#### Part of popular assay cell line list developed at ProBio

Category	Cat. No.	Target	Product	Category	Cat. No.	Target	Product
ADCC	RD00830	CD16a 158V	GS-J2C/CD16a 158V TCE TRD00812 CD3 GS-		GS-J2C		
ADCP	RD00941	CD32/CD64	ADCP reporter Cytokine RD01027 IL-22 IL-		IL-22 Reporter cell		
Immune checkpoint	RD00901	4-1BB	GS-H3/4-1BB	B Cytokine RD01026 IL-21 (		GS-U2/IL-21R	
Immune checkpoint	RD00718	CD40	GS-H3/CD40	Cytokine	RD00956	RANK	GS-H3/RANK
Immune checkpoint	RD00940	LAG3	GS-J4B/LAG3	-J4B/LAG3 Cytokine RD00934 IL-12R		GS-E9/IL-12R	
Immune checkpoint	RD01036	BTLA	GS-J2B/BTLA	Cytokine	RD00932	IL-23R	GS-E8/IL-23R
Immune checkpoint	RD01034	Trem2	GS-J2C/DAP12/Trem2 Cytokine RD00930		GHR	GS-E7/GHR	
Immune checkpoint	RD01005	KIR3DL3	GS-J4B/KIR3DL3	Cytokine	RD00908	TSLPR	GS-E6/IL-7Ra/TSLPR
Immune checkpoint	RD00841	PVRIG	GS-J2C/PVRIG	Cytokine	ne RD00869 EPOR GS-E6/E		GS-E6/EPOR
Immune checkpoint	M00695	TIGIT/PD-1	GS-J2/TIGIT/PD-1 Cytokine RD00704 VEGF		GS-E3/VEGFR2		
Immune checkpoint	RD01038	BTLA/PD-1	GS-J2B/PD-1/BTLA	GPCR	RD01035	GCGR	CHO-K1/CRE-Luc/GCGR
Immune checkpoint	RD00942	CTLA4/PD-1	GS-J4B/CTLA4/PD-1	GPCR	RD00938	GIPR	HEK293/CRE-Luc/GIPR
Immune checkpoint	RD00899	4-1BB/PD-1	GS-J7/4-1BB/PD-1	GPCR	RD00918	GLP-1R	CHO-K1/CRE-Luc/GLP-1R

#### Research grade virus packaging and testing platform

**One-stop solution**: end-to-end services, including **gene synthesis**, **virus packaging**, **and functional evaluation**, all tailored to your gene of interest.



Proposal Plas	aration	irus/vector	Virus/vector	In vitro functional
design prepa		backaging	purification (optional)	analysis
2-4 weeks		1 week	1 week	1-2 weeks

#### CGT product functional characterization - CAR-T/NK in vitro assay service

Comprehensive CAR-T/NK in vitro pharmacology platform offering a range of services to support clients in developing CAR cell therapy

#### Highlights:

- High-throughput screening: high throughput and cost-effective platform for CAR lead screening
- Experience and expertise: versatile in vitro bioassays to validate CAR-T/NK cell function, backed by our extensive experience and expertise

#### Work-flow

Generation of CAR encoding vector	T/NK cell activation Expansion of CAR-T/NK cells	Functional assay for cytotoxicity and cytokine release
<ul> <li>FTO CAR vector</li> </ul>	Non-virus transfection     High fold expansion	Time-based killing assay
	Lentivirus	Cancer cell lines
	Retrovirus	Overexpression cell lines
		<ul> <li>Luciferase target cells</li> </ul>

#### GPCR drug screening and evaluation

- 57 families covering 153 GPCR targets and providing HTS screening assays
- > 200 GPCR functional assays
- 4 assay types (cAMP, calcium flux, beta-Arrestin and binding), covering almost GPCR signaling pathways
- 3 analysis of pharmacological MoAs (Agonist, Antagonist, Allosteric modulation)

#### Contact us

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# In Vitro Pharmacology Solutions

Acquiring a holistic grasp of your molecules



- Bioactivity of monoclonal Ab
- Bispecific Ab characterization
- ADC characterization
- Cell line engineering for assays



# **Highlight of In Vitro Pharmacology Platform**



# **Assay Platform**



# **Bioactivity of monoclonal Ab**

#### Aspects for monoclonal Ab evaluation



Fc domain MOA: Fc mediates effects

#### **Bioactivity analysis of Fab domain**

- Functional assays Binding assays Agonist activity Ligand blockade activity Neutralization assays
- Preclinical safety assessment Cytokine release syndrome assessment Species cross reactivity assessment
- More customized assays

#### **Bioactivity analysis of Fc domain**

- Functional assays Antibody-dependent cellular cytotoxicity (ADCC) assays Antibody-dependent cellular phagocytosis (ADCP) assays Complement-dependent cytotoxicity assay (CDC) assays
- Preclinical safety assessment Antibody-dependent enhancement(ADE) test Induction of cytotoxicity in non-targeted cells

## In Vitro Pharmacology Solutions

# **Bispecific Ab characterization**

#### Aspects for BsAb evaluation



# **ADC** characterization

#### Aspects for ADC evaluation

Payload

**MOA**: a cell-killing cytotoxic drug conjugated in Ab, inducing target cell death Cell based assay: cytotoxicity assay

Antibody internalization assay

Antigen-mediated internalization is a

• pH-indicator based internalization

• Toxin-conjugated mAb based

tion

critical characteristic of ADC candidates.

• Live-cell imaging based internalization

cytotoxicity assay (DT3C/FabFc-ZAP)

• Temperature shift based internaliza-



# Cytotoxicity assay

Cytotoxicity assays, measuring the killing potential of ADCs are a necessary first step to triage ADC molecules.

- CellTiter-Glo<sup>®</sup>
- CCK8
- LDH • Luminescence/fluorescence-based

### By-stander effect

\_ \_ \_ \_ \_ \_ \_ \_ \_ \_

Bystander cell is the surrounding cell of tumor, which could be Ag+ or Ag-. When the tumor cell is lysed, the uptaken toxin will be released to kill neighboring cells.

Fab Region

internalization assay

• Medium transfer or co-culture assay using live cell imaging or Flow cytometry

#### In Vitro Pharmacology Solutions

	Scope	Internalization assay					
	Cell based assay	Live-cell imaging based internalization	Toxin-conjugate cytotoxicit	d mAb based ty assay	pH-indicator based internalization	Temperature shift based internalization	
	General designation	Incucyte	DT3C	FabFc-ZAP	pHrodo	Temperature Shift	
 et	Diagram	Live-Cell Analyzer Incucyte®	Andory DTSC + CED C statisfies Instruction 3:C Pointings Instruction 3:C Pointings - Construction 3:C Pointings - C Poin	Le Protect	Mandade for Y Provide instants and the Y Provide instants Instants of the Provide instants Instants	Antibody 4 °C - Antibody Briding Target Antigen 37 °C-Antibody Internalization Secondary antibody Secondary antibody	
	Molecule	Naked Antibody					
ay	Analyzer	Live-Cell Analyzer	Microplate	Reader	FACS	FACS	
У	Feature	Real-time and kinetics analysis	High sens High throu Close to the cyt	sitivity ughput totoxic MOA	High sensitivity Low throughput	Cost-effective Susceptible to multiple factors	
	Application	Most versatile Best choice	Alternative choice	Optional	Optional	Optional	
	Case study	+ HEDSH' + LCOR + LCOR + LCOR + Henrical + H	+ MEGN/ + 100 	- HEOSH7 - TCO - TCO - TCO - TCO - TCO - Homesgri - Hom	+ MECHAF + DC08 + Homesupot -	+ HESH/ + LOR + LOR + Henry(2) - Henry	

# **Cell line engineering for assays**

#### High quality of cell line development

Substantial cell library: 70+ in-house reporter cell lines; 450+ overexpression cell lines Clear tracebility and construction records, ensuring the compliance with ICH and FDA guidelines Efficient method development process: meets the requirements throughout both of the ADD and CMC stages, with a 100% success rate in method qualification. Cases for lot release (ADCC, PD-1, 4-1BB, PVRIG, VEGF etc.)

#### Work-flow of bioassay cell line development



**MOA**: Fab region binds to target antigen, triggering endocytosis Cell based assay: antibody

