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Technical Sheet

In Vitro 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 (MOA) with that of a control preparation. The data generated by bioassays are typically analyzed using biostatistical methods.

At Charles River, we understand that bioassays are central and critical for product development and manufacturing. These assays are necessary to ensure the continued quality, safety and efficacy of biopharmaceutical products and also when developing new biosimilars. In turn, these assays must be reliable, standardized and relevant to reflect the product's mode of action. The experts at Charles River have developed the assays mentioned in this sheet and welcome discussions with clients regarding which assays would be most appropriate to fulfill the testing needs related to a particular product.

Product Specific Bioassays

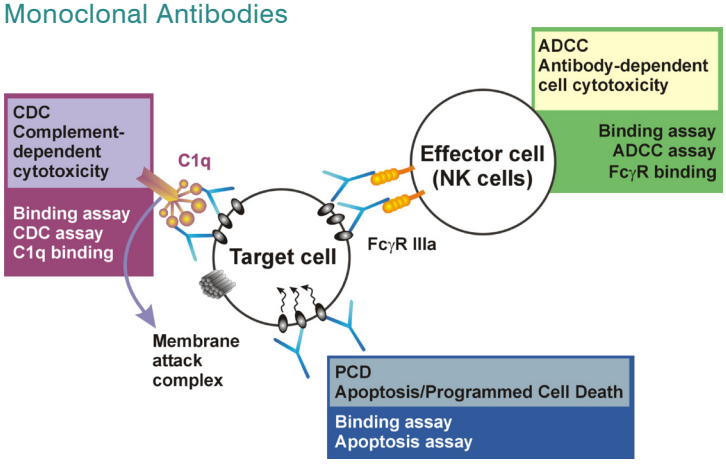
Monoclonal Antibodies

ADCC: Antibody-dependent cell cytotoxicity (ADCC) is measured by LDH release using NK effector cells freshly isolated from peripheral blood mononuclear cells (PBMCs) or with a luminescence-based reporter bioassay. The target cell line is selected based on the product.

CDC: Complement-dependent cytotoxicity (CDC) is measured by flow cytometry using a live-dead-discriminating dye or luminescence-based. 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.

Mode of Action Assay for Monoclonal Antibodies



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Antiviral Compounds (Interferons)

Compendial bioassays on various interferon products (IFN-α, IFN-β) have been performed for more than a decade. These bioassays are based on the inhibitory activity of interferons on the cytopathic effect of a virus on a susceptible cell line. 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).

Bioassay Technology

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.

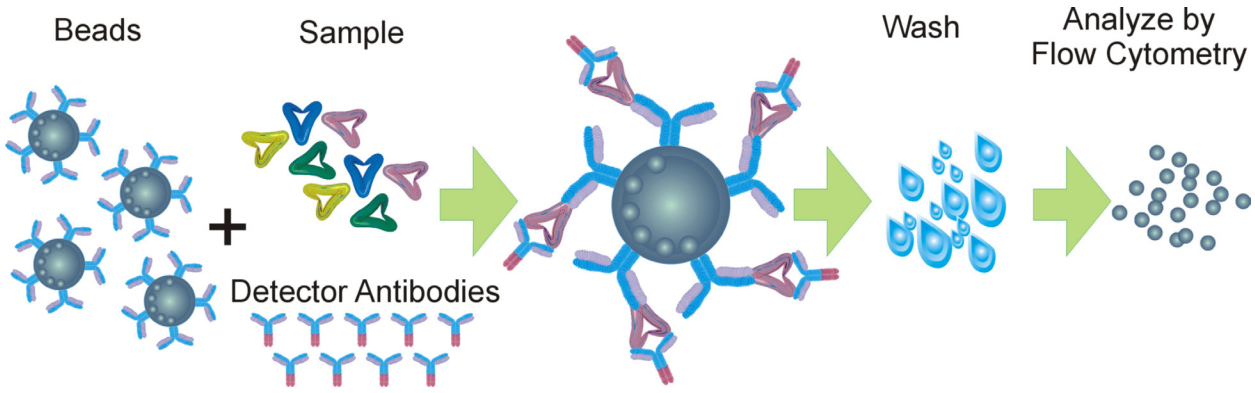
Applications

- Mode of action assays for monoclonal antibody therapeutics
- Antigen, receptor or ligand density (e.g., binding assays)
- Multiplexing analyses of cytokines (CBA technology)
- Cell-based immunogenicity
- 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
- Various combinations (DNA/surface antigens, etc.)
- In-process quality control of primary cells

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.

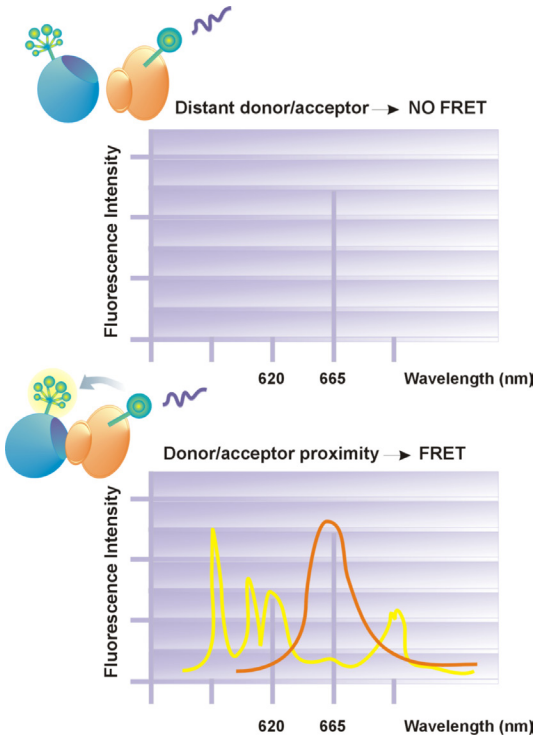
Cytometric Bead Array



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 activeate/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.

Homogenous Time-Resolved Fluorescence



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.

Summary

In vitro bioassays allow for the determination of the potency of products which is necessary for regulatory submission and lot release of biopharmaceutical products. It is important to carefully evaluate the type of bioassay used in testing to ensure that it is appropriate for the product type being tested. In addition, *in vitro* mode of action assays are a powerful tool for the confirmation of biocomparability of innovator products and follow-on biologics.

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