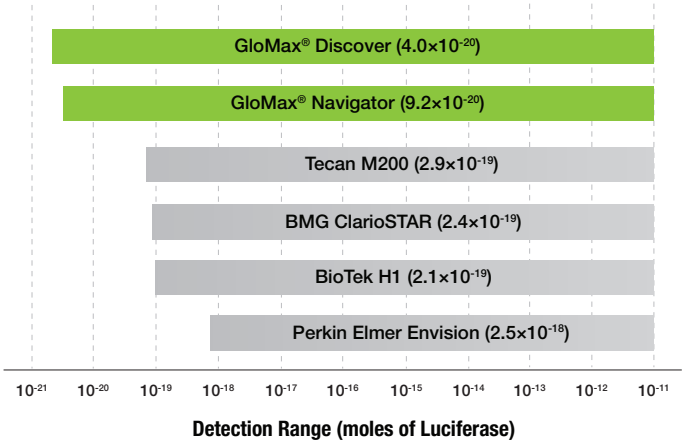

Figure 3. Sensitivity of Bio-Glo™ detection on GloMax® Systems and other commercially available plate readers.

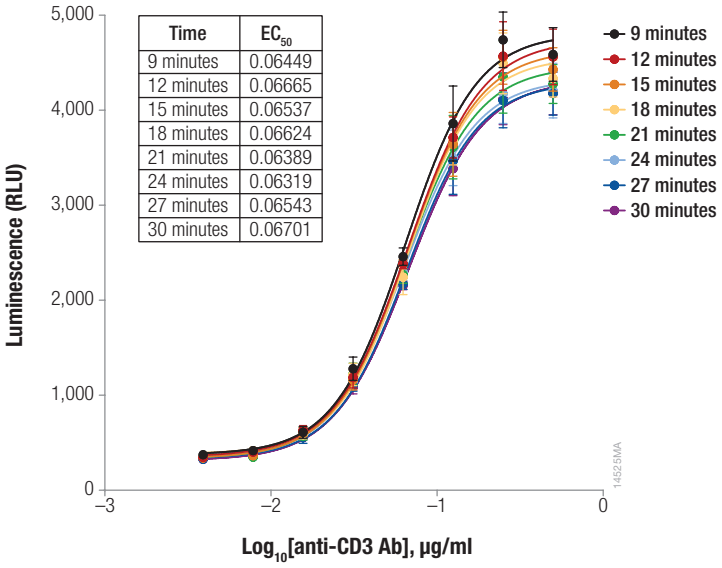

Figure 4. Sequential reads of T cell activation (IL2) Bio-Glo™ Assay with anti-CD3 antibody using the GloMax® Discover System.

Reproducibility: Read-to-read reproducibility was also tested to determine instrument reliability. Because GloMax® instruments exhibited the best limit of detection, GloMax® Discover was selected to evaluate read-to-read reproducibility. The IL2 pathway assay was performed within a single 96-well plate and luminescence measurements collected every three minutes for approximately 30 minutes. EC_{50} was determined for each plate read (Figure 4).

Conclusion

Here, we demonstrate use of the T Cell Activation (IL2) Bioassay and the GloMax® Discover System to detect and measure IL2 pathway expression in response to Blinatumomab Bispecific Antibody, as shown in Figure 2. Response time is dependent on the number and type of cells being studied, but the assay can easily detect IL2 pathway activation using 1×10^5 cells per well, resulting in a 10-fold signal-to-background induction. Thaw-and-use cells are frozen cells that can be plated right after thawing to eliminate the need to culture cells. This provides a convenient assay workflow, high reproducibility and low variability due to end-user plating.

GloMax® instruments were the most sensitive plate readers tested, exhibiting 10 to 100-fold greater sensitivity (Figure 3). GloMax® Discover also gave excellent reproducibility, with EC_{50} values remaining consistent after eight measurements collected over a 30-minute period (Figure 4). The small variation in the second half of the time course is attributed to the half-life of the Bio-Glo™ reagent, due to each time point within each curve containing very tight error bars.

In addition to these performance advantages, GloMax® instruments provide ease-of-use, pre-loaded Promega Bioassay protocols, Instrument and Operational Qualification services (IQ and OQ), the technical elements to comply with part 11 regulations (user authentication and authorization, data integrity and protection, electronic signatures and audit trails), and multiple data export formats for use in both research and manufacturing environments.

The IL2 Pathway assay was developed and optimized using the GloMax® Discover System due to the superior performance for assay sensitivity, dynamic range, cross-talk, ease-of-use, and reproducibility. This integrated bioassay provides confidence that even low-level IL2 pathway responses can be assessed successfully. Together, the T Cell Activation Bioassay (IL2) and the GloMax® Discover System provide a simple, sensitive assay for assessing immune response pathway responses.

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