PD-1+TIGIT Immune Checkpoint Detection with Bio-Glo™ Luciferase Assay System and GloMax® Discover

Promega Corporation

Materials Required

- PD-1+TIGIT Combination Bioassay (available by custom order)
- GloMax® Discover System (Cat.# GM3000)
- White, 96-well assay plates (Corning Cat.# 3917)
- Fetal Bovine Serum
- Anti-PD-1 antibody (Cat.# J1201)
- Anti-TIGIT antibody (eBioscience Cat.# 16-9500)

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

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

Immune checkpoint pathways are common targets for immunotherapy drug development. PD-1 (programmed cell death protein 1) and TIGIT (T cell immunoreceptor with Ig and ITIM domains) are immune checkpoint receptors that inhibit T cell activation. Binding of PD-1 to its ligand, programmed death ligand 1 (PD-L1), inhibits activation signals from the T cell receptor. TIGIT binds CD155, the ligand for the activating receptor CD226, but with greater affinity. The presence of TIGIT attenuates T cell activation by inhibiting the activation of CD226. Combination bioassays to block both PD-1 and TIGIT are an effective method for determining the potency of PD-1+TIGIT-targeted therapies during drug development.

The PD-1+TIGIT Combination Bioassay is a non-radioactive, plate-based, homogeneous bioluminescent method for measuring functional activity of PD-1 and TIGIT inhibitors. The assay consists of two cell lines—a PD-1+TIGIT Effector Cell line and an artificial antigen-presenting cell (aAPC) line. PD-1+TIGIT Effector Cells stably express PD-1, TIGIT and a luciferase reporter. Luminescence generated in the assay corresponds to T cell activation level. The aAPC cells stably express the ligands PD-L1 and CD155, as well as a T cell activator protein, which activates the PD-1+TIGIT Effector Cells in an antigen-independent manner. Co-incubation of the two cell lines results in low-level T cell activation (determined by luciferase activity). Addition of antibodies that block PD-1, TIGIT, or both, will enhance T cell activation and lead to increased luciferase activity.

This Application Note describes a protocol for measuring PD-1+TIGIT immune checkpoint inhibitor response using the PD-1+TIGIT Combination Bioassay and the GloMax® Discover System. 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.

PD-1+TIGIT Combination Bioassay Protocol

For detailed instructions and assay notes for various assay volumes and plate formats, see the PD-1+TIGIT Combination Bioassay Technical Manual. The following protocol is performed in 96-well plates.

1. Plate 100µl thaw-and-use PD-L1+ CD155 aAPC/CHO-K1 Cells into white 96-well assay plates. Incubate overnight at 37°C.
2. Remove media from plate.
3. Add 40µl of serially-diluted anti-PD-1 and anti-TIGIT antibodies and 40µl PD-1+TIGIT Effector Cells to wells containing PD-L1+CD155 aAPC/CHO-K1 Cells.
4. Incubate at 37°C for 6 hours.
5. Add 80µl of Bio-Glo™ Luciferase Assay Reagent and incubate for 10 minutes.
6. Measure luminescence on the GloMax® Discover using the Bio-Glo protocol.

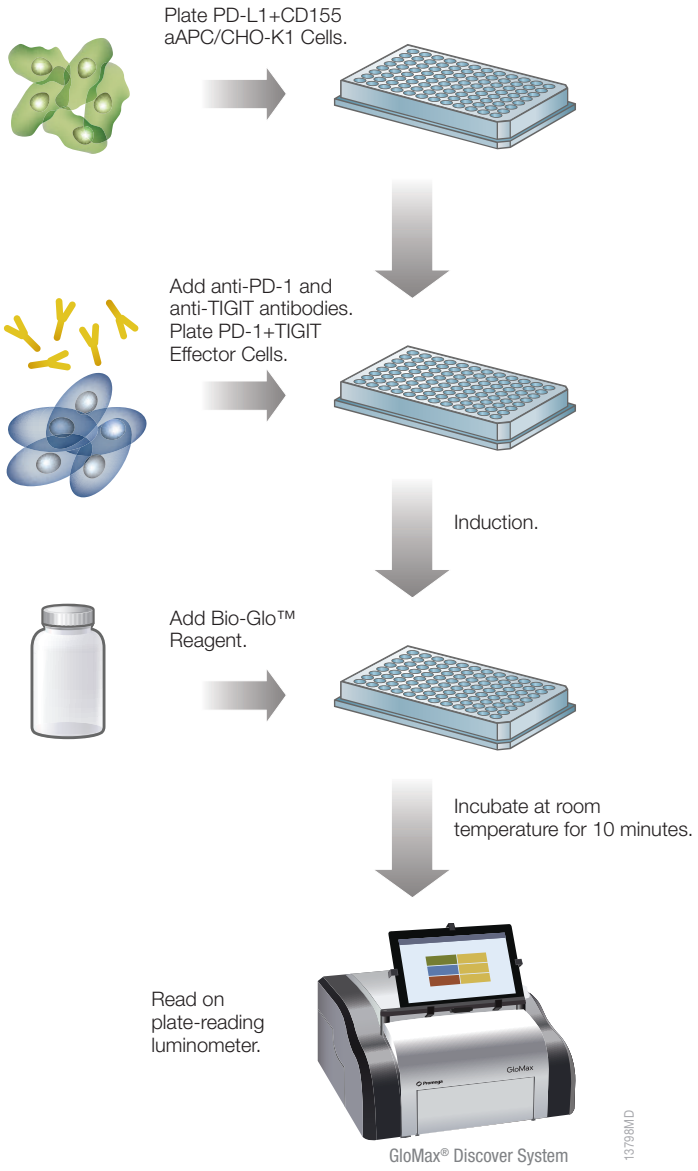

Figure 1. Schematic workflow for the PD-1+TIGIT Bioassay.

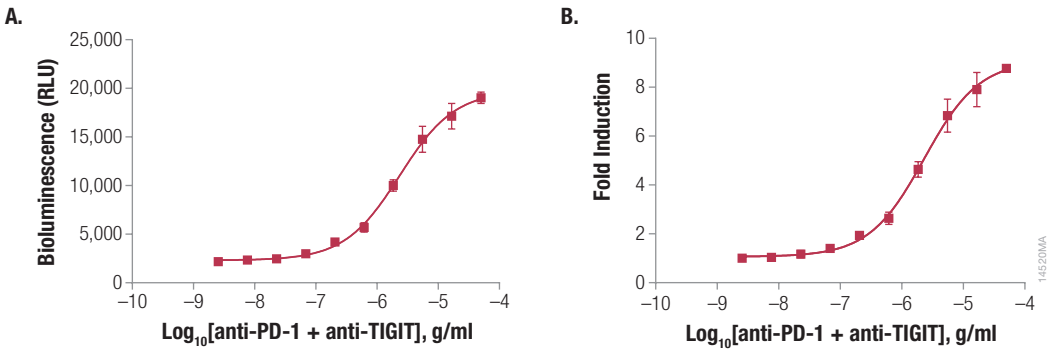

Figure 2. PD-1+TIGIT Combination Bioassay response to anti-PD-1 and anti-TIGIT antibodies. Luminescence was determined using the GloMax® Discover. Four-parameter logistic curve analysis was performed with GraphPad Prism® software..

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, then luminescence was measured (Figure 3).

The limit of detection (LOD) was determined for each instrument based on a concentration of $4.1x10^{-18}$ 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:

$$LOD = \frac{[4.18E\text{-}18]}{\text{Mean RLU at 4.1E-18} - \text{Mean RLU at blank}} \times (3 * \text{StDev of blank})$$

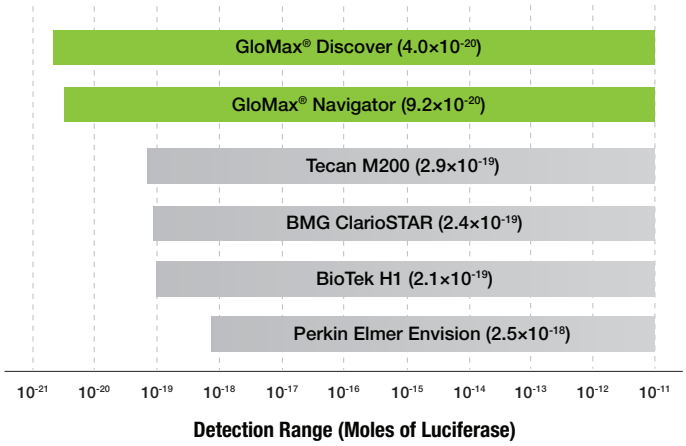

Figure 3. Sensitivity of Bio-Glo™ detection on GloMax® Systems and other commercially available plate readers.

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 PD1+TIGIT Bioassay was performed within a single 96-well assay plate and luminescence determined. Eight measurements were collected every three minutes for approximately 30 minutes. EC50 was determined for each plate read (Figure 4).

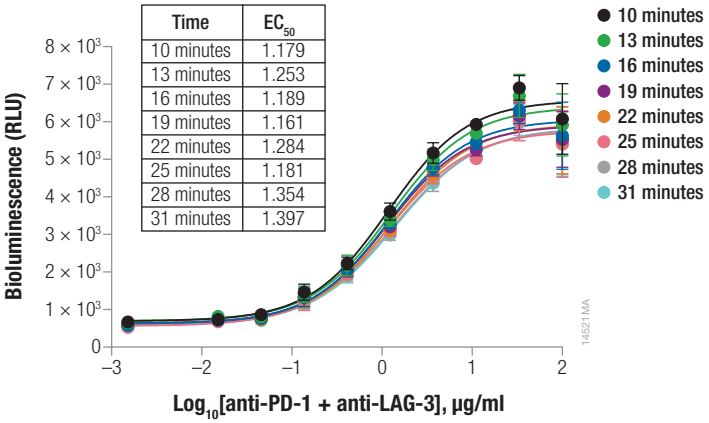

Figure 4. Sequential reads of the PD-1 + TIGIT Bio-Glo™ Assay using the GloMax® Discover System

Conclusion

The PD-1 + TIGIT Combination Bioassay measures the expected cellular response to anti-PD-1 and anti-TIGIT antibodies (1:1 molar ratio) as shown in Figure 2. A ninefold assay window is observed when performing a 6-hour assay using the GloMax® Discover System. Thaw-and-use cells are frozen cells, which can be plated right after thawing to eliminate the need to culture cells in the laboratory. This provides users with a convenient assay workflow and high reproducibility, ensuring 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 PD-1+TIGIT assay was developed and optimized using the GloMax® Discover System due to superior performance for sensitivity, dynamic range, cross-talk, ease-of-use, and reproducibility. This integrated bioassay provides confidence that even low level PD-1 + TIGIT cellular responses can be measured successfully. Together, the PD-1+TIGIT Combination Bioassay, and the GloMax® Discover System provide a simple, sensitive assay for immune checkpoint responses.

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