

Assessing Effector Function in Biologics

What is Effector Function & Why is it Important?

Antibody effector function is an important part of the immune response and forms an essential link between innate and adaptive immunity.

The success of many therapeutic antibodies lies not just in their ability to bind to specific targets, but also in their ability to induce effector function including ADCC, ADCP and CDC and to kill the cells expressing the target. By way of contrast, for certain modalities, effector function may be undesirable and even pose a safety risk. Thus, performing an appropriate assessment of effector function is crucial before progressing a biological drug into the clinic. Indeed, many regulatory bodies now require an assessment of Fc receptor binding and functional ADCC, CDC, and ADCP assays.

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 provide options for choosing the most suitable target cell lines, effector cells and appropriate controls.

Types of Effector Function

The most well-known Fc-mediated antibody effector functions are antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC). Most effector functions are mediated via the constant (Fc) region of the antibody through interactions with C1q or specialized Fc gamma (Fc γ) receptors. The human Fc γ R family consists of:

- Activating receptors (hFc γ RI, hFc γ RIIA, hFc γ RIIC, and hFc γ RIIIA)
- Inhibitory receptor (hFc γ RIIB)
- Receptor with unknown function (hFc γ RIIB)
- Receptor involved in recycling and transport of IgG (hFcRn)

Antibody-dependent cell cytotoxicity (ADCC): ADCC is a cell-mediated immune mechanism where an effector cell actively lyses and kills the antibody-bound target (e.g. a tumour cell). ADCC requires an effector cell, such as natural killer (NK) cells that interact with IgG antibodies via Fc γ RIIIa receptors.

Antibody-dependent cellular phagocytosis (ADCP): ADCP is a mechanism of elimination whereby antibody-opsonized target cells recruit macrophages via binding to Fc γ IIa to induce phagocytosis, resulting in internalization and degradation of the target cell.

Complement-dependent cytotoxicity (CDC): CDC is an effector mechanism where antibodies trigger the complement system to kill target cells. When antibodies bind to antigens on a target cell, the classical complement pathway is triggered by binding C1q, resulting in the formation of a membrane attack complex (MAC) which creates pores in the cell membrane and ultimately causes cell lysis.



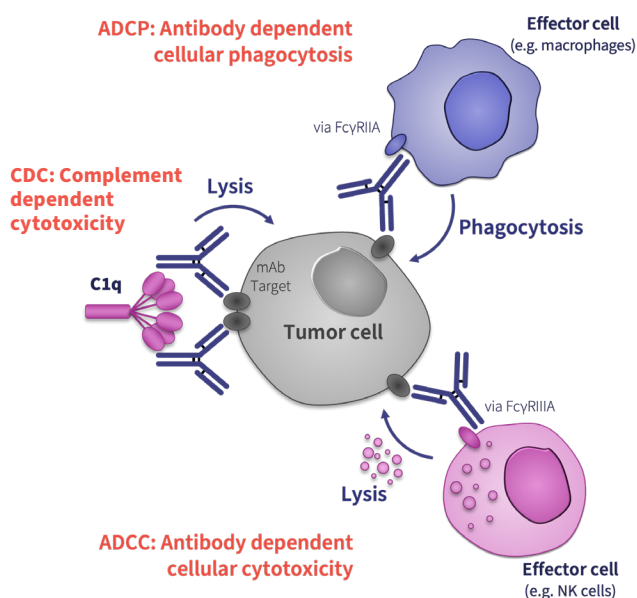


Figure 1: Effector function mechanisms of therapeutic monoclonal antibodies

How to Select the Right Assay?

Selecting the right assay can be complex and depends upon what question you are trying to answer and what stage of development you are at. At Abzena, we have a suite of assays available ranging from higher throughput, lower cost binding assays to more complex (but with increased biological relevance) cell-based assays.

Tiers of Assays

Binding: Binding assays represent the simplest types of assays and are typically used in the early stages of discovery and development as they are low cost and provide a rapid method of assessing the ability to bind to different Fc receptors—useful if testing e.g. different Fc modifications.

- **Biacore™ Assessment of mAb Binding to FcRs:** Assesses binding to individual or a full range of Fc gamma receptors (e.g. human FcγRI, FcγRIIa (H131 and R131 allotypes), FcγRIIb, FcγRIIIa (V158 and F158 allotypes), FcγRIIIb as well as FcRn). FcRs from different species can also be assessed (murine, human, cyno etc.)
- **C1q Binding:** Uses an ELISA-based format to assess the binding of test samples to C1q protein

Reporter Assays and Engineered Cell Lines: Reporter assays provide a good balance of cost and relevance as they use a cell-based readout to assess effector function. They are both sensitive and reproducible and are useful at earlier stages of discovery and are typically recommended for ranking and screening.

- **Engineered effector reporter cell lines** are incubated with the appropriate target cell line and FcR engagement is monitored by a luminescence readout. Reporter assays include: FcγRIIIaV158 and FcγRIIIaF158, Mouse FcγRIV (ADCC) and FcγRIIIaH131, FcγRIIIaR131 and FcγRI (ADCP).
- **Engineered target cell lines** using HiBiT or KiLR® technologies are incubated together with primary human effector cells and target cell lysis is monitored by a luminescence readout.

PBMC-Based Assays: These assays aim to provide a meaningful and relevant readout using primary effector cells to mimic the physiological response in vivo and are typically used for lead candidate selection as well as for safety assessment of mAbs.

- **In vitro primary ADCC assays** assess the ability of a therapeutic to mediate lysis of target-expressing cells by primary human effector cells (PBMC or freshly isolated NK cells)
- **In vitro primary ADCP assays** assess the ability of a therapeutic to mediate phagocytosis of target cells by either a monocytic cell line (using flow cytometry) or macrophages (using the Incucyte® S3 Live cell imaging system).
- **In vitro CDC assays** assess the ability of a therapeutic to mediate lysis of target-expressing cells by normal human serum.

Advanced / Complex Assays: Beyond the standard PBMC-based assessments, more advanced complex assays are increasingly becoming used. Whilst often considered as being less “off-the-shelf” and requiring a degree of optimization, these assays aim to be the most representative of complex cellular environments as well as providing an in-depth understanding of the mechanism of action.

- Kinetic ADCC assay via live cell imaging to assess kinetics of tumor elimination via distinct mechanisms (e.g. payload- and immune-mediated killing in the case of ADCs)
- ADCC assay using 3D spheroids to model solid tumour environments
- ADCC assay using mixed target populations to model heterogeneous tumour environments



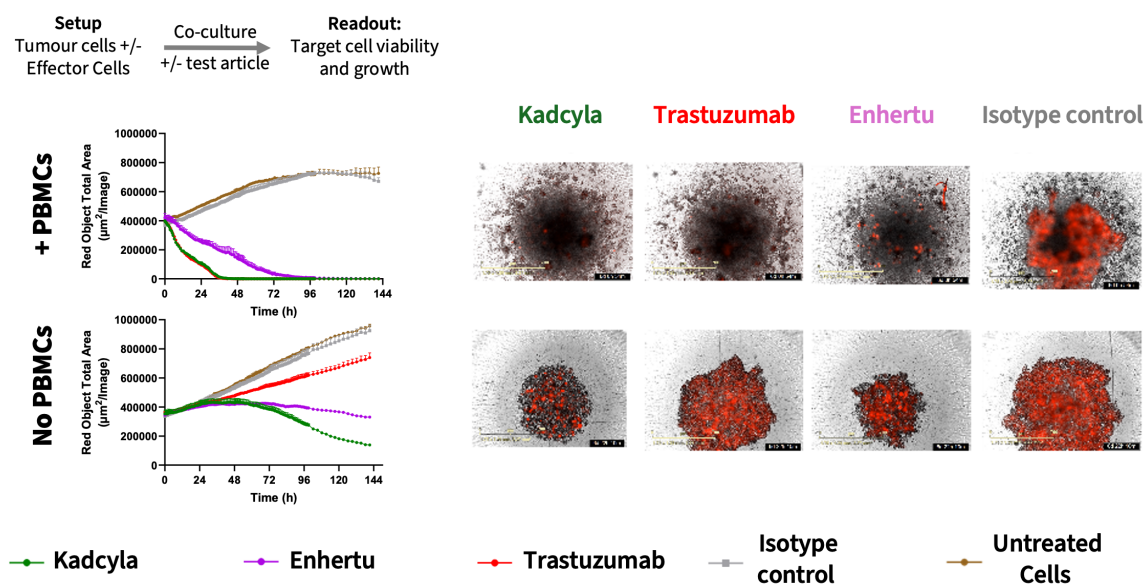


Figure 2: Time course of payload and immune-mediated tumour cell killing using 3D tumour spheroids.
In the presence of immune cells, Trastuzumab, Kadcyla and Enhertu induce ADCC, whereas in the absence of immune cells, tumour cell killing is only observed with the two ADCs, Kadcyla and Enhertu.

Safety Assessment Using Cytokine Release Assays: In rare cases, antibody therapeutics can promote the release of pro-inflammatory cytokines in a phenomenon known as cytokine release syndrome (CRS). This excessive release of cytokines can lead to endothelial damage and multi-organ failure and so clearly poses a major safety risk. Several well documented cases have provided clear examples of how crucial it is to broadly evaluate the impact on FcR binding and on wanted and unwanted Fc-mediated effector functions.

- Abzena's **Cytokine Screen™** Cytokine Release assay is used to assess the likelihood of drugs to stimulate cytokine release using either a whole-blood or a PBMC-based approach.

FcRn Binding: The neonatal receptor (FcRn) binds to the Fc region of IgG antibodies at acidic pH within endosomes and is involved in recycling of IgG. This recycling is the primary reason for the extended half-life (several weeks) of IgG in serum, one of the critical factors in the success of therapeutic antibodies. Therefore, the FcRn-IgG interaction is a key parameter to optimize and track throughout the antibody drug development process.

- Biacore® assessment of mAb binding to FcRn:** Assesses binding of IgG to FcRn at both neutral and acidic pH, mimicking differences in the extracellular and intracellular environments. Different species are also available (murine, human, cyno etc.)

Trusted Data

Quality data generation requires a deep understanding of the assays since assays can be complex with many different factors influencing assay performance. Within Abzena we use our many years of experience to provide data you can trust.

- Quality Data generation:** Data trending and continual monitoring of assay performance
- Effector cells:** Abzena is Human Tissue Authority (HTA) licenced and has an extensive in-house cell bank of PBMC from healthy donors which are pre-screened for suitability for ADCC and ADCP activity
- Target cells:** Experience with diverse panels of tumour cell lines.
- Target binding confirmation:** As part of any assay establishment, binding of the therapeutic to the tumour cells will be determined using flow cytometry to confirm target binding and establish antigen expression levels.
- A wide range of assay platforms available:**
 - Biacore®
 - Flow cytometry
 - Imaging (Incucyte® S3 live cell imaging, confocal and super-resolution microscopy)
 - Plate readers (luminescence, absorbance, fluorescence)

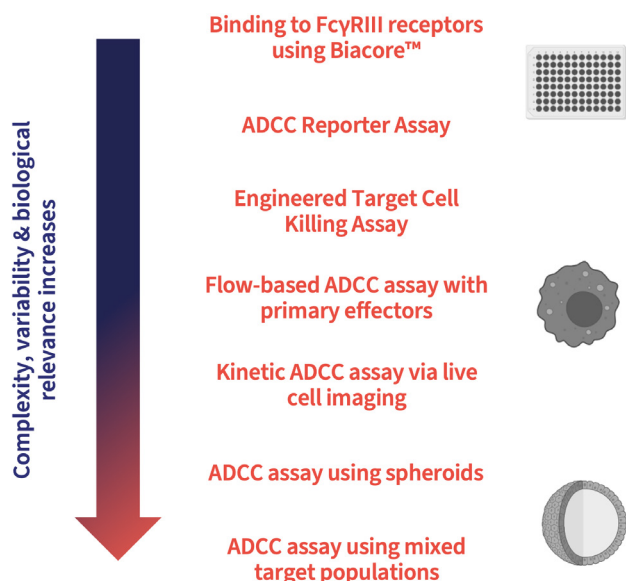


Figure 3: Example assay hierarchy for assessing ADCC

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 your 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.

Effector Function & Fc Engineering

Intricately associated with the ability to assess effector function is the ability to design molecules with different effector properties. Besides the choice of the isotype (e.g. IgG1, IgG4 etc), protein engineering of the Fc domain can be used to further fine tune the antibody effector function, as well as to improve safety and biodistribution through:

- Altered pharmacokinetics, either increasing or decreasing half-life through modulation of FcRn binding;
- Enhanced or reduced/removed effector function;
- Improved stability

Abzena has significant experience in Fc engineering to design antibodies with the appropriate properties.



Take your project to the next level

At Abzena, we appreciate that every biologic and every scenario is different. With our extensive experience, we guide customers through the complexities of antibody development, from early identification of potential challenges to successful product creation. Our tailored strategies and innovative approaches aim to meet your objectives and ensure the development of effective, safe, and high-quality therapeutic biologics. To learn how our comprehensive suite of assays can help your project reach its next target inflection point with greater success, visit **Abzena.com**.

CAMBRIDGE, UK | BRISTOL, PA, USA | SAN DIEGO, CA, USA

ABZENA.COM