RoukenBio

Assays for Biosimilar Characterisation

Biosimilar capabilities overview

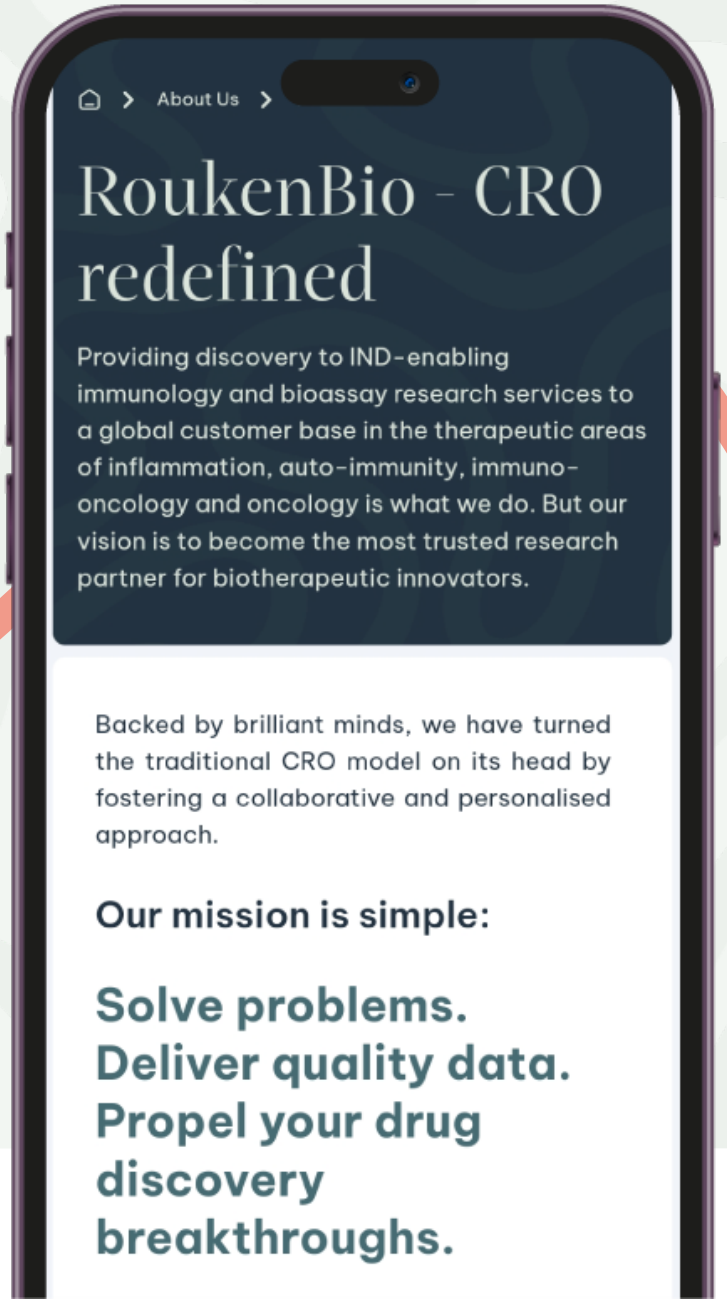
rouken.bio

hello@rouken.bio

RoukenBio

Our Vision

Become the most trusted research partner for biotherapeutic innovators.



Propelling Drug Discovery Breakthroughs Since 2015

2015
Founded in Scotland, as Antibody Analytics

2017
Biosimilar characterisation Services launched

2018
Immunology research Services launched

2019
R&D department established

2020
Cell Line engineering services launched

2022
2000m^2 Discovery Centre opened and 2 patents filed

2023
IndEx-2 launch & NorthEdge investment

2024
100+ employees, laboratory footprint doubled and **RoukenBio** is born

NPS 79
Net Promotor Score - our clients love working with us

27
countries with customers globally

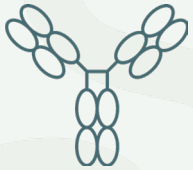
2000m^2
of lab space with state-of-the-art instruments

100+
employees and growing

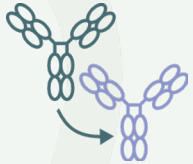
Novel Therapeutic Focus

Advancing Novel Therapies

We focus on established and emerging therapeutic classes, including:

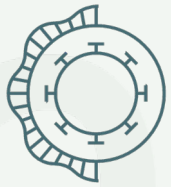


mAbs, BsAbs and multi-specifics



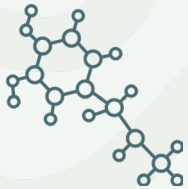
Biosimilars

Immunocytokines



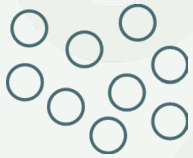
Cell therapies

Targeted protein degraders

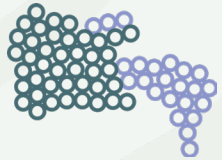


Small molecules

Oligonucleotide-based



Nanoparticles



Complex biologics

Peptides

Expertise in oncology, immuno-oncology, autoimmunity, allergy and inflammation

Adaptable assay platforms designed to address the unique challenges of these therapeutic areas

With expertise spanning a range of disease areas, we develop innovative assays to support the development of cutting-edge treatments.

Tools & Resources

Embracing Change, Driving Innovation

Our extensive in-house resources and cutting-edge technologies provide the foundation for delivering high-quality data and accelerating your drug development journey.

Human PBMC banks
(1000s of vials stored on-site)

Fresh whole blood access
with genotyped donors

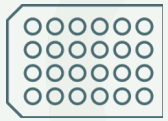
Access to disease-state PBMCs
and patient-derived material

Hundreds of cell lines (non-engineered), target cells (engineered) and reporter cell lines available

Recallable donors

Access to best-in-class
technologies and instrumentation

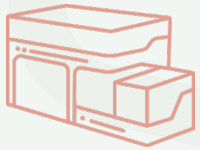
In-house cell line engineering
capabilities utilising unique platforms



'GMP-ready' assay
development and technical transfer

RoukenBio – CRO Redefined

Thought partners, grounded in scientific empathy



Advanced Instrumentation Ensures High-Quality Data Every Time:

Multi-parameter flow cytometers (NovoCyte Quanteons)

xCELLigence RTCA MPs (impedance)

High throughput Biacore 8K SPR systems

Luminex MAGPIX® System

NEW: heliX^{cyto} (scIC)

NEW: Maestro TrayZ (impedance)

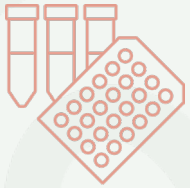
SpectraMax i3x Plate Reader

Automated Cell Isolation

Hidex Sense Plate Readers

Collaborative Expertise and a Consultative Approach

Our team of cross-functional experts works closely with you to understand your challenges and design tailored solutions that deliver results, taking your project to the next milestone.



Custom and Off-the-Shelf

We offer the flexibility of both custom-designed and readily-available assay formats, ensuring we can meet your specific project needs and timelines.

Biosimilar Services

Functional analysis for exploratory and analytical similarity studies

RoukenBio

Our biosimilar experience

We have supported 18 biosimilar developers to deliver global market authorisations.

Nivolumab: PD-1
Pembrolizumab: PD-1
Bevacizumab: VEGF-A
Aflibercept: VEGF-A
Palivizumab: F protein (RSV)
Tocilizumab: IL-6R

Etanercept: TNFα
Adalimumab: TNFα
Infliximab: TNFα
Golimumab: TNFα
Denosumab: RANKL
Rituximab: CD20

Ocrelizumab: CD20
Trastuzumab: Her2
Pertuzumab: Her2
Ustekinumab: IL-12/23
Risankizumab: IL-23
Dupilumab: IL-4/13

Binding and Functional Work Packages

Tailored approaches for biosimilar characterisation

Work Package	Purpose	Key Assessments	Applications	Data Quality & Outputs
Primary MoA Characterisation	Characterise product critical quality attributes	Key binding and biological activity measurements (typically Fab-directed). SPR, ELISA and/or simple (i.e. reporter) bioassay(s).	Range finding, early development, analytical similarity, CMC	Qualified methods of high performance and robustness employing accepted statistical analysis approaches to report quantitative outputs.
Extended MoA Characterisation	Validate CQA findings through application of wider suite of assessments	Orthogonal methods, extending to use of primary cells or advanced models (e.g. co-culture models).	Analytical similarity	Method-specific variations in accuracy/precision assessments to provide deeper understanding of analytical similarity within relevant systems.
Negative Effector Function Characterisation	Characterise binding activity and confirm the absence of functional activity	FcγR, FcRn, C1q binding (SPR) and ADCC, CDC, ADCP assessments using methods of the highest sensitivity.	Analytical similarity	Qualified methods employing accepted statistical analysis approaches to report quantitative/qualitative outputs with significant emphasis on method sensitivity.
Positive Effector Function Characterisation	Characterise binding and functional activity	FcγR, FcRn, C1q binding (SPR) and ADCC, CDC, ADCP assessments using methods of “varying” sensitivity.	De-risking in early development , analytical similarity	Qualified methods employing accepted statistical analysis approaches to report quantitative outputs with significant emphasis on method sensitivity and correlation of activity across methods.
Investigational Studies	Address observed differences between the biosimilar and originator	Typically, effector function assessments evaluating the role of Fc-glycans, donor genotype and effector preparation type of observed activity together with assessment of target antigen expression levels.	Confirmation that observed differences are not clinically meaningful	Investigatory methods, with to support decision-making regarding the clinical impact of biosimilar-originator differences.

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MoA = Mechanism of Action, CQA = Critical Quality Attributes, ADCC = Antibody dependent cell-mediated cytotoxicity, CDC = complement-mediated cytotoxicity, CMC = chemistry, manufacturing and controls, ADCP = Antibody dependent cell-mediated phagocytosis, FcγR = Fc receptor gamma, FcRn = neonatal Fc receptor, SPR = surface plasmon resonance

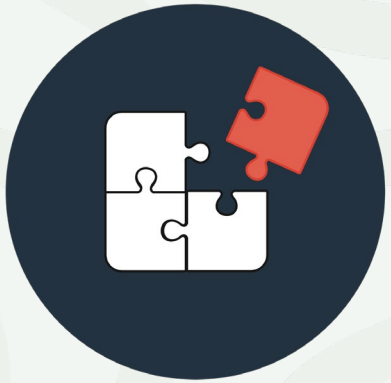
We offer

Extensive Experience

We have in-depth experience in binding and functional characterisation of biosimilar therapeutics.

Cell Engineering

We have a library of reporter and target cell lines, and cell engineering experts ready to provide custom solutions.



Primary Tissue

Access to a wide variety of primary cell types including pre-screened cryopreserved PBMC material, large lot sizes stored on site allowing control over critical reagents.

Expert-Guided Assay Design

We provide expert-guided assay design and access to collaborative teams in the fields of immunology and bioassay design.

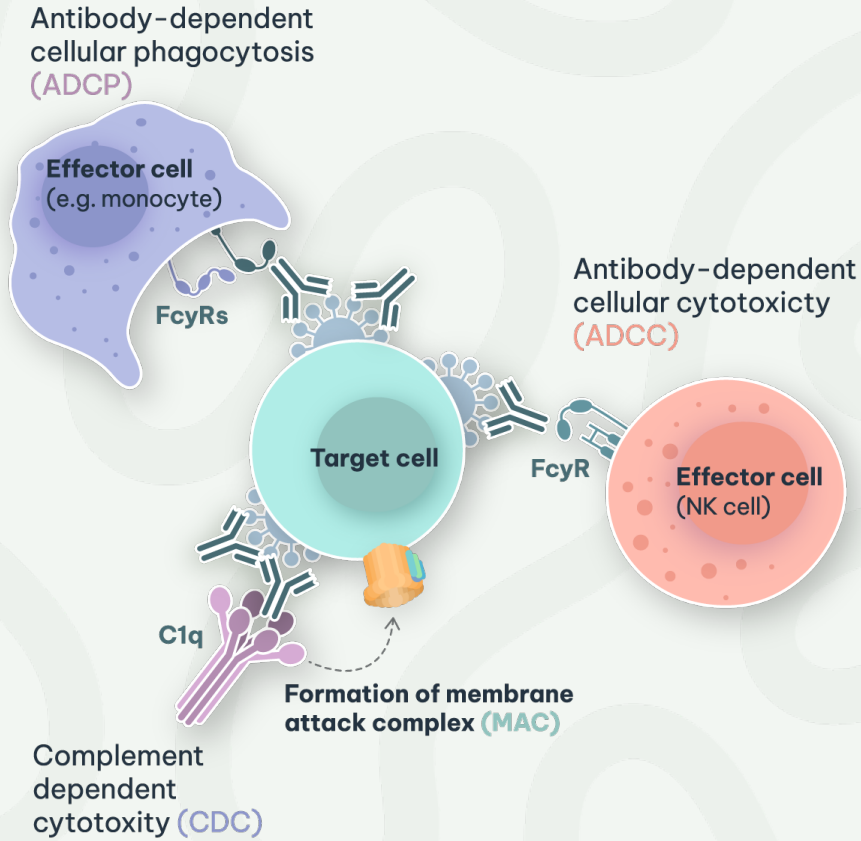
Effector Function Capabilities

RoukenBio

Exploring Effector Function Services

Functional Activity Assessments

Phagocytosis (**ADCP**) by flow cytometry. Primary monocyte or macrophage effector cells.



ADCC by plate-based luminescence methods. Primary NK cells or PBMC as effector cells.

CDC by plate-based luminescence methods. Human and rabbit serum as effector preparation.

Exploring Effector Function Services

Relevant Binding Interactions

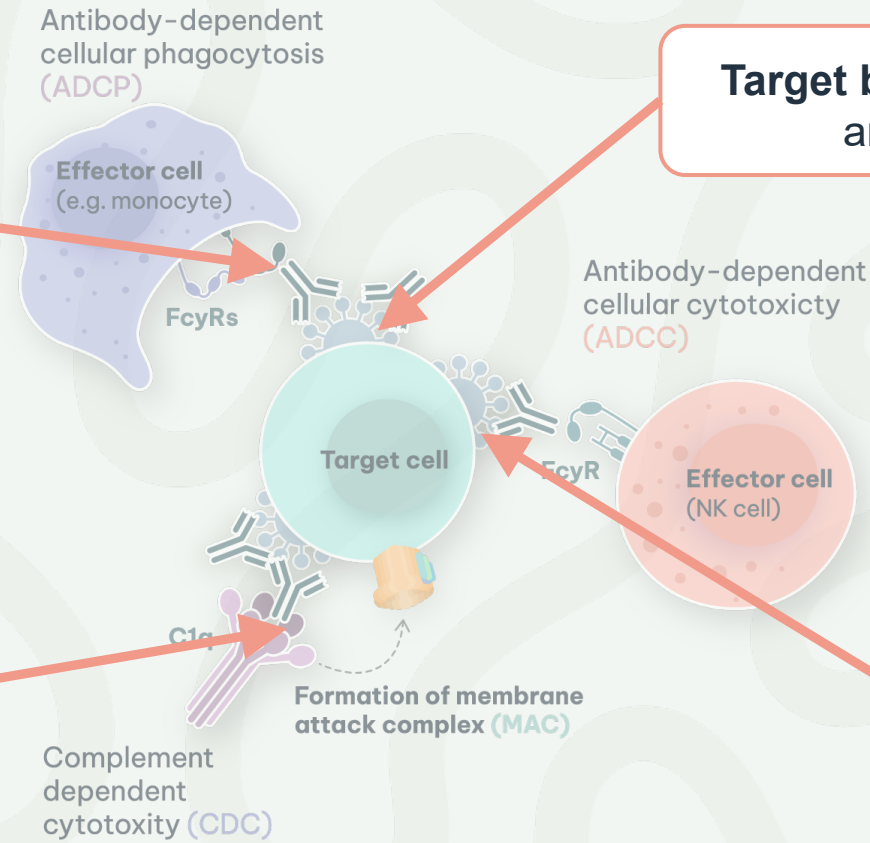
Fcγ receptor binding by SPR

FcγRI (CD64), FcγRIIa (CD32a H variant), FcγRIIa (CD32a R variant), FcγRIIb (CD32b), FcγRIIIa (CD16a V variant), FcγRIIIa (CD16a F variant), FcγRIIIb (CD16b), FcRn

C1q binding by SPR and ELISA

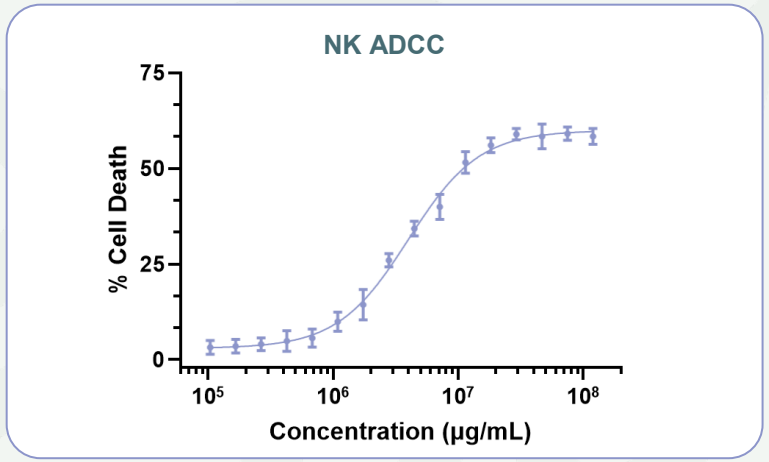
Target binding by SPR and ELISA

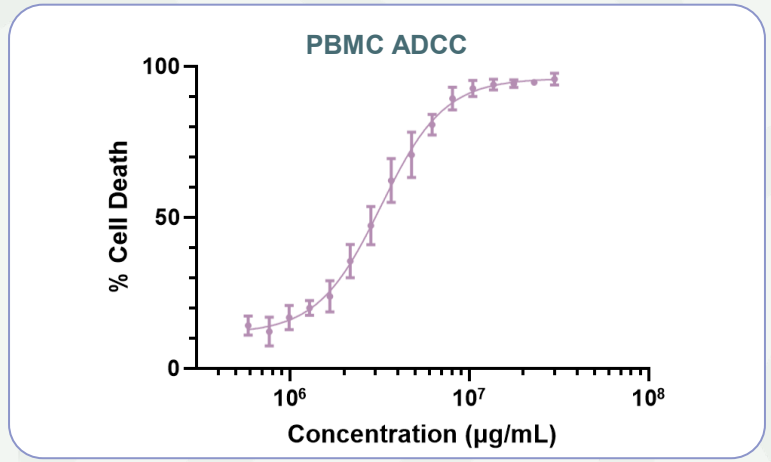
Cell-based target binding by flow cytometry

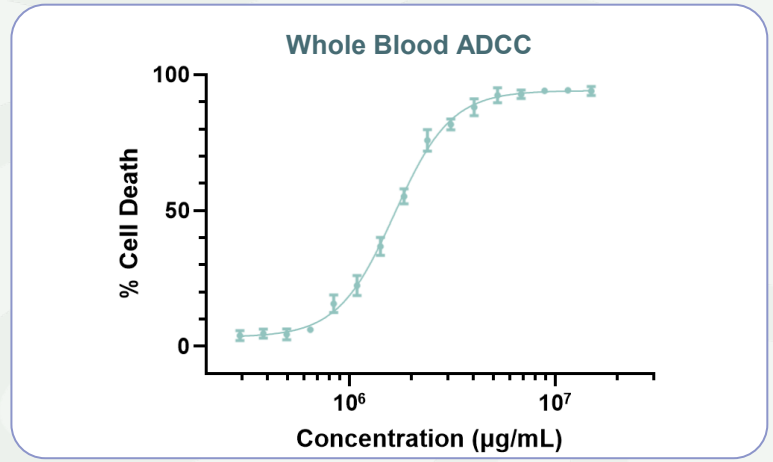


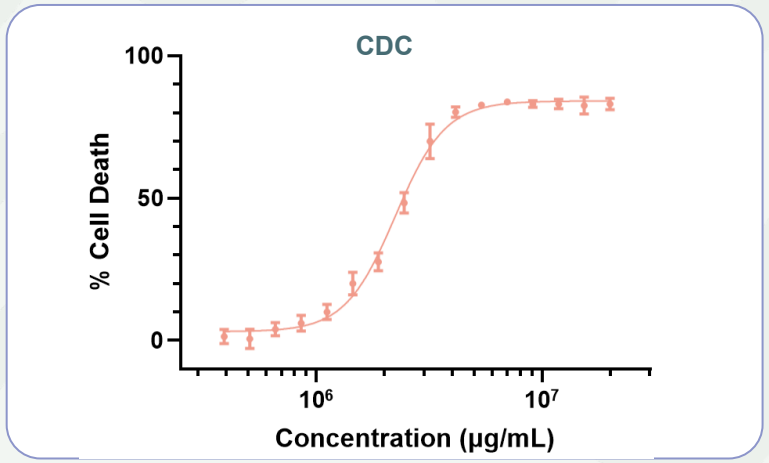
Effector Function Bioassays

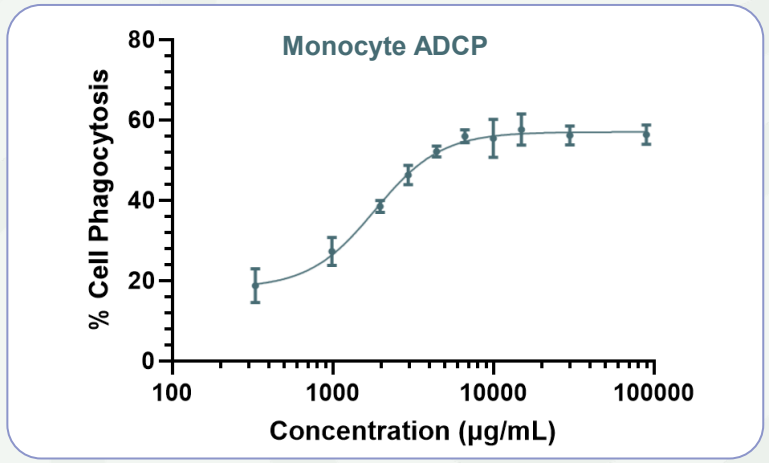
Orthogonal approaches for extended characterisation



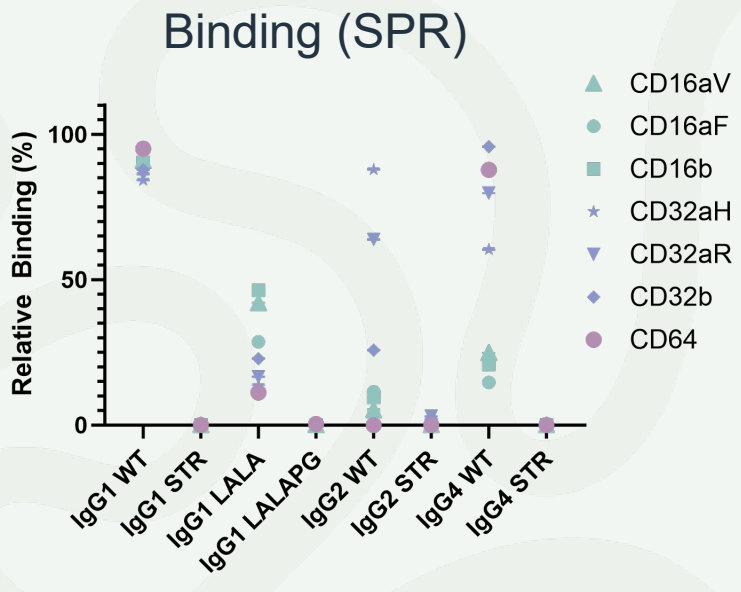


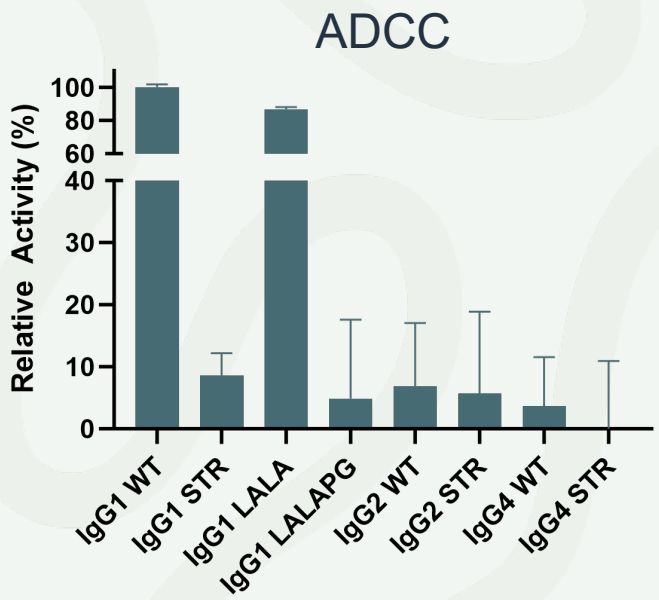


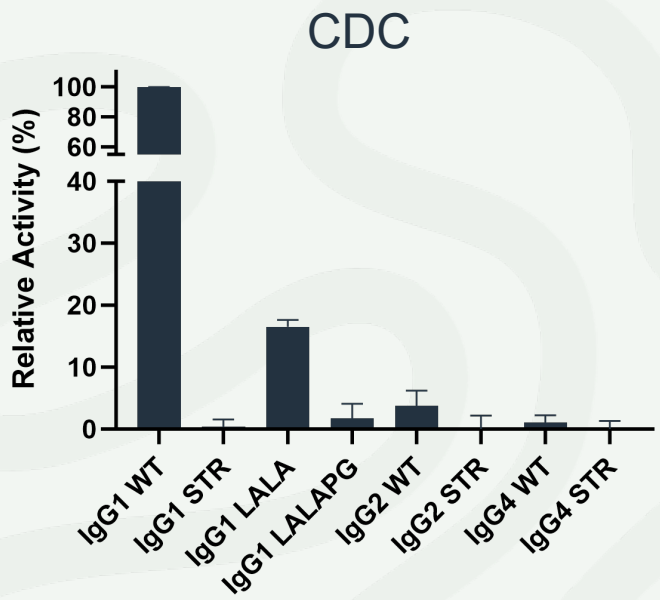




Example data – FcγR binding and effector function for ‘silent’ antibodies







Evaluation of the FcγR binding profile and functional activity of a suite of antibodies directed against CD20. IgG1, IgG2 and IgG4 backbones were assessed alongside STR mutation-silenced counterparts. The results for each antibody were calculated relative to the IgG1 Wild Type. The data demonstrates that use of IgG2 or IgG4 backbones, or use of the LALA mutation, do not result in complete silencing of effector binding and functional activity.

SPR Capabilities

RoukenBio

SPR Technology and Expertise

- **Two** Biacore 8K instruments
- Gold standard for SPR measurements
- 8 needle, **high-sensitivity**, high-capacity instrument
- Housing 4 microplates (96 or 384 well) allows for **high throughput assays**
- Data rich outputs – **KD, ka, kd**

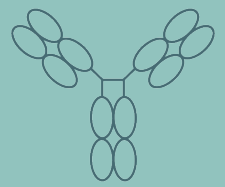
RoukenBio

Advanced SPR Solutions

RoukenBio has extensive experience in assessing binding interactions of biosimilars. Assays include:

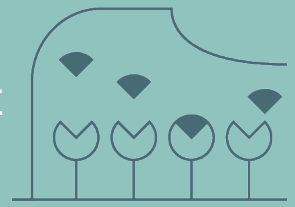
Fab binding

- Off-the-shelf ligand binding assays
- Ligand-analyte interactions
  - High throughput format
  - High accuracy format
- Antibody screening
- Binding dependency
- Custom assay development



Fc binding

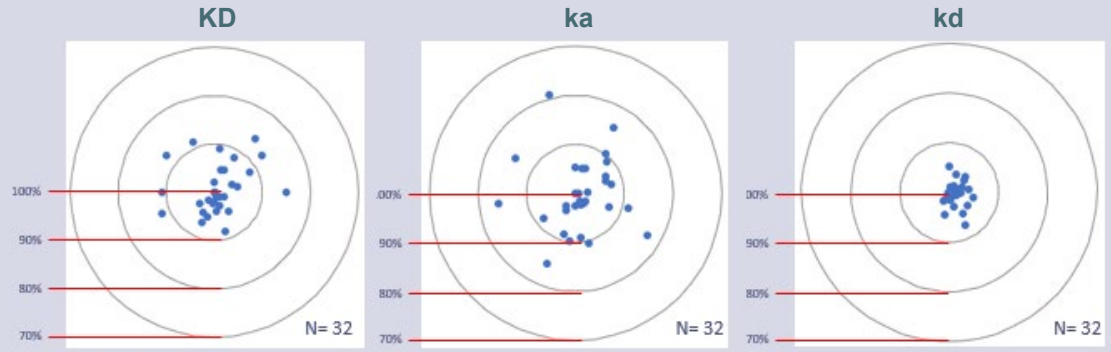
- Standard off-the-shelf assays; human, cynomolgus & mouse FcRs produced & quality controlled in-house
- Multiple receptor and Ab combinations
- Custom assay development



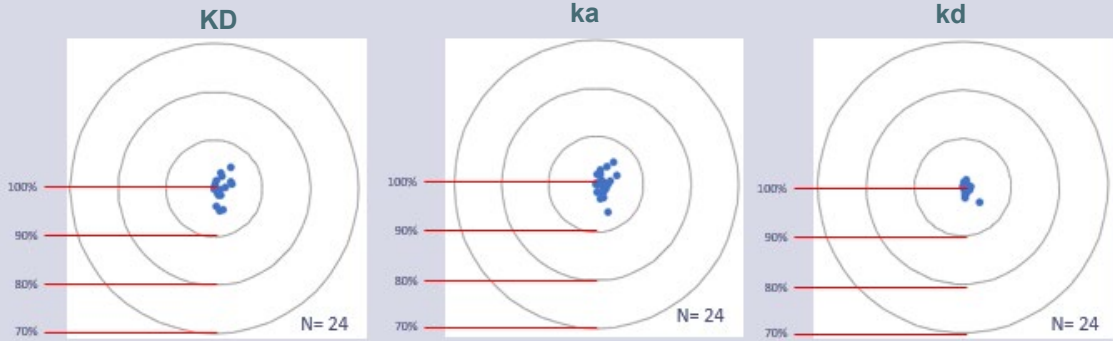
Ligand – Analyte Interactions

Biacore 8k Performance: Trastuzumab binding HER2

High throughput procedure accuracy



High performance procedure accuracy



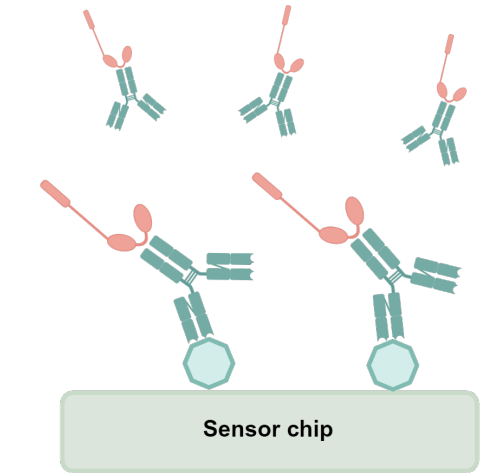
High Throughput		
Reportable Value	**Accuracy**	**Precision**
KD (M)	87.6 – 114.9%	7 %CV
ka	84.0 – 120.8%	9 %CV
kd	94.9 – 107.4%	3 %CV

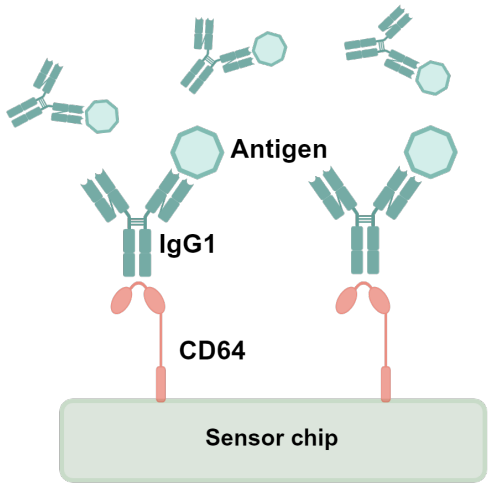
High Performance		
Reportable Value	**Accuracy**	**Precision**
KD (M)	94.5 – 104.8%	3 %CV
ka	93.8 – 105.9%	3 %CV
kd	95.2 – 102.2%	2 %CV

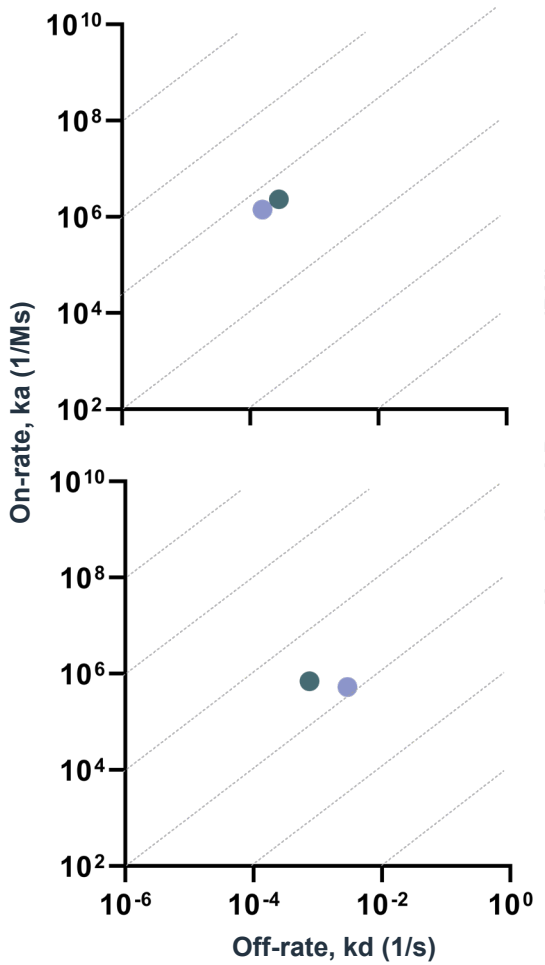
Standardised SPR assays focusing on high throughput or high performance depending on project needs.

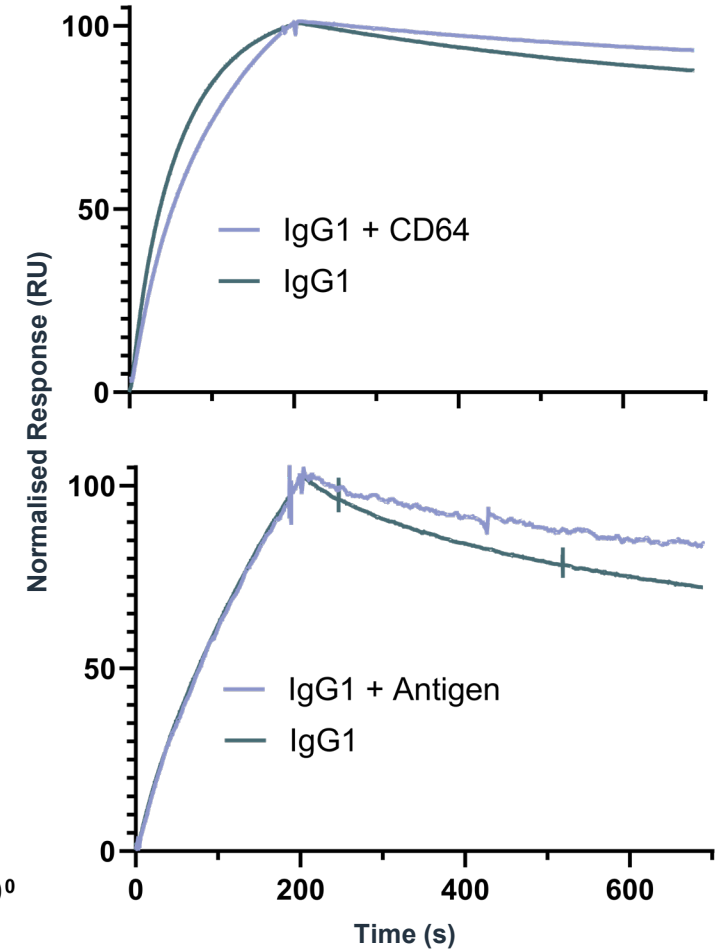
Example data - Fab-antigen-FcR dependency

- **IgG1-CD64** complexes bind PD1 with **similar affinity**
- **IgG1-Antigen** complexes bind CD64 with **differential affinity**









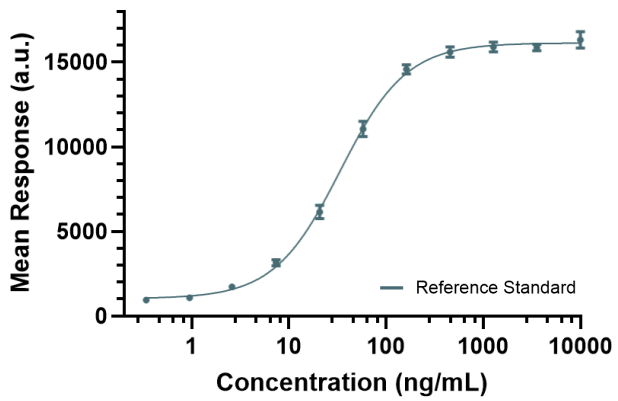
Primary MoA Characterisation

RoukenBio

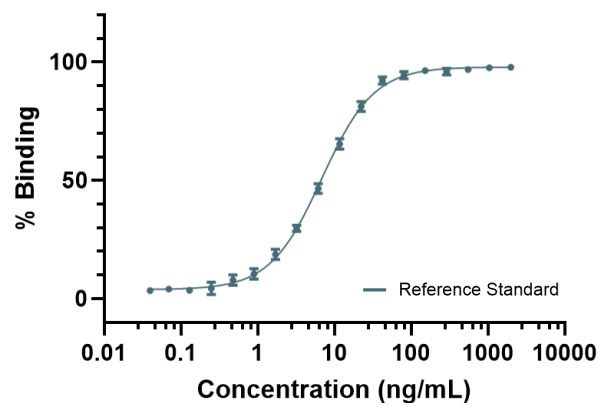
Primary MoA – ELISAs & cell-based bioassays

Example of Anti-TNF-α Characterisation Assays

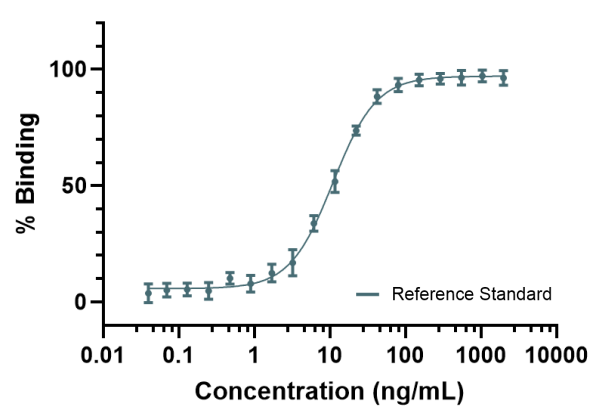
TNF-α Binding



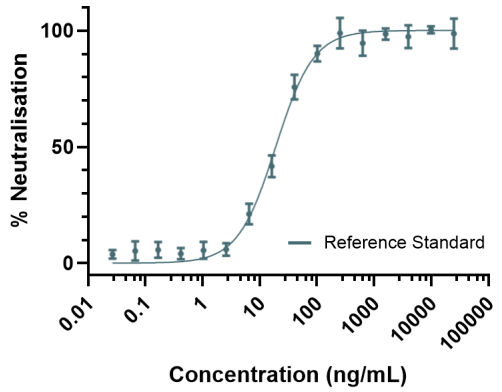
TNF-α Blocking ELISA for TNFR1



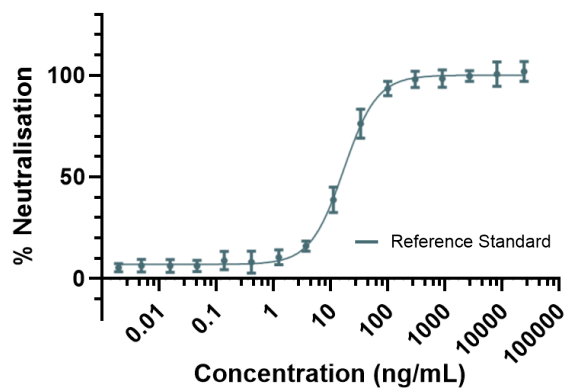
TNF-α Blocking ELISA for TNFR2



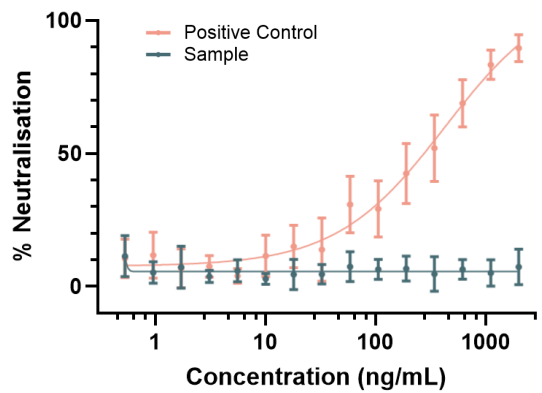
Potency – Neutralisation of Cell death (L929)



Potency – Neutralisation of Apoptosis (L929)



Potency - LTα Neutralisation of Apoptosis (U937)



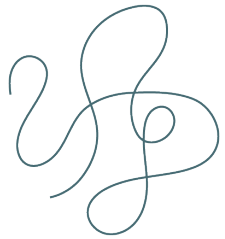
This data demonstrates the high-quality functional assays developed in-house for biosimilar characterisation including target interactions, neutralisation of TNF-α and LTα activity in relevant functional models.

Pre-developed reporter cell lines available

Created by RoukenBio

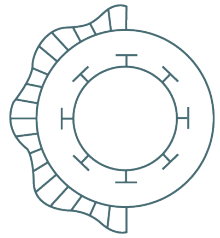
Reporter Assay Formats Available

- Jurkat FcγRIIIa (CD16) V176-NFAT-RE Luc RCB
- Jurkat FcγRIIIa (CD16) F176-NFAT-RE Luc RCB
- Jurkat FcγRIIa (CD32a) H167-NFAT-RE Luc RCB
- Jurkat FcγRIIa (CD32a) R167 NFAT-RE Luc RCB
- Jurkat FcγRI (CD64)-NFAT-RE Luc RCB
- PD-1 Jurkat NFAT Luc (PD1/PDL1 Reporter assay)
- TIGIT NFAT Luc
- LAG-3 NFAT Luc
- CTLA-4 NFAT Re Luc
- HEK-293 TSLPR Reporter (Luc)



Engineered Target Cells

- mTNFα CHO Luc
- IL6R CHO Luc
- IL12 CHO Luc
- HER2 CHO Luc
- CD20 CHO Luc
- CTLA4 CHO Luc
- PD-L1 CHO
- EGFR CHO Luc



In addition to our pre-developed cell lines, RoukenBio has extensive experience in the creation of custom reporter and target cell lines for biosimilar projects.

Case Study:

A MOA-reflective IL-23 inhibition assay for ustekinumab

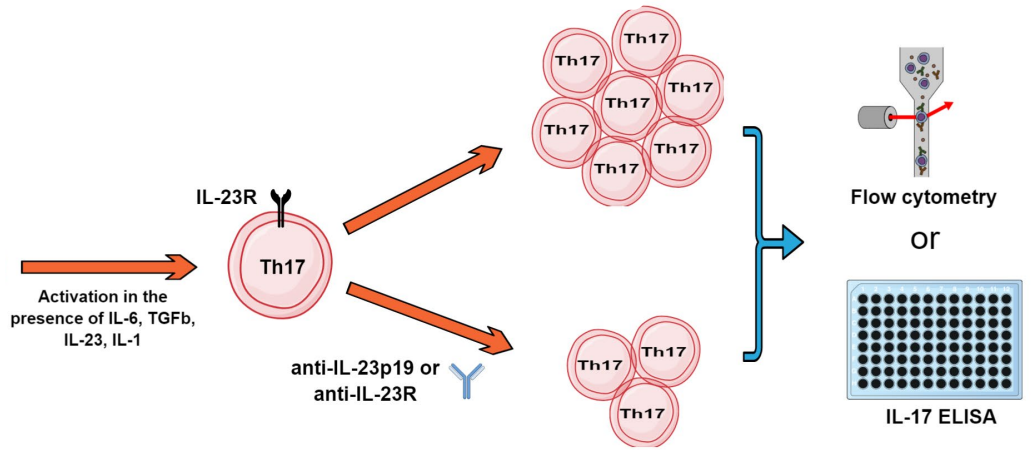
RoukenBio

MOA-Based Functional Confirmation Using Primary Human Cells

Biologically relevant assay for ustekinumab neutralisation of IL-23 mediated t cell activation

Biosimilar developers must confirm that their candidate blocks IL-23 activity in a physiologically relevant system.

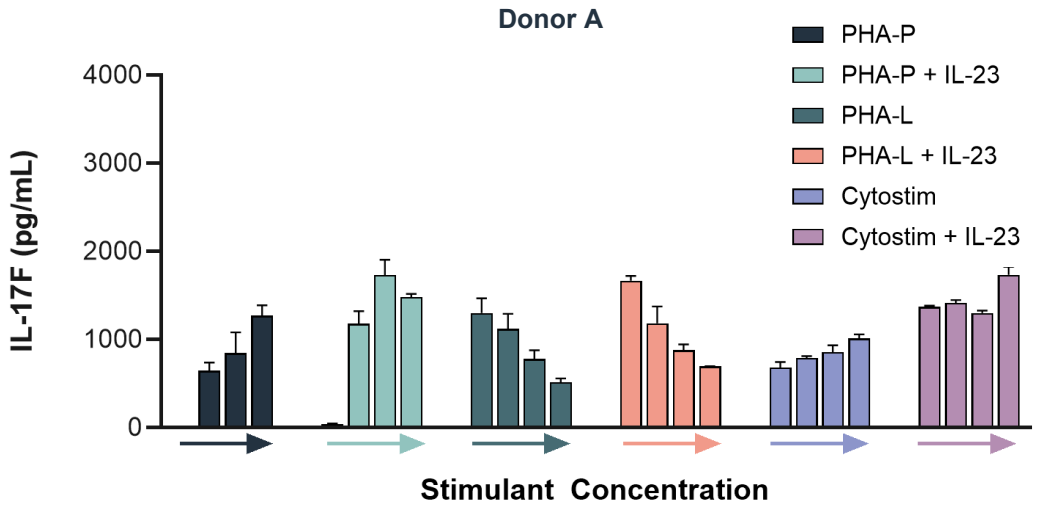
However, primary cell-based assays often exhibit high variability, making quantitative comparisons difficult.

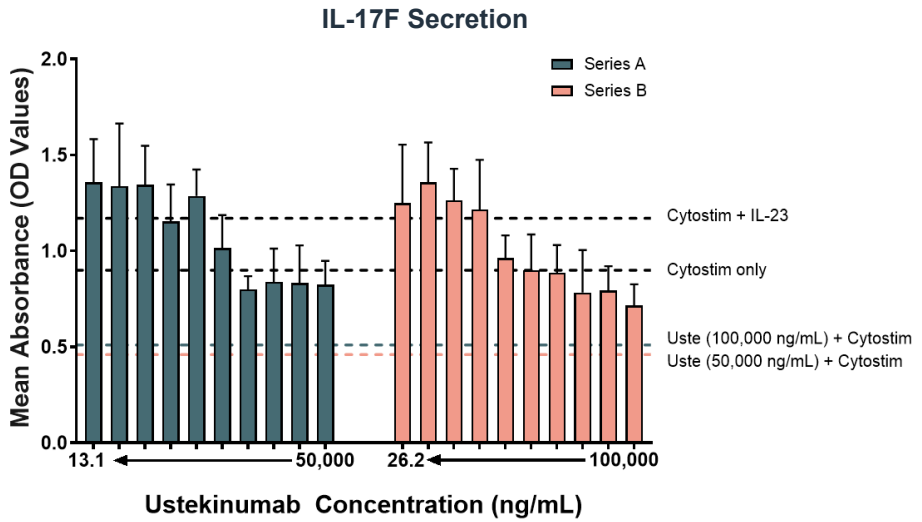


- IL-23 stimulates Th17 activation, driving IL-17 production.
- Blocking antibodies target IL-23, reducing IL-17 levels.

MOA-Based Functional Confirmation Using Primary Human Cells

Assay development for a reliable neutralisation of IL-23 mediated t cell activation





- Evaluation of PHA-P, PHA-L, and Cytostim-induced activation of Th17 cells in presence/absence of IL-23.
- IL-17A, IL-17F, IL-10, IL-22, IL-6, IFN-γ, and TNF-α secretion determined via flow cytometry).
- 3 donors assessed with illustrative IL-17F data shown for Donor A.

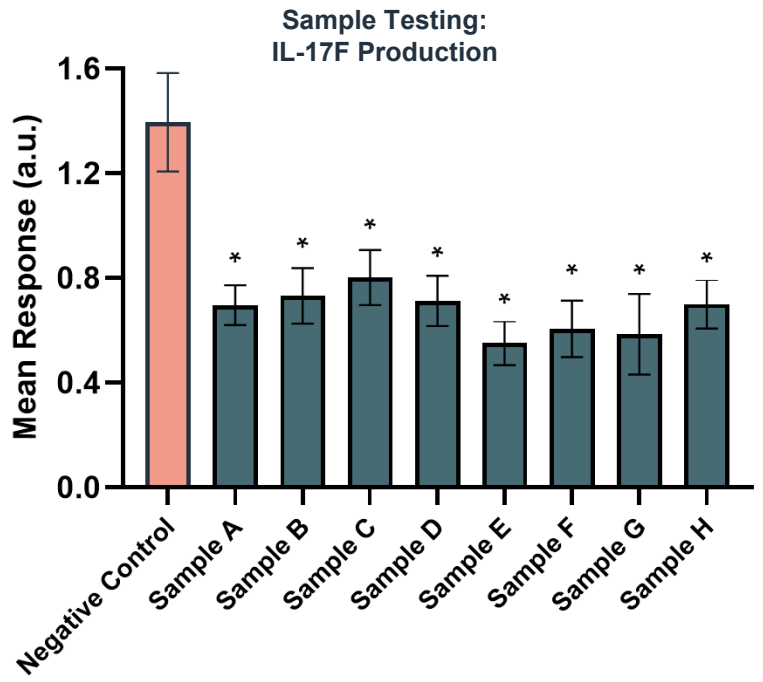
- Assay endpoint transferred to an ELISA format.
- Effective neutralisation of Ustekinumab-mediated inhibition of IL-23 stimulated IL-17F production.
- Variability was observed at all dilutions causing challenges with implementation of standard relative potency design.

MOA-Based Functional Confirmation Using Primary Human Cells

Reliable and controlled assay for the neutralisation of IL-23 mediated t cell activation

System Suitability Criteria

Parameter	Criteria
All Control Sample replicate variability	≤ 30% CV
	If >30% CV, application of Grubb's test to removal a single outlier for each control sample assessment
Negative Control	Mean Absorbance ≥ 0.6AU
Negative Control QC	Not Statistically different to Negative Control
	If statistically different to negative control: Mean absorbance > Negative Control mean absorbance
Positive Control	Statistically different to Negative Control
	Mean absorbance < Negative Control mean absorbance



This assay maintained physiological relevance with reduced variation to provide a controlled format to confirm the functional inhibition of IL-23 activity by Ustekinumab biosimilars.

Why Choose RoukenBio?

RoukenBio

What some of our customers have shared

Testimonials

alvotech

“We appreciate the consultative approach that RoukenBio uses, working closely with us to truly understand our needs before engaging. This model results in a highly customised offering, quick response times when challenges arise, and timely delivery of high-quality research services.”

Dr Claudia Wiza
Manager,
Functional Assays

NextPoint Therapeutics

“We were impressed right away by the team’s depth of knowledge and appreciated their input on our proposed experimental plans; the RoukenBio team excelled at communication and executing the work. I would highly recommend working with RoukenBio.”

Dr Bijan Etemad-Gilbertson
Vice President,
Antibody Technology

Larkspur BIOSCIENCES

“Collaborating with RoukenBio is like a natural extension of our internal team. The level at which RoukenBio inputs and buys into the project, along with their attention to close communication and detail, is not something we experience with other CROs and are key factors that drive project success”

Emily Corse, Ph.D
Executive Vice President,
Biology & Translation

igm biosciences®

“We have worked with RoukenBio for 3 years to run various immune cell based functional assays that our teams did not have the capabilities or capacity to run. We have been delighted with the quality of data, the clear collaborative communication & troubleshooting that RoukenBio has provided over the years and we look forward to continuing to work with them in our drug development processes.”

Dr Angus Sinclair
Executive Vice President,
Research

Collaborate with RoukenBio

RoukenBio is here to support your complex assay development for biosimilars.

Contact our experts and accelerate your drug development journey with confidence.

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Biosimilar Analysis Service Models

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Service Models: Key Benefits

The right service model to accelerate your development

	Full-Time Equivalent (FTE)	Fee for Service (FFS)
Suited to	• Complex, iterative development studies • Running multiple projects in parallel	• Well-defined projects • Stand-alone studies
Key benefits	• One contract for multiple projects with a single monthly invoice. • Ability to rapidly redeploy resource in line with your requirements • Freedom to change experimental direction and switch priorities	• Clarity of scope, milestones and timelines defined upfront at project start • Ready deployment of off-the-shelf methods and pre-developed platforms
The delivery	• Resource allocated to you over a pre-agreed (flexible) time period • Recurring meetings to facilitate data-driven decisions	• Timeline estimate provided for study completion • Ability to define “go / no-go” points in study