A Homogenous PBMC ADCC Bioassay Enables Bridging Studies with ADCC Reporter Bioassays in Immunotherapy Monoclonal Antibody Development

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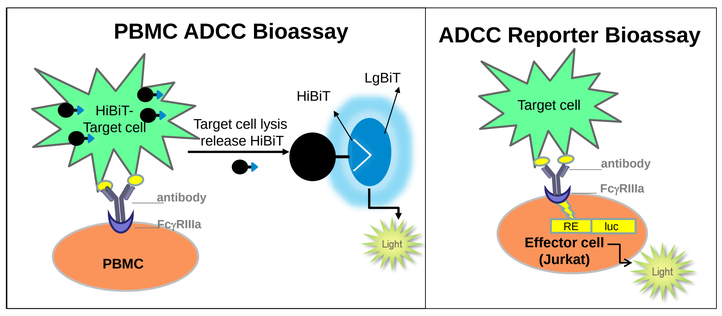
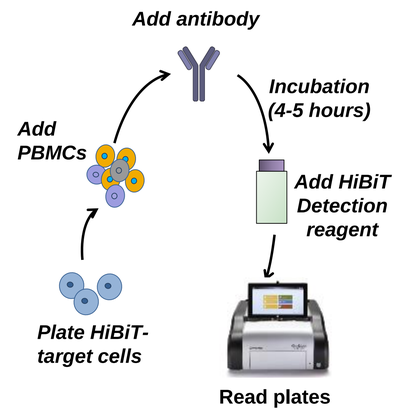
1. Introduction

- Antibody-dependent cellular cytotoxicity (ADCC) is a key mechanism of action for therapeutic antibodies.
- Previously, we developed a surrogate ADCC Reporter Bioassay using engineered reporter effector cells and demonstrated its suitability for antibody product release and stability study.
- Here we developed an improved ADCC assay using PBMC and engineered HiBiT-target cells for use in antibody early characterization and enable ADCC method bridging studies.



	PBMC ADCC Assay	ADCC Reporter Bioassay
Effector cells	PBMCs, ADCC-prequalified	ADCC Reporter Effector cells
Target cells	Antigen+ tumor cell lines expressing HiBiT	Antigen+ tumor cell lines
Read-out	Extracellular HiBiT activity from target cell lysis	Luciferase activation in Effector cells
Application	Antibody discovery and characterization	Lot release, stability study

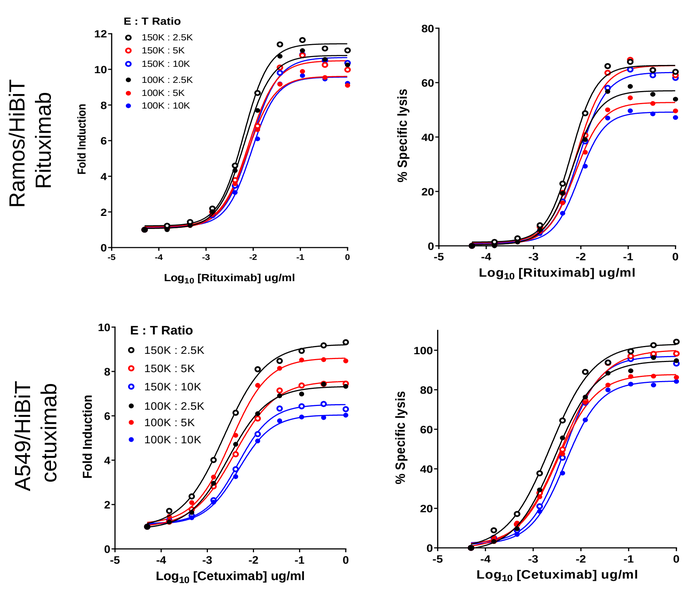
2. PBMC ADCC Bioassay Workflow and Features



Assay procedure
1. Plate HiBiT target cells
2. Add PBMCs
3. Add test antibodies
4. Incubate for 4-5 hours
5. Add HiBiT Detection reagent
6. Read plates

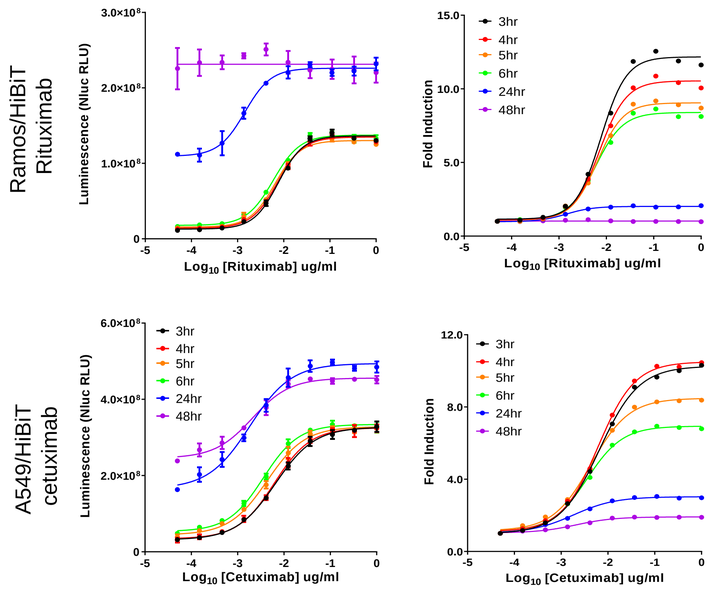
Features
- ADCC-prequalified PBMCs
- Low spontaneous release (<10% MR)
- Measurement of target cell-specific killing
- Simple, homogenous and fast
- Sensitive and robust

3. E:T Ratio Optimization



PBMCs were incubated with Ramo/HiBiT and rituximab, or with A549/HiBiT and cetuximab at the E:T ratio and cell density per well as indicated.
- Fold of induction was determined as RLU(antibody)/RLU(no antibody).
- % Specific lysis was determined as % [specific lysis-spontaneous release)/(maximum lysis- spontaneous release)].

4. Incubation Time Optimization



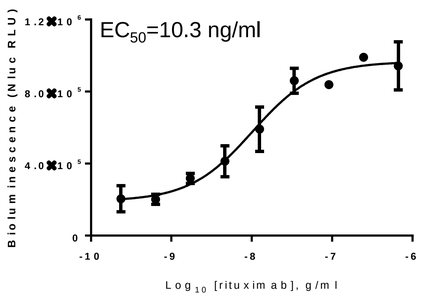
PBMCs were incubated with Ramo/HiBiT and rituximab, or with A549/HiBiT and cetuximab at the time points as indicated. The E:T ratio was 20:1. Fold of induction was determined as RLU(antibody)/RLU(no antibody).

5. ADCC Method Comparison in Relative Potency Determination and Stability Study

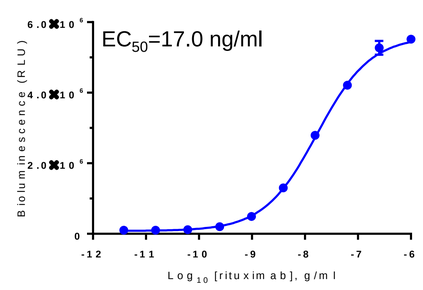
Both assays showed similar trend in to measuring antibody relative potencies and potency changes for heat-stressed antibody samples.

6. ADCC Bridging Study for anti-CD20 Antibody Rituximab

A. PBMC ADCC Bioassay using Raji Cells (HaloTag-HiBiT)



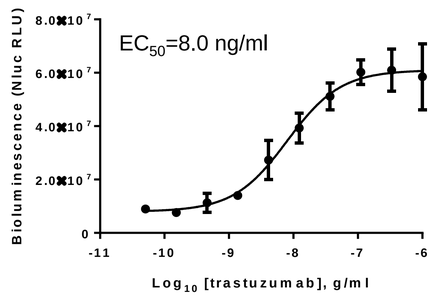
B. ADCC Reporter Bioassay using Raji cells



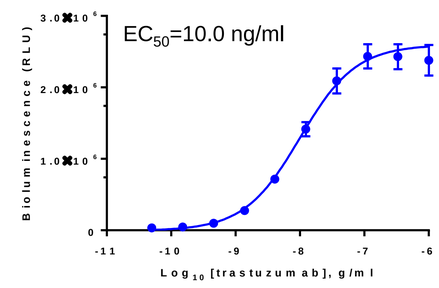
Potency determination for anti-CD20 mAb rituximab in PBMC ADCC Bioassay using Raji Cells (HaloTag-HiBiT) **(A)** and ADCC Reporter Bioassay using Raji target cells **(B)**.

7. ADCC Bridging study for anti-HER2 Antibody Trastuzumab

A. PBMC ADCC Bioassay using SK-BR-3 Cells (HaloTag-HiBiT)



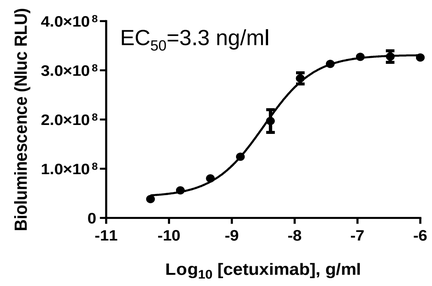
B. ADCC Reporter Bioassay using SK-BR-3 cells



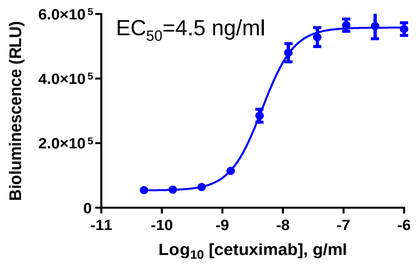
Potency determination for anti-HER2 mAb trastuzumab in PBMC ADCC assay using SK-BR-3 Cells (HaloTag-HiBiT) (**A**) and ADCC Reporter Bioassay using SK-BR-3 target cells (**B**).

8. ADCC Bridging study for anti-EGFR Antibody Cetuximab

A. PBMC ADCC Bioassay using A549 Cells (HaloTag-HiBiT)



B. ADCC Reporter Bioassay using A549 cells



Potency determination for anti-EGFR mAb cetuximab in PBMC ADCC assay using A549 Cells (HaloTag-HiBiT) (**A**) and ADCC Reporter Bioassay using A549 target cells (**B**).

9. Conclusions

We developed an improved ADCC assay using primary PBMC and engineered HiBiT target cells for antibody characterization and drug development.

- **ADCC-prequalified PBMCs**
- **Measurement of target cell-specific killing**
- **Optimized assay protocol, easy-to-implement**
- **Sensitive and robust assay window**
- **Showed similar trend in measuring antibody relative potency and potency change for heat-stressed antibody samples.**
- **Quantitative readout of antibody potency comparable with ADCC Reporter Bioassay**
- **Enables ADCC method bridging studies from antibody discovery and characterization to lot release**

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