Streamlining the Development of Relative Potency Assays for ADCs

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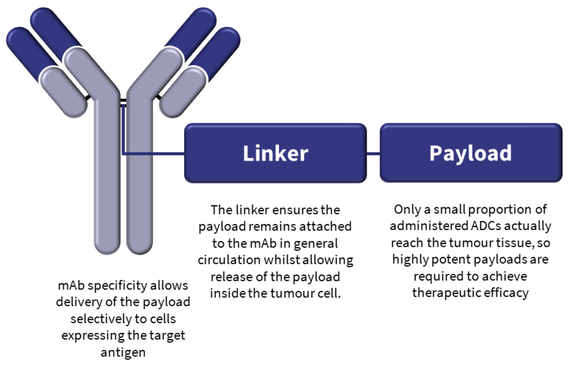
Abstract

Relative potency assays are a critical part of a drug's lifecycle. They are designed to ensure that the efficacy and potency of manufactured batches, drug substance and drug product is maintained throughout drug development and once the drug is in the market. This is particularly important for biologics with complex MoAs. For example, a successful antibody drug conjugate (ADC) requires multiple steps including binding to its target, internalization, release of payload intracellularly, and elimination of tumour cells via the payload's mode of action. An ideal relative potency assay captures all of these processes, and reports on the final biological outcome.

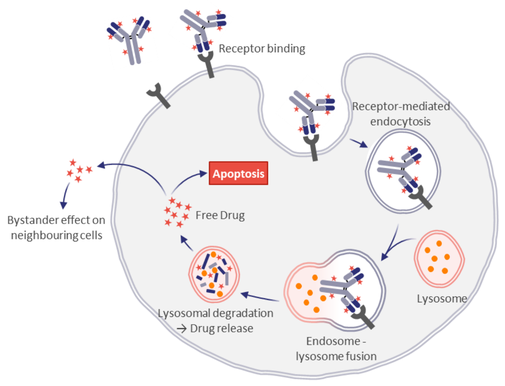
At Abzena, we offer a phase-based approach for relative potency assay development based on the needs of our customers and where they are in their drug development lifecycle and provide GMP-ready testing once the assay is validated. Here we present Abzena's approach on developing relative potency assays for an ADC biosimilar. At the initial stages of the project, an antigen-binding ELISA was developed to support the testing of early batches and formulation samples for pre-clinical testing. To progress the project for clinical testing, a cell-based cytotoxicity assay was developed to support lot release testing as well as stability and toxicology studies. We present our approach for early feasibility studies, assay development, as well as validation. This streamlined process minimizes the risks, timelines and costs to develop and validate phase-appropriate relative potency bioassays for ADC biosimilars.

Antibody Drug Conjugates

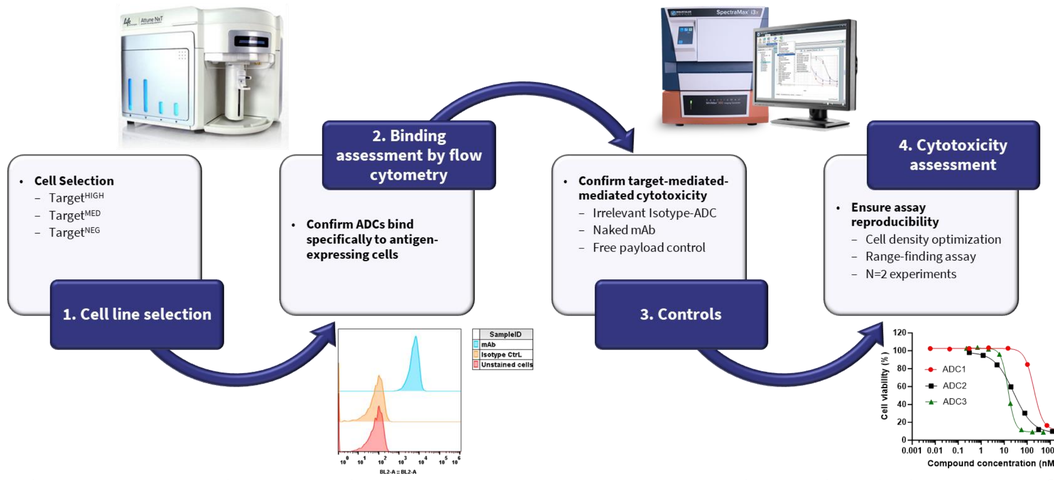
ADCs combine the key design elements of small & large molecules:



ADCs have a multi-step mechanism of action with specific requirements for each step / component.



Thorough Development Strategy for Assay Design



Abzena has a comprehensive development process captured in detailed reports for IND submissions.

The data package Abzena provides validates the use of target cell lines for MoA reflective cytotoxicity

Phase-Appropriate Assay Development

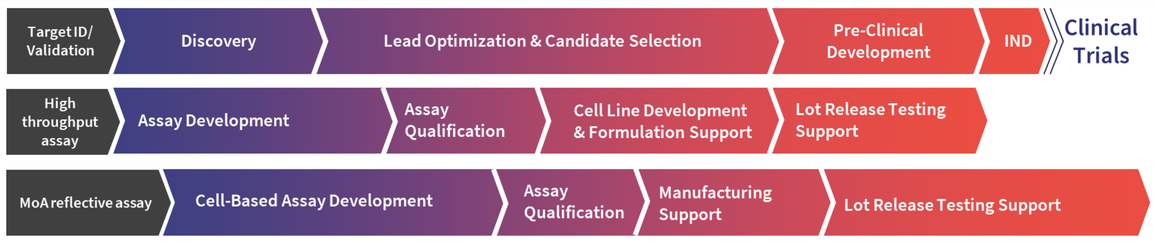


Figure 1: A timeline of Abzena's phase-based approach to assay development to ensure a suitable bioassay is available at the appropriate stage of drug development.

Report Provided for IND Data Package

- *In vivo* assay
- Cell-based assay – primary cells
- Cell-based assay – cell line
  - Phenotypic assay
  - Reporter / activation assay
- Binding assays
  - Cell-based
  - Biochemical
- Physiochemical assays

Clinical Relevance

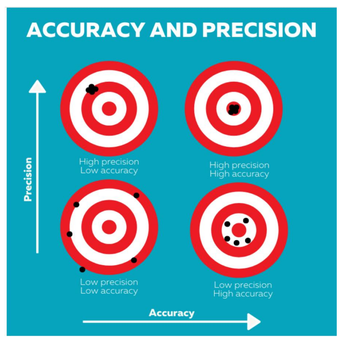
Suitability for QC

To reduce timelines and provide the critical support needed throughout drug development, Abzena develops bespoke assays to meet the exact needs of its clients.

As needs require, Abzena develops more complex assays to satisfy regulatory requirements to proceed to clinical trials.

Abzena strikes a balance when developing an assay that is:

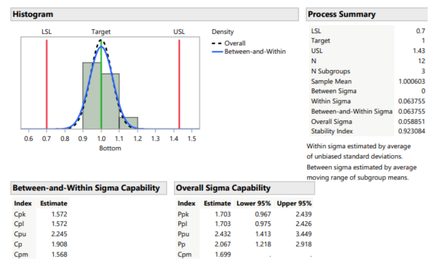
- Representative of the critical quality attributes (CQAs) required at the given stage of drug development.
- Accurate and precise to satisfy regulatory requirements

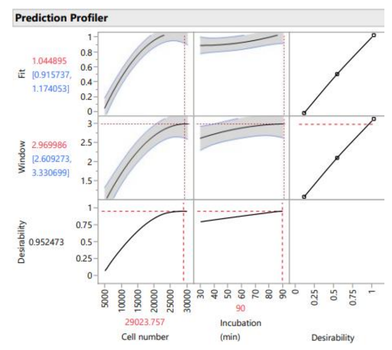


Data Driven De-Risking During Assay Development

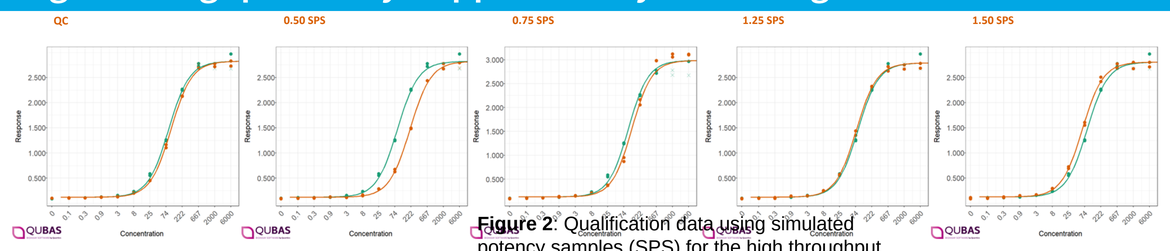
Abzena uses Design of Experiments (DoE) to efficiently optimise assays.

Abzena trends all data generated throughout development and testing to de-risk assays.



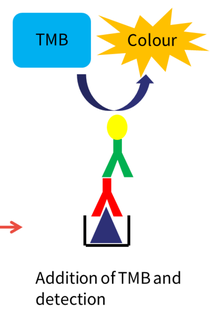


High Throughput Assay Supports Early Screening Studies



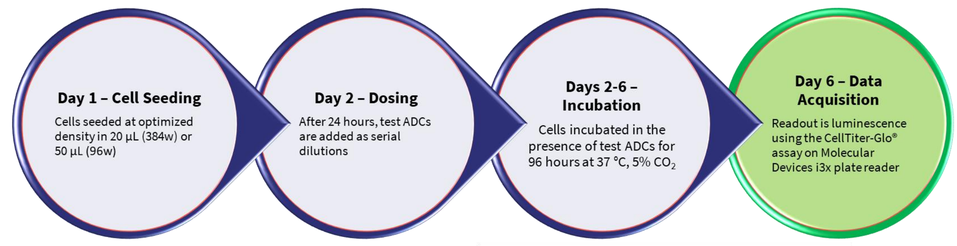
Expected RP (%)	Actual RP (%)				Potency		Relative Bias		
	N=1	N=2	N=3	N=4	Average	%GCV	RB	RBL	RBU
50	41.2	42.5	44.9	41.7	42.55	3.3	-14.90	-19.24	-10.32
75	70	67.5	65.5	64.4	66.82	3.2	-10.91	-15.30	-6.30
100	79	92.8	106.6	82.1	89.50	12.4	-10.50	-25.72	7.84
125	108.7	126.9	128.7	100.4	115.01	11.4	-7.99	-22.48	9.21
150	137.2	131.7	160.6	117.8	135.97	11.8	-9.35	-24.04	8.18

Figure 2: Qualification data using simulated potency samples (SPS) for the high throughput assay developed at Abzena.



Early high throughput assay qualified to ensure CQAs are maintained during early development of the drug.

Mechanism of Action Reflective Cytotoxicity Assay

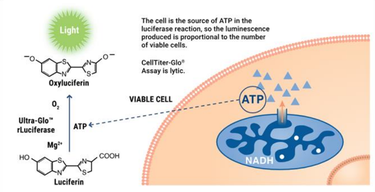


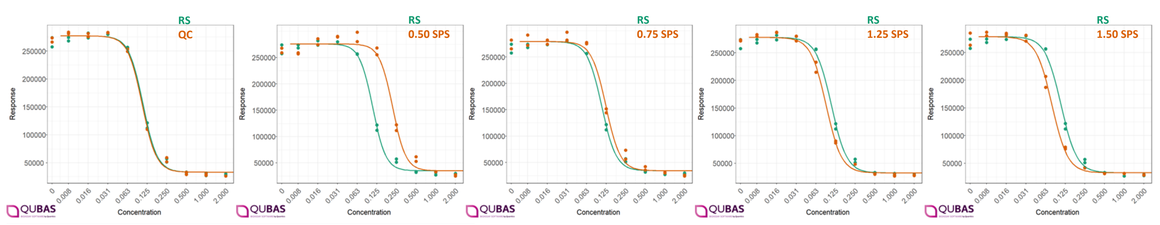
CellTiter-Glo™ Assay Principle:

- Determine the number of viable cells in culture based on quantitation of the ATP present

Sensitivity:

- Capable of <10 cell sensitivity
- Up to 5 log linear range





Expected RP (%)	Actual RP (%)				Potency		Relative Bias		
	N=1	N=2	N=3	N=4	Average	%GCV	RB	RBU	RBL
50	58.05	49.5	42.0334	50.1	49.60	12.1	-0.80	13.47	-13.29
75	63.04	85.9	55.50474	87	71.51	21.5	-4.65	19.94	-24.21
100	100.09	103.4	85.40741	106.1	98.41	8.8	-1.59	8.68	-10.89
125	101.89	120.6	128.9737	119.6	117.34	9.0	-6.13	3.94	-15.22
150	124.24	137.5	129.0015	138.2	132.10	4.6	-11.93	-7.18	-16.44

Figure 3: Qualification data using simulated potency samples (SPS) for the MoA reflective bioassay developed at Abzena.

MoA reflective assay demonstrates killing of target cells in the presence of ADC.

Assay demonstrates potency within qualified ranges pre-defined for our projects.

Summary

At Abzena, our assay development process allows us to:

- Streamline design, development and manufacturing of ADCs all under one roof. Abzena provides phase-appropriate potency testing throughout.
- Efficiently develop assays using a data driven DoE approach with robust justifications for each decision taken.
- Provide qualified relative potency assays to support the drug development life cycle through to clinical trials.

For additional information, or to discuss your project, please contact **info@abzena.com** or visit **www.abzena.com**