



Bioassays

Potency determination is necessary for regulatory submission and lot release of all biopharmaceutical products. Bioassays are used to determine the potency of a biopharmaceutical by comparing the biological response related to its mode of action with that of a control preparation. The data generated by bioassays are typically analyzed using biostatistical methods.

With extensive experience in establishing, validating and conducting routine bioassays to Good Manufacturing Practice (GMP) standards, Charles River Biopharmaceutical Services (BPS) has the capability to perform a comprehensive array of both *in vitro* and *in vivo* bioassays for a variety of biologically active molecules.

Services

- Method development
- Method transfer
- Method optimization
- ICH-compliant method validation
- Lot release testing for drug substance and drug product
- Stability testing

Bioassays and Products

Monoclonal Antibodies

ADCC: Antibody-Dependent Cell Cytotoxicity is measured by LDH release using NK effector cells freshly isolated from Peripheral Blood Mononuclear cells (PBMCs). The target cell line is selected based on the product.

CDC: Complement-Dependent Cytotoxicity is measured by flow cytometry using a live-dead-discriminating dye. An appropriate target cell line is marked by the antibody and attacked by the complement cascade.

Apoptosis/Programmed Cell Death (PCD): This mode of action is typically addressed by a reporter gene assay or by flow cytometry-based assays.

Assays Available

- Cell proliferation assays
 - Cell-based potency assays for EPO, PTH, G-CSF and GM-CSF
 - Antiviral cell-based assays for measuring the potency of interferons (IFN- α , IFN- β)
- Assays for monoclonal antibodies
 - Mode of Action (MOA) assays: ADCC, CDC, apoptosis
 - Binding assays
 - Potency assays
 - Competitive assays
 - Neutralization assays
- Immunogenicity testing
 - Anti-Drug Antibody (ADA) assays: binding and neutralizing antibodies
 - T-cell proliferation assays
 - Multiplex cytokine analysis by flow cytometry
- *In vivo* potency assays

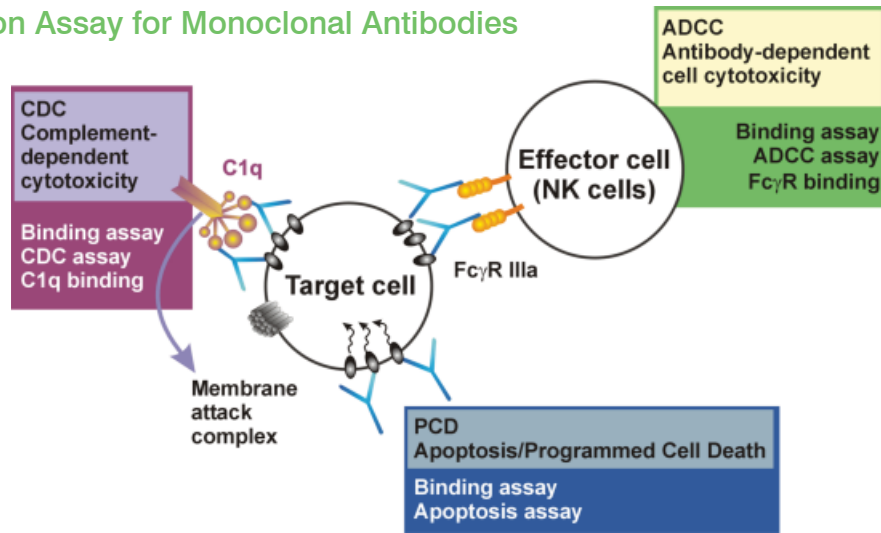
Assay Readouts

- Fluorescence
- Time-resolved fluorescence
- Absorbance
- Luminescence
- Analysis by flow cytometry

Statistical Evaluation

- Parallel line analysis
- Four-parameter fit
- Five-parameter fit
- EC₅₀ determination

Mode of Action Assay for Monoclonal Antibodies



Antiviral Compounds (Interferons)

Compendial bioassays on various interferon products (IFN- α , IFN- β) have been performed for more than a decade. Antiviral assays for human interferons are based on the induction of a cellular response in human cells, which prevents or reduces the cytopathic effect of an infectious virus. All assays comply with the requirements of the European Pharmacopoeia and have been validated according to ICHQ2(R1).

Growth Factors

The potency of human growth factors, such as EPO, GM-CSF and G-CSF, is measured with classical proliferation assays. These assays have been successfully applied on originators as well as first- and second-generation biosimilar products. If applicable, the assays comply with the requirements of the European Pharmacopoeia, and all assays have been validated according to ICHQ2(R1).

Hormones

For the parathyroid hormone (PTH), a cell-based assay is performed based on the determination of cyclic AMP (cAMP) release, detected by ELISA. The method has been validated according to ICHQ2(R1). Alternatively, time-resolved fluorescence is used to measure cAMP release.

Flow Cytometry

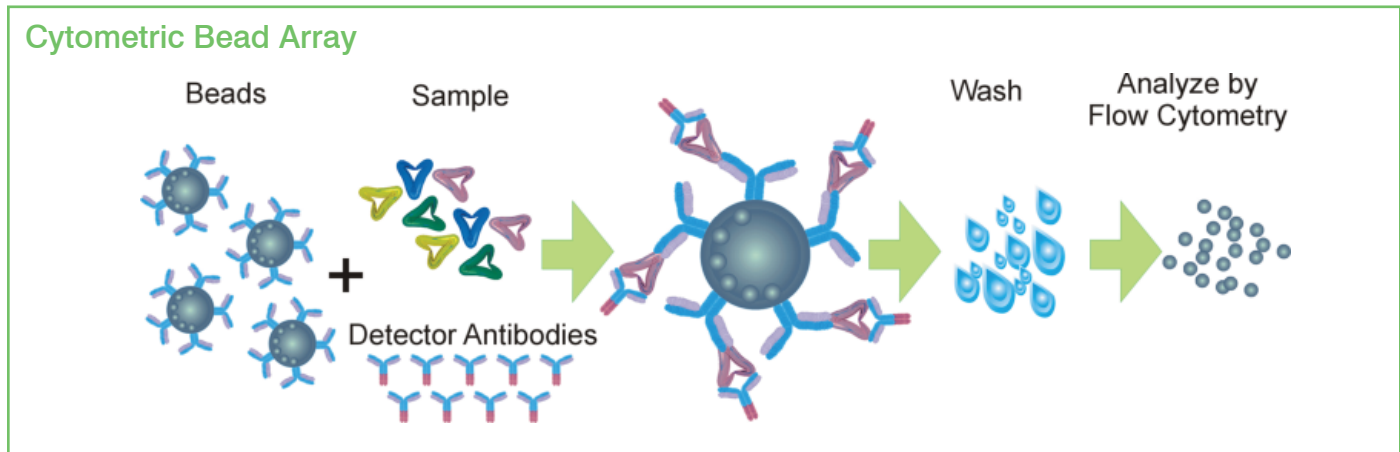
In addition to traditional cell-based bioassays, flow cytometry provides a fast, highly specific and accurate, quantitative readout tool, especially for complex heterogeneous samples. It allows simultaneous, multiparametric and fast analysis of the physical and chemical characteristics on a single cell level in real time (several thousand particles per second). Complex heterogeneous samples can be tested, and multiple markers can be correlated.

Applications

- Antigen, receptor or ligand density (e.g., binding assays)
- Multiplexing analyses of cytokines in sera (CBA technology)
- Intracellular protein expression
- Transgenic products *in vivo* [e.g., green fluorescent protein (GFP)]
- Enzyme activity
- Phosphoprotein analysis
- Apoptosis
- Viability
- Cell cycle analyses
- Changes in intracellular pH, calcium and glutathione
- Detection of soluble analytes by bead array technology
- Various combinations (DNA/surface antigens, etc.)

Cytometric Bead Array

The determination of drug side effects on cytokine expression is necessary and required by authorities, especially in the preclinical (e.g., rodent model or cell line model) or early clinical phases. The classical determination of cytokine expression by ELISAs is time-consuming and expensive, and big sample amounts are necessary. The new approach is multiplexing. The CBA method for the flow cytometric analyses of cytokine panels is fast and economical, and small sample volumes are sufficient. In addition, international standards are available, and the method is highly sensitive with a range from low ng/ml up to pg/ml. The method is suitable for cell supernatants, cell lysates and sera.



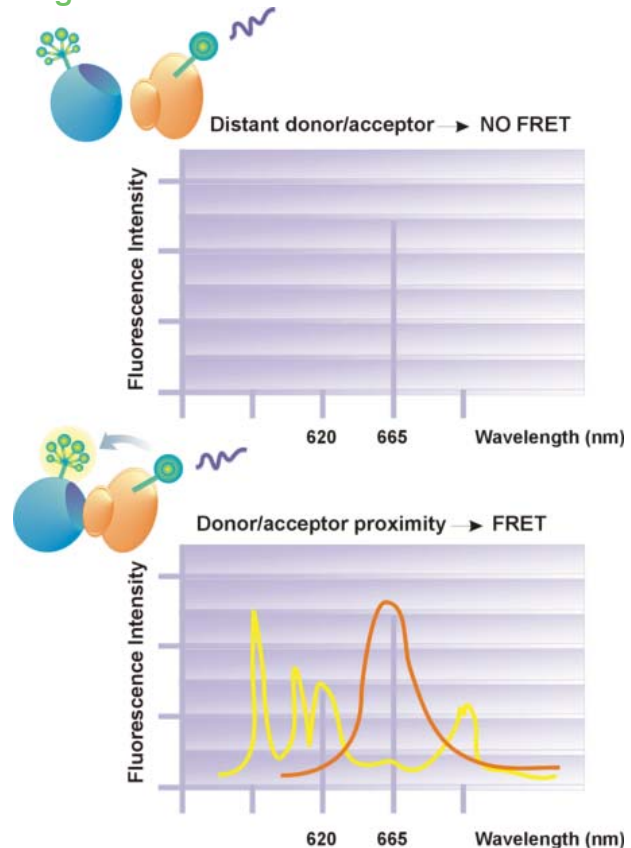
Time-Resolved Fluorescence

The time-resolved fluorescence method is based on FRET (fluorescence resonance energy transfer) in a microtiter plate. It is often used for third-generation anticancer and anti-inflammatory drugs, which tend to activate/act on specific phosphorylation pathways in the target cells. For the proof of the mode of action of such drugs, the assay must reflect the effect on the phosphorylation of key mediators of the involved pathway.

Advantages of the method are low background, increased assay sensitivity compared to classical approaches for the determination of phosphorylation (e.g., ELISA), fewer false-positive or false-negative results, and suitability for cell-based assays.

The homogeneous time-resolved fluorescence (HTRF) technology is an interesting new approach that might be used as an alternative mode of action assay.

Homogenous Time-Resolved Fluorescence





In Vivo Potency Assays

We can aid in the development of an *in vivo* potency assay through range-finding studies by investigating parameters such as dose level and route of administration, followed by validation and implementation. We have experience conducting *in vivo* bioassays for the purposes of showing efficacy and safety. These assays include adjuvant assessment, lot release potency, bacterial and viral challenge studies, and stability testing for a diverse range of products, including:

- Hormone potency assays, such as FSH, FSH-LH and hCG (performed to either Ph. Eur. or USP)
- Vaccines (performed to Ph. Eur. or USP, or new assays can be established)
- Neurotoxins
- Allergens
- Antivenoms
- Bacteria
- Blood products

