

Flow Cytometry Case Study

Background:

Antibody immunotherapy leverages the binding of antibody drugs to receptors on specific immune cell subpopulations to achieve therapeutic objectives by modulating immune cell functions. Evaluating these therapeutic drugs' binding efficacy and biological impact on their targets is crucial. Receptor occupancy assays, utilizing high-parameter flow cytometry technology, are instrumental in assessing receptor expression and drug occupancy on specific cell subpopulations. These assays provide critical data to support antibody pharmacodynamics, dose selection, safety, and other vital aspects in clinical trials for various drugs and analytical purposes.

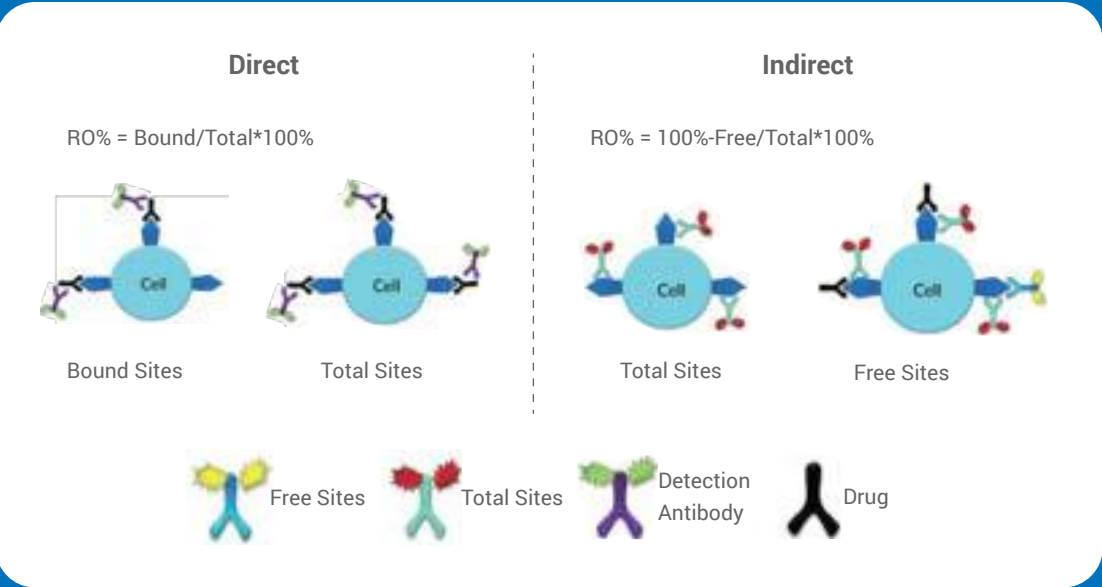
Assay Design:

Selecting the appropriate analytical strategies for receptor occupancy assays depends on the target drugs, target cell subpopulations, and specific analytical goals. Classical direct or indirect methods are recommended for common monoclonal antibodies. However, for complex analyses involving bispecific or tri-specific antibodies, where classical methods may fall short, it's essential to evaluate the feasibility of detecting bound, free, and total receptors. This evaluation considers the availability of detection reagents and the characteristics of target cell subpopulations.

Free Receptor: Detect receptors on target cells not bound by the drug using antibodies that compete perfectly with the drug or specifically labeled drugs.

Bound Receptor: Detect receptors on target cells bound by the drug using specifically labeled anti-drug antibodies.

Total Receptor: Detect the total receptors on target cells using antibodies that do not compete with the drug or by adding samples with saturated concentrations of the drug and detecting them with labeled anti-drug antibodies.



Project Experience:

Targets: PD-1, PD-L1, Tim-3, OX-40, CD3, CD19, CD20, CD47, TGF- β , 4-1BB, LAG-3, PVRIG, CD38, CD40, TNFR2, etc.

Antibody Types: monoclonal antibodies, bispecific antibodies, and tri-specific antibodies.

Target Cells: lymphocytes (CD3+ T cells, CD4+ T cells, CD8+ T cells, B cells, NK cells, Treg cells, etc.), monocytes, neutrophils, red blood cells, platelets.



Our Advantages



Global Integrated Platform: We provide one-stop services to global sponsors, from preclinical to clinical stages, supported by multiple BAS sites worldwide.



Customized Services: Our sponsor-centric professional project management team delivers tailored services to meet your specific needs.



Experienced Team: Our core technical team members boast over 10 years of experience, with our management team having more than 20 years.



Platform Expertise: We have successfully executed more than 100 projects utilizing flow cytometry technology. These projects span a range of clinical trials, including those for small molecule inhibitors, targeted degraders, monoclonal antibodies, bispecific antibodies, multifunctional antibodies, fusion proteins, protein-based drugs, cell therapies, mRNA vaccines, and other innovative drug modalities.

Common Industry References

- Guidelines for Validation of Quantitative Analysis Methods for Biological Samples, Chinese Pharmacopoeia, 2020 edition
- Guidelines for Clinical Development of Anti-Tumor Antibody-Drug Conjugates by the Center for Drug Evaluation of the National Medical Products Administration, "Bioanalytical Method Validation and Study Sample Analysis M10," International Council for Harmonisation
- Bioanalytical Method Validation, U.S. Food and Drug Administration
- Clinical Pharmacology Considerations for Antibody-Drug Conjugates, U.S. Food and Drug Administration
- Immunogenicity Testing of Therapeutic Protein Products—Developing and Validating Assays for Anti-Drug Antibody Detection, U.S. Food and Drug Administration



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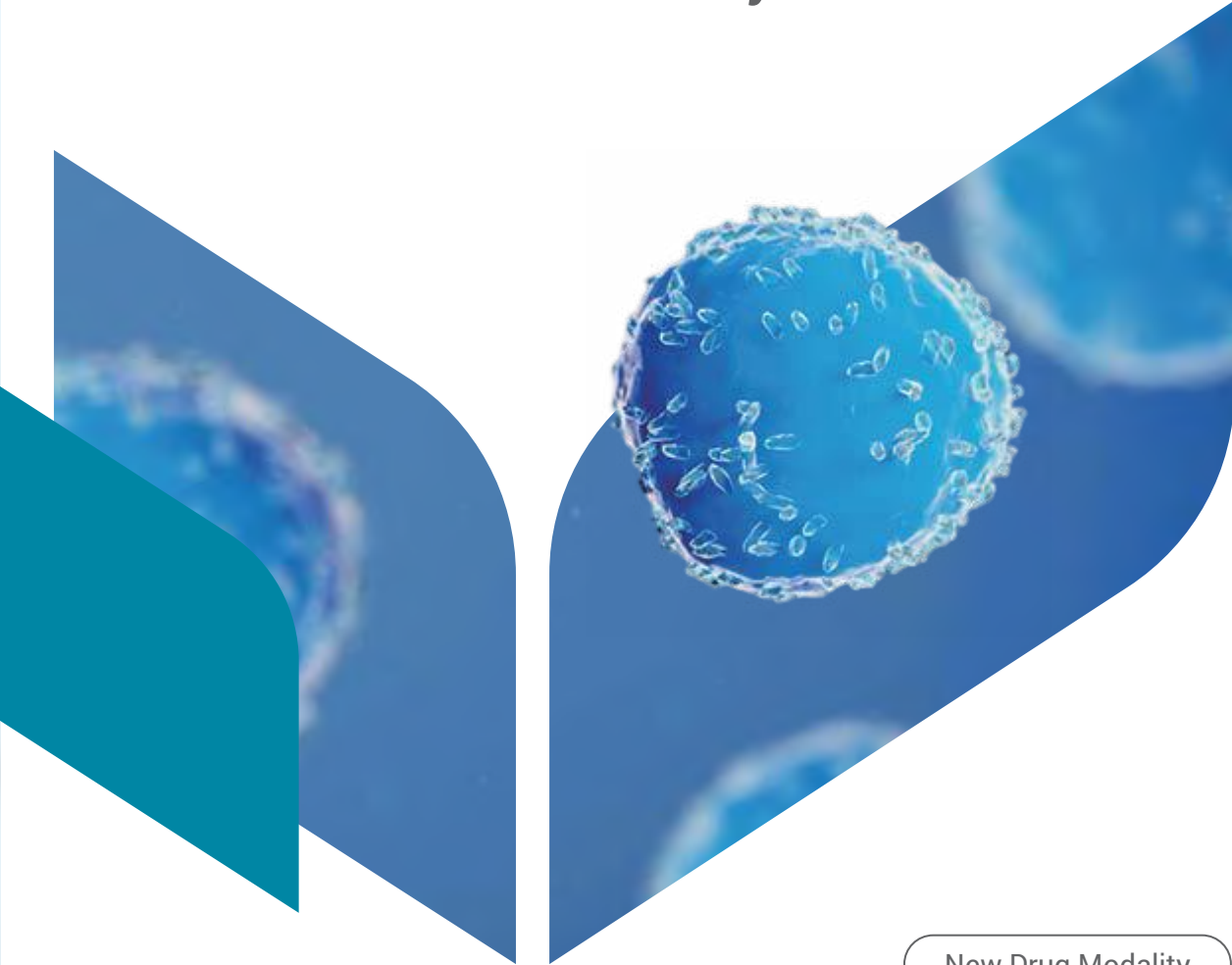
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Laboratory Testing Division
Bioanalytical Services



Flow Cytometry

Bioanalysis



New Drug Modality

Flow Cytometry

Bioanalysis

Bioanalytical Services

Global Integrated Bioanalytical Platform

WuXi AppTec Bioanalytical Services operates globally and has leading integrated laboratories in China and the U.S. We adhere to GLP and GCP principles and strictly follow high industry regulatory standards. Our comprehensive platform, equipped with advanced instruments and staffed by experienced experts, provides professional one-stop services in preclinical/clinical mass spectrometry, immunochemistry, molecular/cellular, and central laboratory services to global clients.



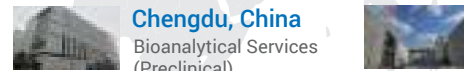
Shanghai, China
Headquarters
Bioanalytical Services
(Clinical)
Central Lab



Chengdu, China
Bioanalytical Services
(Preclinical)



Suzhou, China
Bioanalytical Services
(Preclinical)
Antibody Service



Nantong, China
Bioanalytical Services
(Preclinical)



New Jersey, USA
Bioanalytical Services
(Preclinical/Clinical)
Central Lab



5,000+ small molecule validated methods
2,800+ large molecule validated methods
2M+ samples analyzed per year

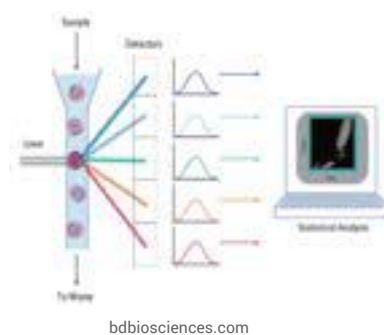


Global Regulatory Standards

Successfully passed global regulatory inspections, comply with GLP, GCP, CAP quality standards
200+ China NMPA
30+ International regulatory agencies (U.S. FDA, OECD, EMA, PMDA, CAP)

Flow cytometry is a cutting-edge technique for quantitatively analyzing and sorting cells or biological particles in a rapid, linear flow. This technology integrates laser technology, electronic physics, cell fluorescence chemistry, monoclonal antibody techniques, and more, supporting immune monitoring, drug efficacy research, biomarker analysis, and other critical aspects of drug development.

We have experience in more than 50 preclinical and 70 clinical projects involving various drug types; we bring extensive experience in method development. Our unique flow cytometry platform and systematic development strategy for biological analysis enable us to help sponsors accelerate their drug development projects. We provide comprehensive flow cytometry testing and analytical services for drugs at any clinical research stage, facilitating faster global submissions for IND, NDA, and BLA.



Flow Cytometry Platform



BD FACSCanto™ II (3 Lasers, 8 Colors, 10 Parameters)

The BD FACSCanto™ II system is designed to provide high-quality data and convenient operation. It enables easy and fast operation while maintaining highly accurate testing results based on the innovative six-color fluorescence analysis technology. Additionally, the system's unique design allows for quick adaptation to a wide range of clinical applications, supporting clinical diagnosis and monitoring.



BD LSRFortessa™ X-20 (5 Lasers, 18 Colors, 20 Parameters)

The BD LSRFortessa™ X-20 cell analyzer is mainly used for supporting the growing demand for multi-color flow cytometry. It provides high-dimensional flow data for drug studies and greater application flexibility, allowing users to easily transfer detection and data analysis from one platform to another.



Beckman CytoFLEX (3 Lasers, 13 Colors, 15 Parameters)

The Beckman CytoFLEX integrates optical components and the flow cell to minimize losses during transmission, resulting in optimal instrument sensitivity.

Platform Advantages

Experience with more than 100 molecule detection projects, including robust and GLP-compliant data flow management for ensuring data integrity.

Rich experience in experimental design based on drug/pharmacology/disease characteristics.

Extensive experience in sample handling and flow cytometry data analysis for different diseases/species.

Professional and experienced team with more than 10 years of experience and GLP/CAP bioanalytical experience.

Introduction to Flow Cytometry Bioanalytical Projects

Preclinical:

Species: Cynomolgus monkeys, Rhesus monkeys, rats/mice, dogs, and minipigs.

Drugs: antibody drugs, ADC drugs, cell therapy drugs, small molecule innovative drugs, and other types of novel drugs.

Assay Types:

- Immunophenotyping:
 - T cell, B cell, NK cell phenotyping
 - Treg cell, macrophage, dendritic cell, monocyte, and other cell subsets

Receptor Occupancy:

- Target receptors: PD-1, CD3, CD4, CD36, CD47, CD137, LAG-3, and other targets
- Target cells: T cells, B cells, NK cells, red blood cells, platelets, monocytes, and other cells

Cytokine (Cytometric Bead Array (CBA)) :

- Up to 13 cytokine markers in one assay, including IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ, TNF-α, and other factors

Cell Therapy Drug Analysis:

- Analysis of the percentage and absolute count of positive cells in cell therapy products. Distribution analysis of cell therapy drugs in whole blood, spleen, lung, tumor tissues, etc.

Intracellular/Nuclear Marker:

- Intracellular transcription factors, cytokines, etc.

Bacterial Product Concentration Analysis

Clinical:

Drugs: monoclonal antibodies, bispecific antibodies, multi-functional antibodies, cytokines, fusion proteins, and other protein-based drugs; small molecule inhibitors, small molecule targeted degraders, and other small molecule drugs; GCT drugs such as CAR-T and mRNA vaccines.
Disease Types: hematological diseases, neurological diseases, infectious diseases, autoimmune diseases, solid tumors, preventive treatments for healthy individuals, etc.

Assay Types:

- Immunophenotyping:
 - T cell, B cell, NK cell phenotyping
 - Th/Treg cell analysis, macrophage, dendritic cell, monocyte, and other cell subset analysis

Receptor Occupancy:

- Target receptors: PD-1, Tim-3, 4-1BB, CD36, CD47, CD137, LAG-3, and other targets
- Target cells: T cells, B cells, NK cells, red blood cells, platelets, monocytes, and other cells

Cell Therapy Drug Analysis:

- PK analysis of cell therapy drugs in peripheral blood (targets: CD19/BCMA/CD30, etc.)
- Phenotypic and functional analysis of cell therapy drugs

Intracellular/Nuclear Marker:

- Foxp3, Ki-67, Helios, phosphorylated protein p-AKT, etc.

Analysis of Antigen-Specific T Cells in Cell-Mediated Immunity (CMI)

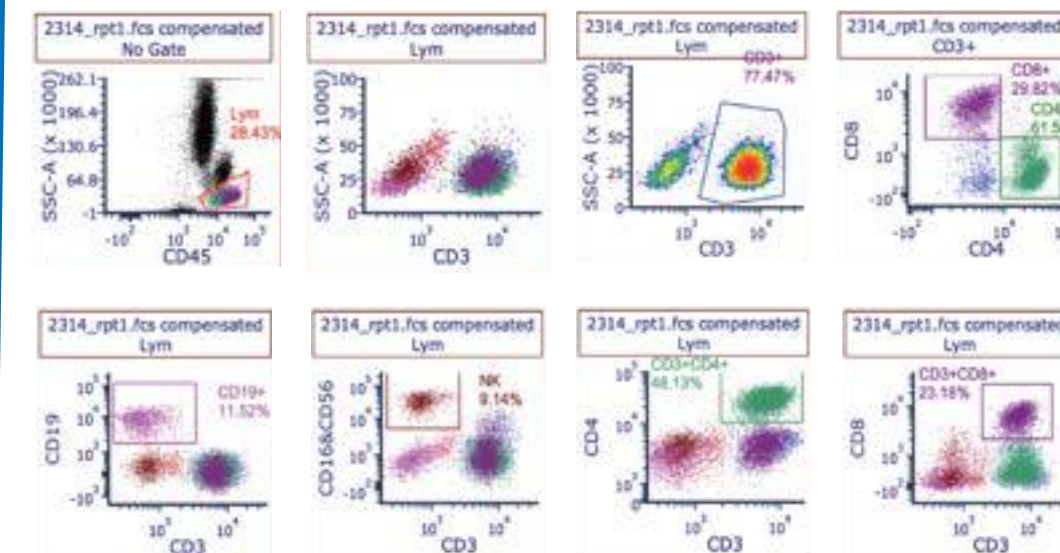
- Antigen-specific T cells

Case Study of Flow Cytometry Analysis

Background:

Determining lymphocyte subsets is a crucial indicator for assessing cellular and humoral immune functions. It provides insight into the body's current immune function, status, and balance. This analysis is essential in diagnosing autoimmune diseases, immunodeficiency disorders, malignancies, hematological conditions, and hypersensitivity reactions. It also plays a significant role in understanding pathogenesis, evaluating treatment efficacy, and predicting prognosis, making it invaluable in clinical and research settings.

6-Color Panel for Human TBNK Detection



Considerations for MD and MV Parameters:

Method Development:

- Antibody combination
- Antibody selection and conjugation
- Antibody titration
- Sample collection, storage, and transportation
- Quality control material selection and evaluation
- Fluorochrome and flow cytometry reagent screening
- Instrument quality control and compensation settings

Method Validation:

- Precision and accuracy
- Specificity and selectivity
- Sensitivity
- Detection limit
- Stability and robustness
- Personnel validation
- Instrument validation
- Dilution factor