Reporter Bioassays to Assess Therapeutic Antibodies in Development for Immunotherapy Programs

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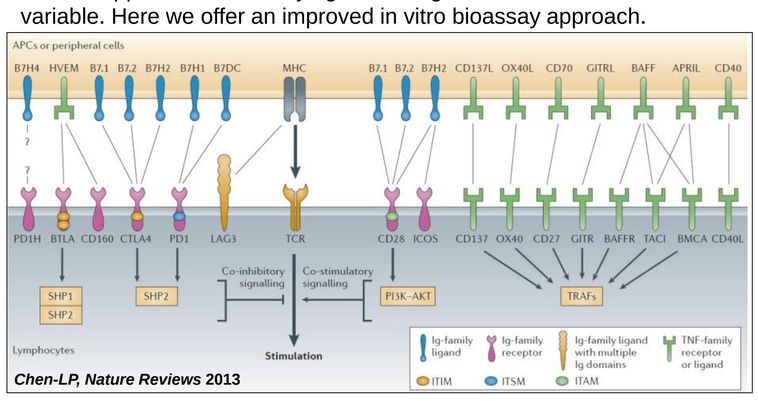
1. Introduction

Immunotherapy, also called *biologic therapy* or *biotherapy,* stimulates certain parts of the immune system to fight diseases such as cancer. Important drug targets in immunotherapy include:

- Co-inhibitory receptors: PD-1/PD-L1, CTLA-4, LAG3, Tim3
- Co-stimulatory receptors: GITR, CD40, OX40, 4-1BB

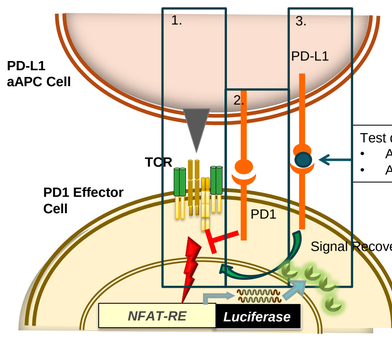
Current approaches to assaying these targets are cumbersome and variable. Here we offer an improved in vitro bioassay approach.



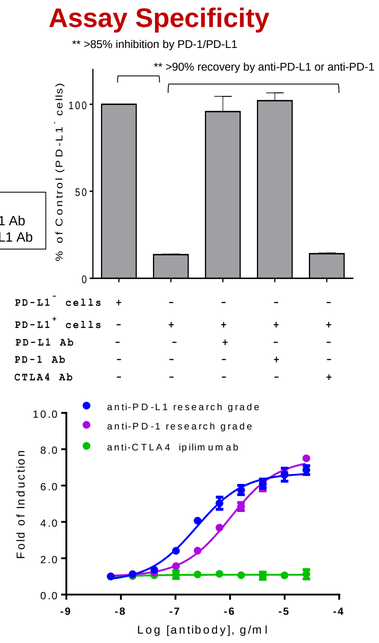
Chen-LP, Nature Reviews 2013

2. PD-1/PD-L1 Blockade Bioassay Principle and Specificity

Assay Principle



Assay Specificity



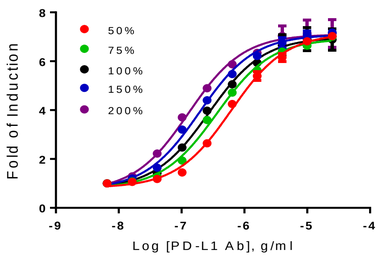
Three major events occur in the Promega homogeneous PD-1/PD-L1 Blockade Bioassay

1. TCR-mediated NFAT induction
2. Inhibition of NFAT signal by PD-1:PD-L1 ligation
3. Recovery of NFAT signal by anti-PD-1 or anti-PD-L1 blocking antibody

3. Assay Repeatability and Linearity Using Thaw-and-Use Cells

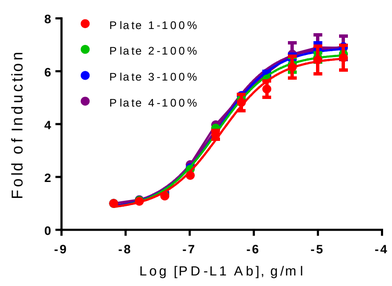
Relative Potency

Plate 1: 100% vs 50%
Plate 2: 100% vs 75%
Plate 3: 100% vs 150%
Plate 4: 100% vs 200%

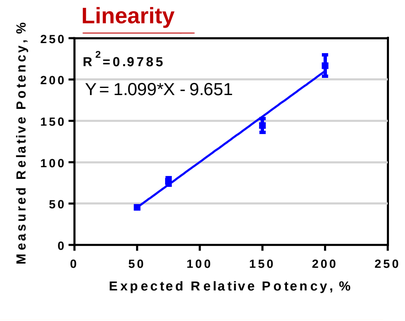


Measuring relative potency for anti-PD-L1 Ab showing assay repeatability and linearity in the range of 50% - 200%.

Repeatability

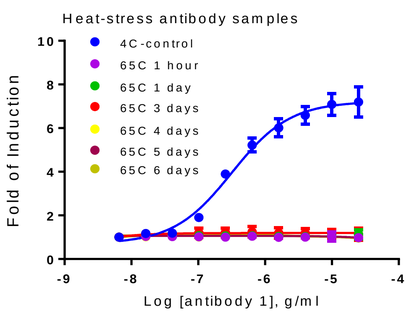


Linearity

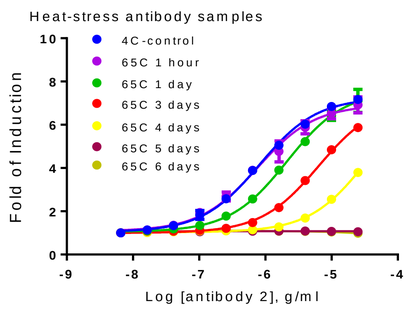


4. PPD-1/PD-L1 Blockade Assay is Suitable for Antibody Stability Study

Antibody #1



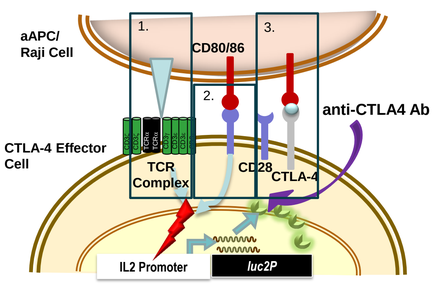
Antibody #2



Detecting loss of reporter response to PD-1 or PD-L1 blocking antibody after heat-treatment: Anti-PD-1/PD-L1 blocking antibody #1 and #2 were heat stressed at either 42°C or 65°C for indicated times before application to thaw-and-use cells in the PD-1/PD-L1 Blockade Bioassay. Loss of reporter response to heat-stressed anti-PD-1/PD-L1 blocking antibody after heat-treatment was detected.

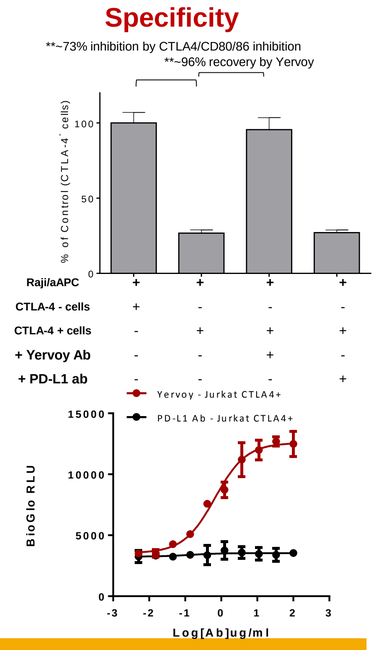
5. CTLA-4 Blockade Assay Principle and Specificity

Assay Principle



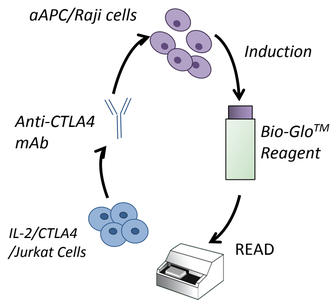
1. By co-cultivating the two cell lines, Jurkat IL2 pathway is activated via antibody crosslinking of TCR activator/TCR complex.
2. CTLA-4 binds to CD80/86 with greater affinity than CD28, resulting in blocking T-cell activation.
3. Blockade of CTLA-4:CD80/86 interaction with anti-CTLA-4 mAb can reactivate IL2 pathway in a dose-dependent manner.

Specificity

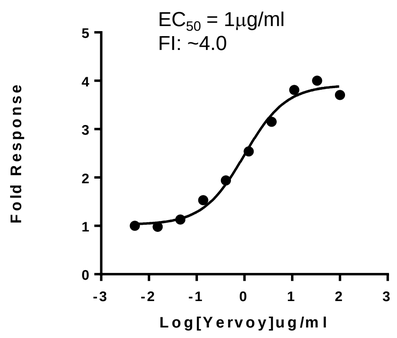


6. CTLA-4 Blockade Bioassay Protocol

Assay Protocol



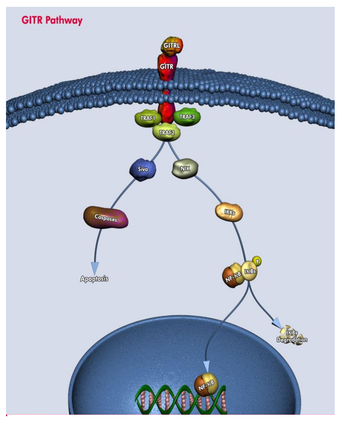
Sample Data



CTLA-4 blockade bioassay: GloResponse™ IL2-luc2P/CTLA4 Jurkat cells were seeded at 100K per well in a solid, white 96-well plate. Next, 3X Yervoy™ CTLA-4 blocking antibody serial diluted 1:3 was added into cell wells at 25µl/well. Finally, aAPC/Raji cells were seeded at 5K/25µl per well. The plate was incubated for 6 hours at 37°C. After 6 hours, 75µl of Bio-Glo™ Luciferase Assay Reagent were added and luminescence was measured using GloMax® Multi+ luminometer. Four-parameter logistic curve analysis was performed with GraphPad Prism software. EC_{50} was calculated at 1µg/ml.

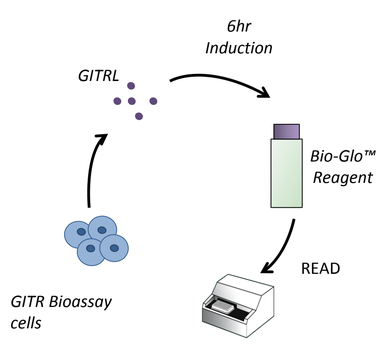
7. GITR/GITRL Assay Principle and Protocol

GITR/GITRL Mechanism of Action



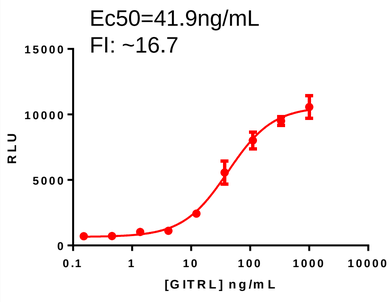
NFκB pathway activation by GITR stimulation

Assay Protocol

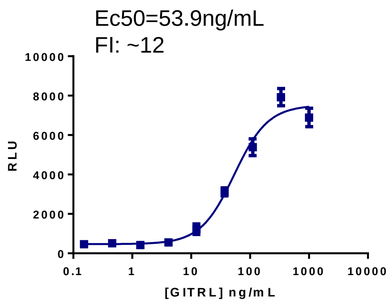


8. Thaw-and-Use GITR Cells Provide Equivalent Assay Performance

Fresh from culture cells



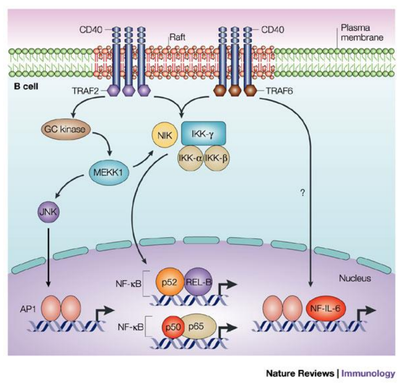
Thaw-and-Use Cells



Detection of GITR ligand-induced NFκB pathway activation: Jurkat/GITR cells were plated in 96-well plate at 100K cells/well. Cells were incubated with various concentrations of cross-linked GITRL. After 6 hours induction at 37°C, Bio-Glo™ Luciferase Assay Reagent was added and luminescence was determined using a GloMax® Multi+ luminometer. Four-parameter logistic curve analysis was performed with GraphPad Prism® software.

9. CD40/CD40L Bioassay Principle and Protocol

CD40/CD40L Mechanism of Action



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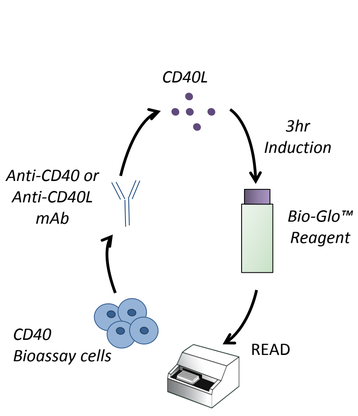
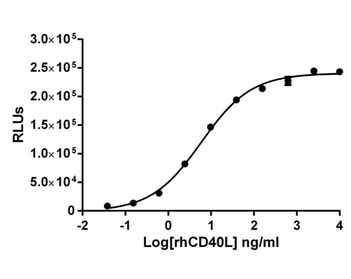
AP1/NFκB pathway activation by CD40 stimulation

Assay Protocol

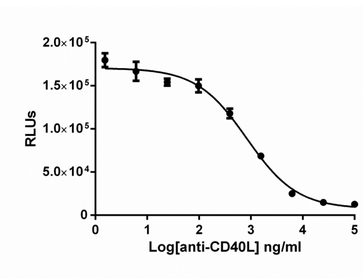


10. CD40 Cells Are Responsive to Ligand induction and Ab Inhibition

Activation by CD40L



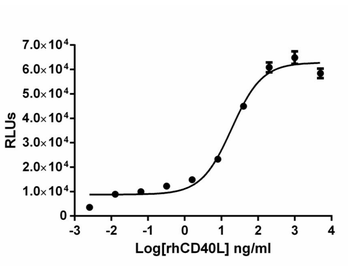
Inhibition by Anti-CD40L Ab



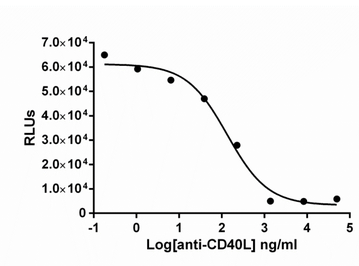
CD40/CD40L bioassay: Cells were incubated with varying concentrations of CD40 ligand (left panel), or EC_{80} of CD40L (R&D Systems) in the presence of varying concentrations of anti-CD40L antibody (BioLegend, right panel). After 3 hours induction at 37°C, Bio-Glo™ Luciferase Assay Reagent was added and luminescence was determined using a GloMax® Multi+ luminometer.

11. Thaw-and-Use CD40 Cells Provide Equivalent Assay Performance

Activation by CD40L



Inhibition by Anti-CD40L Ab



Thaw-and-Use CD40/CD40L assay: Cells were thawed, incubated overnight with varying concentrations of CD40 ligand (left panel), or EC_{80} of CD40L (R&D Systems) in the presence of varying concentrations of anti-CD40L antibody (BioLegend, panel B). Bio-Glo™ Luciferase Assay Reagent was added and luminescence was determined using a GloMax® Multi+ luminometer.

12. Conclusions

The bioluminescent reporter-based bioassays presented here are designed to reflect mode of action with great assay specificity, repeatability and linearity. The assays can be used to measure relative potencies for antibodies targeting many co-inhibitory and co-stimulatory receptors as well as detect potency changes for stressed antibody samples.

- We have built multiple assays using a single bioluminescent, reporter-based platform that can be used to rapidly measure potencies of many different biological immunotherapy drugs in development including PD1/PD-L1, CTLA4, GITR and CD40.
- We demonstrate that these bioassays reflect mode of action of each drug.
- These cell based bioassays can be used for testing antibody stability and to quantify potencies of on-market monoclonal antibody drugs for cancer.

Contact: CAS@Promega.com for additional inquiries