In vitro assessment of Fc functional activity: A broad range of solutions for diverse assay needs

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Introduction

The success of many cancer therapeutics lies in their ability to induce ADCC, ADCP and CDC against specific targets, thus, appropriate assessment is crucial before progressing a drug into the clinic. Demonstrating lack of unwanted effector functions is also important for safety purposes. Here, we consider key aspects for developing successful Fc effector functional assays and weigh up the benefits and challenges of a panel of assay formats for different purposes. We present options for choosing the most suitable target cell lines, effector cells and appropriate controls.

Isotype and effector function

An important consideration for ***any*** mAb or Fc-based therapies is the choice of Ig class/ isotype required.

Different IgG isotypes offer different functions:

ADCC	**G1/G3 > G2 > G4**
Complement	**G3 > G1 > G2 > G4**

Fc regions can also be further modified to modulate their properties through:

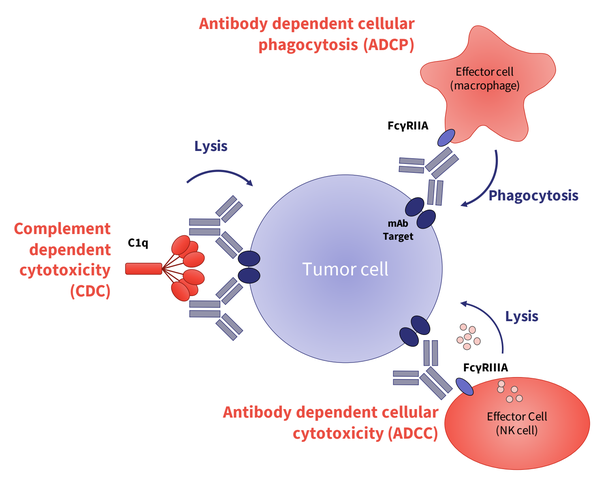
- Altered pharmacokinetics, either increasing (e.g. “YTE”, “LS”) or decreasing half-life through modulation of FcRn binding;
- Enhanced (e.g. “DLE”, “GASDALIE”) or reduced/removed (e.g. “LALA”, “LALA-PG”) effector function;
- Improved stability (e.g. IgG4 S228P).
- Note: Mutations may be covered by 3rd party IP

Assessment of FcγR and FcRn function

Along with the choice of isotype, the ability to assess the capacity of antibodies to bind to FcγRs provides fundamental insights into mechanisms of action, half-life and off-site toxicity.

Effector function is primarily triggered through interactions with C1q or FcγRs. The human FcγR family consists of:

- Activating receptors (hFcγRI, hFcγRIIa, hFcγRIIc, and hFcγRIIIa)
- Inhibitory receptor (hFcγRIIb)
- Receptor with unknown function (hFcγRIIIb)
- Receptor involved in recycling and transport of IgG among other functions (hFcRn)



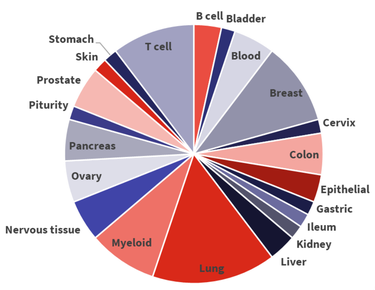
A range of assay options are available to investigate effector function, from binding and reporter assays to flow-based assays with PBMC effectors and Incucyte-based assays with primary macrophages.

Assessment of target cell line suitability

Central to evaluation of effector function is choosing the appropriate target cells

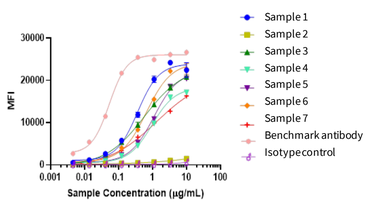
Tumour cell bank

Abzena has access to diverse panel of tumour cell lines. Please enquire for latest list of available cell lines.



Cell line QC

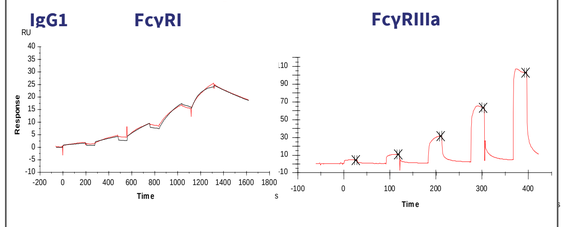
As part of any assay establishment, binding of the therapeutic to the tumour cells will be determined using flow cytometry.



Abzena recommends selecting a target cell line with high antigen expression

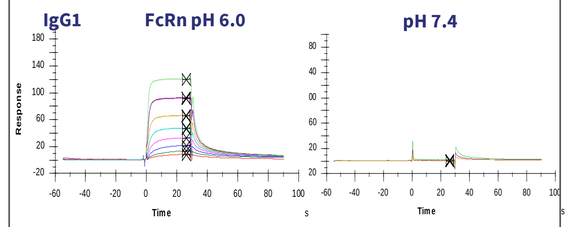
Fc-mediated binding by Biacore assessment

FcγR Biacore



- Fc receptors are captured on an anti-His sensor chip and increasing concentrations of antibody are flowed over
- Trastuzumab is used as positive control for the assay
- Assays can be performed on panels of **human** and **mouse** FcγRs (including IIa and IIIa allotypic variants)

FcRn Biacore

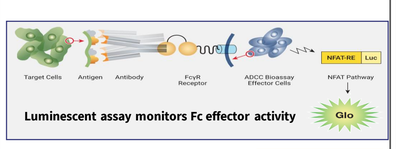


- FcRn is immobilised on a CM5 sensor chip and increasing concentrations of antibody are flowed over at either pH 6.0 or 7.4
- Trastuzumab is used as positive control for the assay
- Assays can be performed on panels of human and mouse FcRn

Utility: Determine/compare binding of candidates to receptors (SPR / Biacore)

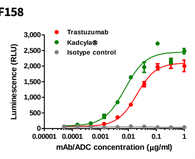
ADCC assays

Reporter Assay

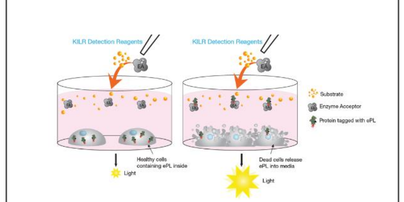


Promega

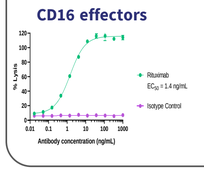
- High affinity **FcγRIIIa$_{V158}$**
- Low affinity **FcγRIIIa$_{F158}$**
- Engineered effectors
- Sensitive and reproducible
- Recommended for screening/ranking



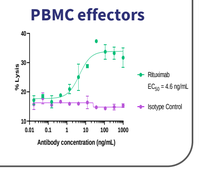
KILR Assay - Reporter/PBMC



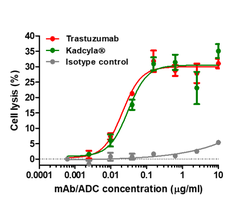
CD16 effectors



PBMC effectors



PBMC Assay – Flow-based

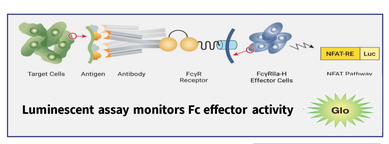


- PBMC effector cells from healthy donors
- Readout dead dye incorporation
- Recommended for safety assessment of mAb

Utility: Determine ability of therapeutics to induce ADCC

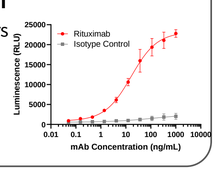
ADCP assays

Reporter Assay

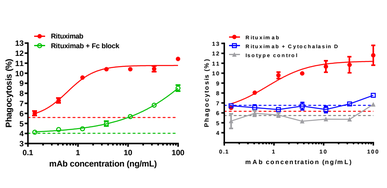


Promega

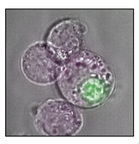
- **FcγRIIa$_{H131}$** or **FcγRI**
- Engineered effectors
- Sensitive and reproducible
- Recommended for screening/ranking



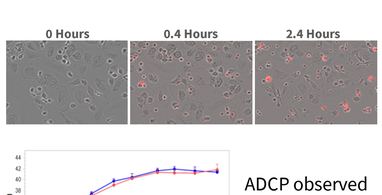
Flow cytometry



- Monocytic cell line effectors
- Readout double positive events
- Recommended for safety assessment of mAb



IncuCyte

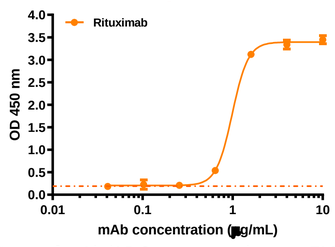


ADCP observed in real time. Unlabelled macrophage and pHrodo-labelled target cells

Utility: Determine ability of therapeutics to induce ADCP

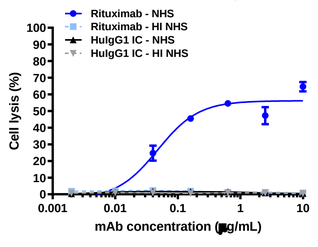
Complement assays

C1q ELISA



- Titrations of mAb/ADC are coated on an ELISA plate.
- Purified human C1q is then allowed to bind before detection using an HRP-labelled anti-huC1q antibody.
- Rituximab is used as positive control for the assay.

Complement Dependent Cytotoxicity (CDC)



- Cancer cell lines are incubated in the presence of human serum containing active complement and a titration of mAb/ADCs.
- Dead-cell protease activity, is measured as an indicator of cell lysis.

Utility: Determine ability of therapeutics to induce CDC

Summary

Understanding the mode of action of a drug candidate is essential for every drug development program.

Abzena has developed a comprehensive suite of assays to support the characterization of the Fc-mediated activity of antibodies, Fc fusions or ADC products.

Each assay can be tailored to the specific requirements of a project and our team of experts will work with you to provide solutions to suit your specific needs, considering aspects such as mode of action, assay sensitivity, complexity and regulatory requirements.