Miniaturization of a Bioluminescent PD-1/PD-L1 Blockade Bioassay Demonstrates Potential Application to Drug Research and Screening in Early Development of Immunotherapeutics

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1. Introduction

Programmed death receptor-1 (PD-1) and its ligand (PD-L1) are promising drug targets for cancer immunotherapy. We have developed a luminescent reporter-based PD-1/PD-L1 Blockade Bioassay, which can be used to measure the potency, during the research and development stages, of therapeutic drugs targeting PD-1 or the PD-L1 ligand. Two engineered stable cell lines were made: the PD-1 Effector cells that express human PD-1 and a luciferase reporter driven by NFAT-response element, and PD-L1+ cells, which are engineered aAPC Cells expressing human PD-L1.

Assay Principle

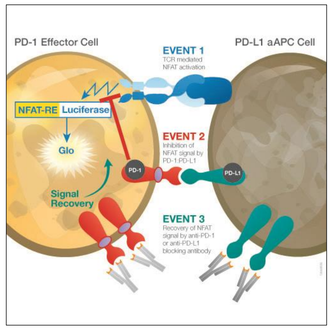
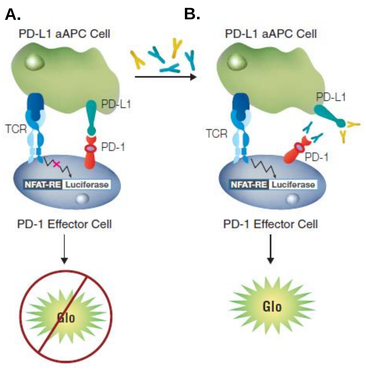


Figure 1. The PD-1 /PD-L1 Blockade Bioassay measures antibodies targeting PD-1 or PD-L1.

2. Luminescence Output Correlates with Presence of Therapeutic Entity

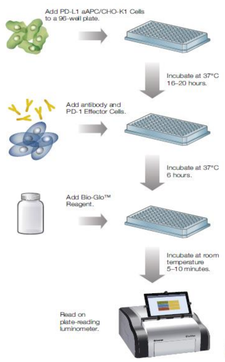


A. In the absence of a blocking antibody, co-culture of PD-L1+ aAPC Cells and Effector cells leads to TCR-mediated transcriptional activation of the NFAT pathway. Simultaneously, signal suppression by PD-1/PD-L1 engagement results in no luminescent signal.

B. TCR-mediated signaling is restored in the presence of a blocking Ab to either PD-1 or PD-L1. Suppression of NFAT pathway activation and generation of luminescent signal are removed.

Figure 2. The effect of blocking antibody on assay signal.

3. Assay Workflow is Amenable to 96 through 1536-well Plate Formats



The PD-1/PD-L1 Blockade Bioassay workflow is compatible with 96- to 1536-well assay plates. PD-L1+ aAPC cells are added to the assay plate and allowed to recover overnight. Antibody, PD-1 Effector cells, and Bio-Glo™ Reagent are added the following day and light is detected with the GloMax® Discover System.

For 1536-well format, PD-L1+ aAPC cells are allowed to attach for 2 hours, followed by addition of antibody and Effector cells and a 24 hour incubation. Bio-Glo™ Reagent is added the following day and light detected with the Tecan M1000.

Figure 3. PD-1/PD-L1 Blockade Bioassay Workflow.

4. Thaw-and-Use Cells Show Equivalent Performance to Cells Grown in Culture

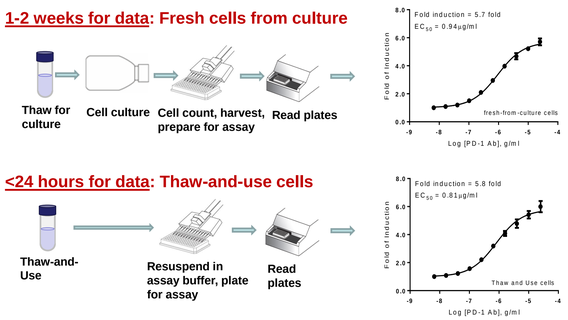


Figure 4. Thaw-and-use cells show equivalent performance to cells grown in culture. Additional benefits of time and consumable savings are also achieved.

5. The Potencies of Commercially Available Therapeutic Antibodies Can Be Quantified

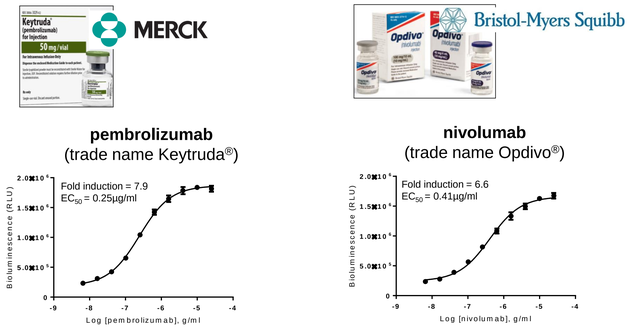


Figure 5. The PD-1/PD-L1 Blockade Bioassay has been verified for use with therapeutic antibodies.

6. Equivalent Antibody Potencies Are Achieved in High Density Plate Formats

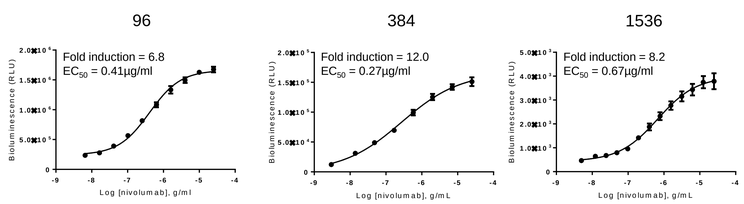


Figure 6. The PD-1/PD-L1 Blockade Bioassay is amenable to volume scaling from 96 to 1536 well plate formats. Antibody potencies are equivalent and fold induction is preserved. For the 384-well assays, a Tecan Freedom EVO® MCA 384 was used to perform antibody titrations and array to the assay plate, and a Thermofisher Multidrop™ Combi nL was used for cell and reagent addition. For the 1536 assay, the Combi nL was used for all additions to the plate.

7. Interleaved Antibody Titrations on the Assay Plate Show No Positional Effects on Assay Quality

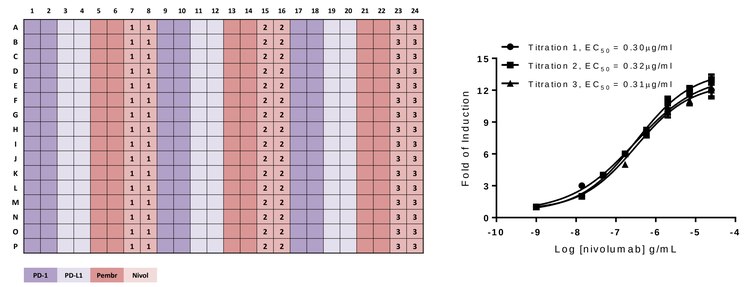


Figure 7. Equivalent EC_{50} for nivolumab was achieved at various locations in the 384-well plate indicating robust assay performance regardless of the position within the assay plate, including edge wells. The inclusion of edge wells enables full use of the assay plate with no constraints to using inner wells only, enabling more assay points for screening. %CV of less than 5% was achieved across each titration.

8. DMSO Tolerance Highlights Potential Use for Small Molecule Inhibitor Screening Applications

PD-1/PD-L1 Blockade Bioassay tolerates DMSO (up to 2.0%)

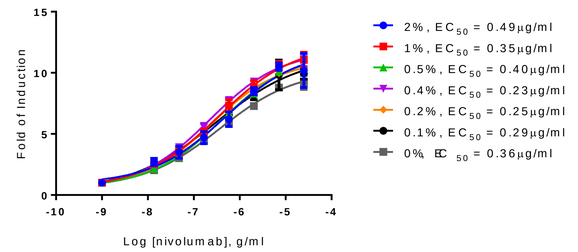


Figure 8. Equivalent antibody potency and fold induction in 384-well format are achieved with nivolumab in up to 2% DMSO. This tolerance suggests that the cells and detection reagent can be used for screening small molecules stored in DMSO, a commonly-used solvent in chemical library preparation.

9. Conclusions

Mode of action is easily quantified

- Simple workflow includes thaw-and-use cells and minimizes dispense steps.
- The effects of both PD-1 and PD-L1 blocking therapeutics can be quantified.

Robust assay performance achieved

- Scalability of assay from 96 to 1536 well format.
- No positional effects noted, enabling full use of the assay plate.

DMSO tolerance up to 2%

- Assay is compatible with a common solvent used in small molecule screening applications.

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The PD-1/PD-L1 Blockade Bioassay is for Research Use Only.