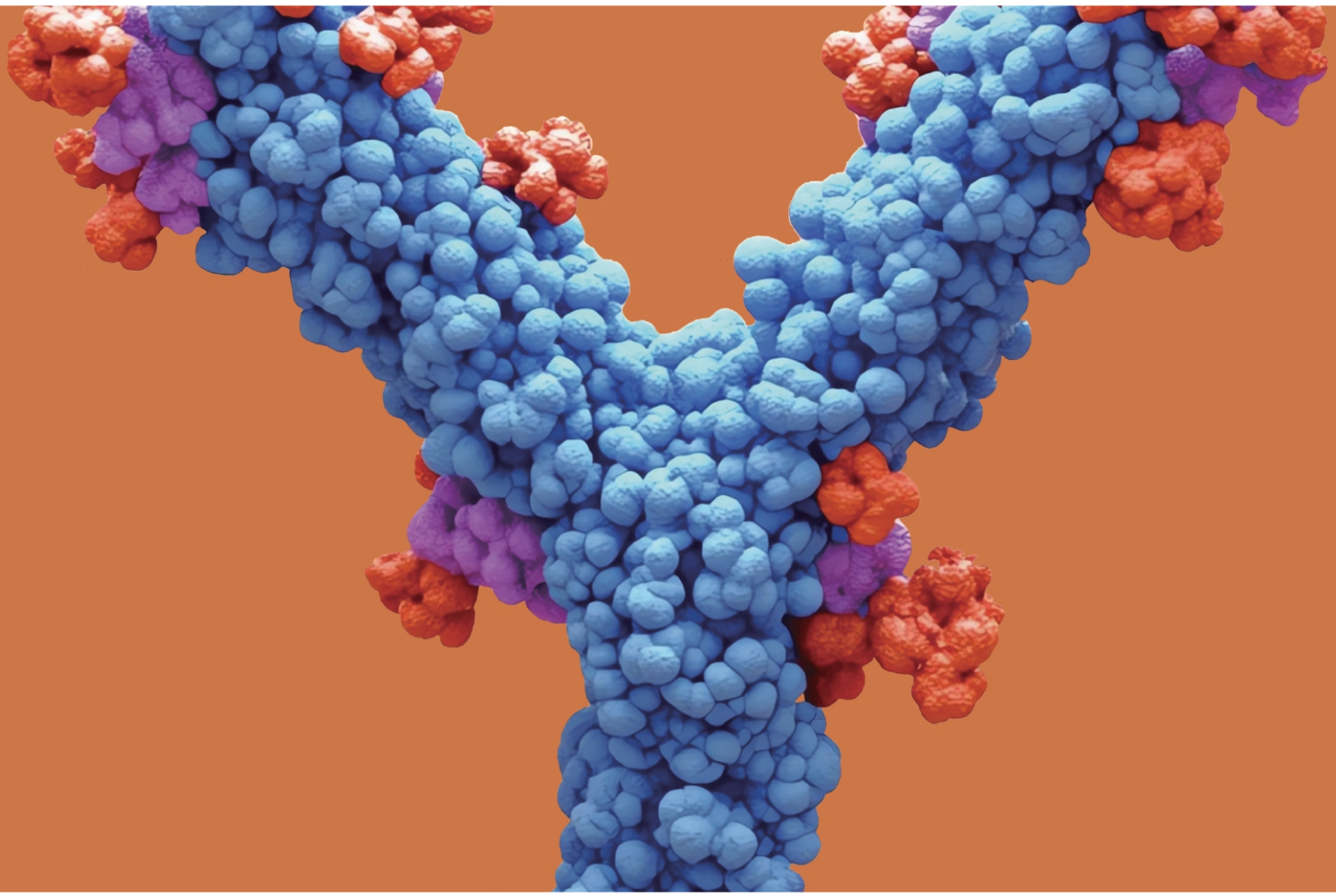
ADC Discovery and Evaluation

Streamlined Solutions for Biology and DMPK



Innovative CRO+Explorer
ICE Bioscience

Summary of Biological Services for ADC Screening and Evaluation

ICE Bioscience offers targeted services for ADC screening and evaluation at every stage of drug development. We provide antibody evaluation, payload screening, and comprehensive ADC characterization to optimize your therapeutic candidates from hit-to-lead through preclinical studies.

	Hit-to-Lead Screening	Lead Optimization or PCC Selection	Preclinical Study
Antibody Evaluation	• Affinity (SPR)-Single conc. • 500+ cancer cell bank, Gene editing cell lines • Absolute/relative expression measurement of membrane antigen • Binding activity • Internalization activity	• Affinity (SPR)-Multiple conc. Kinetics • Cell binding EC_{50} • Internalization activity (different detection methods) • Fc effect assays • Target related downstream MOA verification (if needed)	
Payload (P/L+P) Screening and Evaluation	• 10+ years biological study experience for small molecule drugs • New cytotoxic payload screening • Topoisomerase I inhibition assay • DDR payload screening • TPD payload screening • Immune-modulator payload screening • Cancer cell killing	• Drug-resistant cell line screening • Cell panel screening • Bystander effect	• hERG/cardiac safety • Safety panel • Kinase panel
ADC Evaluation	• ADC characterization • Affinity (SPR) • Binding activity of antibody and ADC • Cancer cell killing	• Binding activity of antibody and ADC • Internalization activity (different detection methods) • Cancer cell killing • Reporter assay (if needed) • Drug-resistant cell line • Bystander effect (*in vitro+in vivo*) • CDX efficacy	• Binding activity of antibody and ADC • Internalization activity (different detection methods) • Antibody and ADC internalization and co-localization • Cancer cell killing • Fc effect assays • Cell cycle detection • Apoptosis • Drug-resistant cell line • Bystander effect (*in vitro+in vivo*) • CDX efficacy

Payload Screening

Our payload screening services are distinguished by our dual expertise in cytotoxic payload and new MOA payload screening. Our capability in both areas ensures an approach for screening effective and safe payload suitable for ADC development.

New MOA Payload Screening

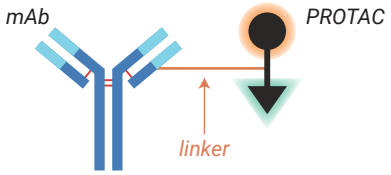
DDR Inhibitors as ADC Payloads

We offer comprehensive screening services designed to identify and evaluate DDR inhibitors that can effectively target and kill cancer cells by exploiting their reliance on DNA repair pathways. (Learn more in our DDR brochure)

DDR Pathways	Targets
HR	ATM, ATR, BLM, RECQ1, RECQ4, RECQ5
NHEJ and TMEJ	DNA-PK, WRN, POLQ
SSB and BER	PARP1/2/3/6/7/10/11/12/14/15, TNSK1/2, FEN1, Topo I, APE1, XRCC1
Cell Cycle	WEE1/2, MYT1, p53 Y220C, CHK1/2
Others	POLA, POLG, POLH, MAT2A, PRMT5, TREX1/2

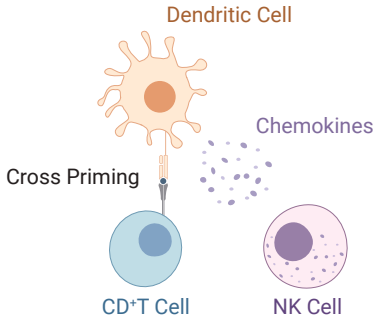
TPD Agents as ADC Payloads

Our targeted protein degradation (TPD) payload screening service is particularly aimed at addressing challenging cancer targets that are otherwise undruggable. (Learn more in our TPD brochure)



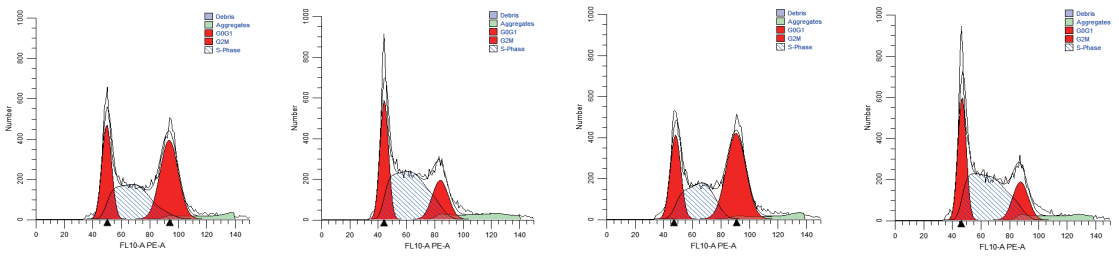
STING Agonists as Immune-Activating Payloads in ADCs

By incorporating STING agonists as payloads, STING-ISAC can activate a robust immune response directly within the tumor microenvironment. We offer comprehensive screening services for both STING agonists and STING-ISAC.(Learn more in our Immunology brochure)



Cell Cycle Arrest Assay

Cell cycle arrest assays are critical in evaluating the efficacy of ADCs, specifically in understanding how the cytotoxic payload affects the proliferation of cancer cells. The principle behind these assays is to determine the ability of an ADC's payload to disrupt the normal progression of the cell cycle, causing the cancer cells to stop dividing and eventually die. This disruption typically occurs at specific phases such as G1, S, or G2/M, depending on the mechanism of action of the payload.



DS-8201 Trastuzumab DXd DMSO @20 nM

Figure 1. Cell cycle of HCC1954 cell line was arrested by treatment with DS-8201 and DXd, compared to Trastuzumab and negative control by flow cytometry.

Apoptosis Assay

Flow Cytometry Analysis allows for the quantification of apoptotic cells in a population based on changes in cell size, complexity, and membrane asymmetry. In this case, Annexin V FITC-A and PI-PE-A are used as markers for apoptosis. Annexin V binds to phosphatidylserine, a molecule that is translocated from the inner to the outer leaflet of the plasma membrane early in apoptosis. PI (Propidium Iodide) is a nucleic acid-binding dye that is impermeant to live cells and early apoptotic cells, but stains late apoptotic or dead cells.

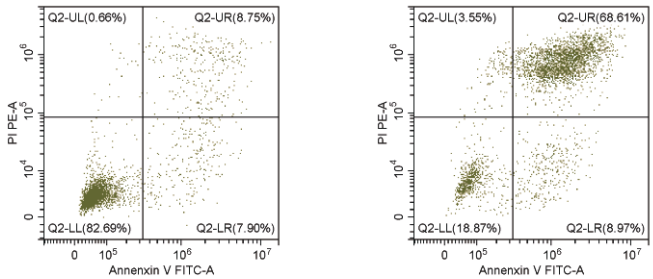
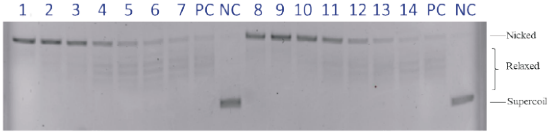


Figure 2. Test compound induced cell apoptosis, indicated by Annexin V/PI staining.

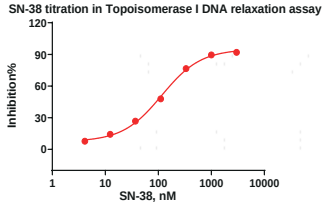
Topoisomerase I–Mediated DNA Relaxation Assay

Topoisomerase I (Topo I) is a crucial enzyme in DNA replication and transcription processes, responsible for alleviating torsional strain in the DNA helix by inducing single-strand breaks and re-ligation. The Topoisomerase I-Mediated DNA Relaxation Assay is used to assess the ability of an ADC's payload to inhibit Topo I, thereby preventing the re-ligation of the DNA strands. This assay helps in determining the potency and effectiveness of the payload as a Topo I inhibitor.

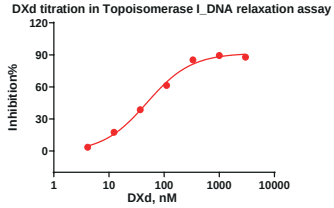


1: 3000 nM SN-38
2: 1000 nM SN-38
3: 333 nM SN-38
4: 111 nM SN-38
5: 37.0 nM SN-38
6: 12.3 nM SN-38
7: 4.12 nM SN-38
PC: Top1+pBR322 with DMSO
NC: only pBR322 with DMSO

8: 3000 nM DXd
9: 1000 nM DXd
10: 333 nM DXd
11: 111 nM DXd
12: 37.0 nM DXd
13: 12.3 nM DXd
14: 4.12 nM DXd



Bottom	7.313	HillSlope	1.178
Top	94.98	IC50	116.2



Bottom	-1.297	HillSlope	1.082
Top	91.66	IC50	48.90

Figure 3. Inhibitory activity of SN-38 and DXd on Topoisomerase I (Top1). SN-38 and DXd prevented recombinant hTop I from converting supercoiled DNA to relaxed closed circular DNA. Gel electrophoresis result (left) and inhibition IC_{50} curve (right) of SN-38 and DXd.

Antibody/ADC *In Vitro* Screening & Evaluation

ADC Conjugation and Characterization

We provide comprehensive ADC conjugation and characterization services, including Size-Exclusion Chromatography (SEC) for analyzing ADC size and aggregation state, Hydrophobic Interaction Chromatography (HIC) for characterizing ADC hydrophobicity and conjugation status, and free payload/linker-payload (LP) detection for identifying and quantifying unbound payloads.

DAR Analysis (Drug-to-Antibody Ratio) quantifies the average number of drug molecules conjugated to each antibody molecule. It is crucial for optimizing the therapeutic efficacy and safety profile of the ADC.

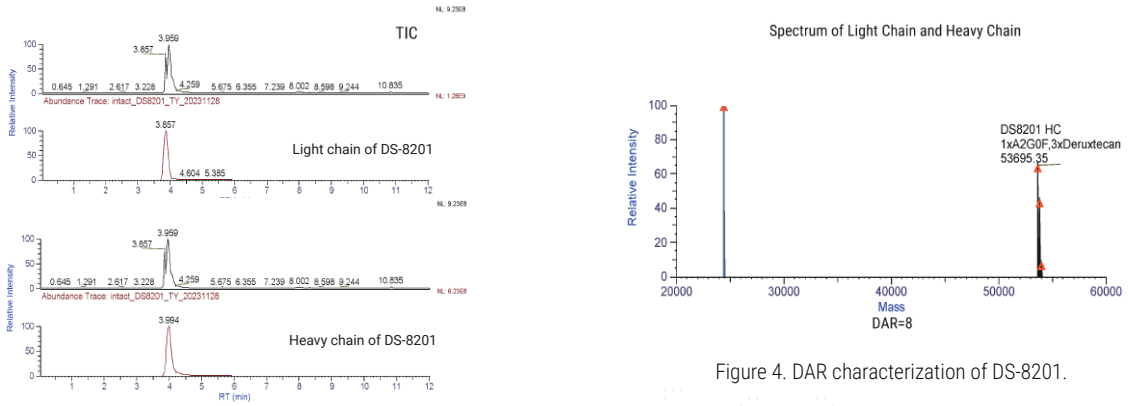


Figure 4. DAR characterization of DS-8201.

Quantitative Analysis of Antigen Membrane Expression

Our service provides precise measurement of target antigen levels on cell surfaces, crucial for identifying and validating new ADC targets. Utilizing advanced flow cytometry, we quantify antigen density on various cell types, ensuring target suitability for ADC development. This detailed analysis supports the selection of high-value targets, enhancing the specificity and efficacy of next-generation cancer therapies.

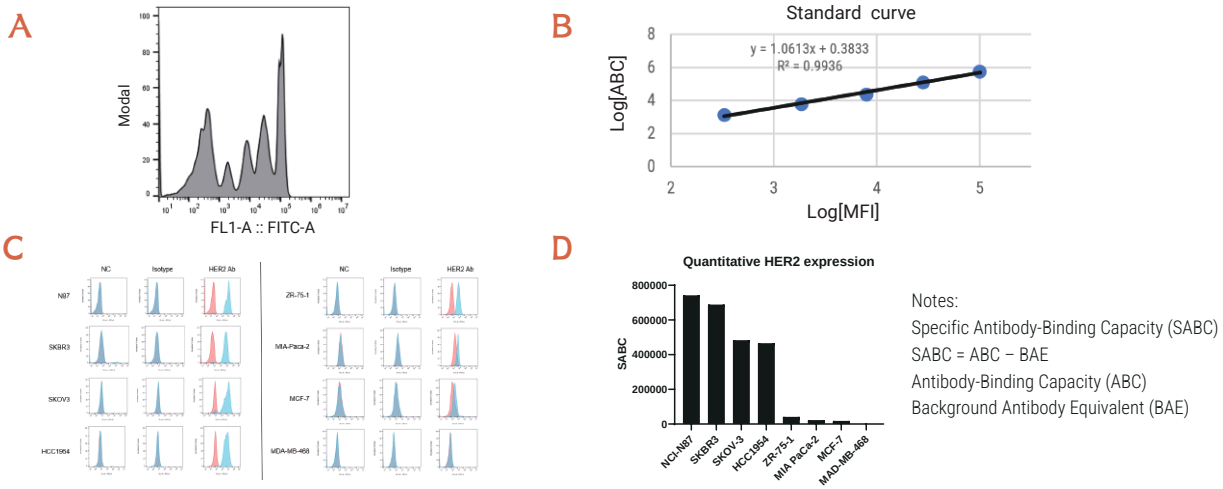
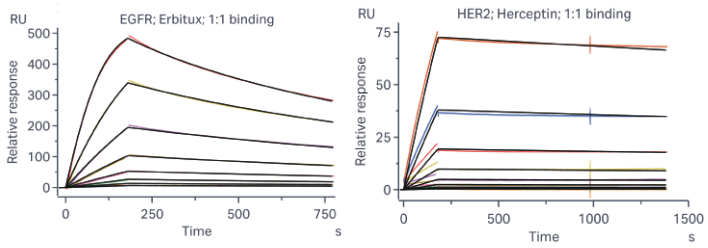


Figure 5. Quantitative analysis of HER2 antigen membrane expression in different cancer cell lines with high/medium/low/negative HER2 expression. A. Histogram of Calibration Bead populations. B. Standard curve. C. Assessment of HER2 expression in different cancer cell lines. D. Quantitative analysis of HER2 membrane expression. SABC, Specific Antibody-Binding Capacity.

Surface Plasmon Resonance (SPR) Services

SPR provides detailed analysis of the binding interactions between therapeutic antibodies and their target antigens, which is essential for designing effective ADCs. The affinity of ADC should be similar as that of naked antibody.



Sample	k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)
Erbitux	9.87E+05	1.47E-03	1.49E-09
Herceptin	2.04E+04	7.28E-05	3.56E-09

Figure 6. SPR analysis of Trastuzumab (Herceptin) binding affinity to HER2-ECD antigen (left) and Cetuximab (Erbitux) binding affinity to EGFR-ECD antigen (right) using Biacore 8K.

Flow Cytometry-Based Antibody Binding Assays

Flow cytometry is a powerful technique for analyzing the binding of antibodies to target antigens, on the surface of different cell lines. This service is essential for evaluating the binding affinity and specificity of antibodies and ADCs across various cell lines. The data is essential for optimizing ADC development, ensuring targeted delivery to specific cancer cells, and minimizing off-target effects.

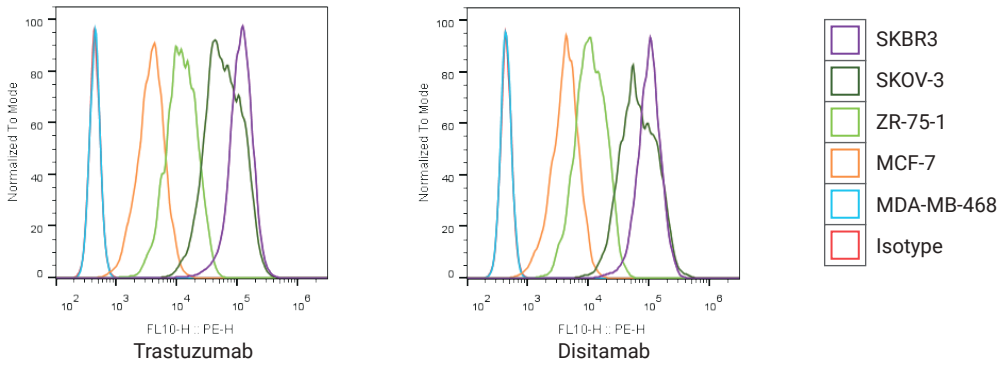
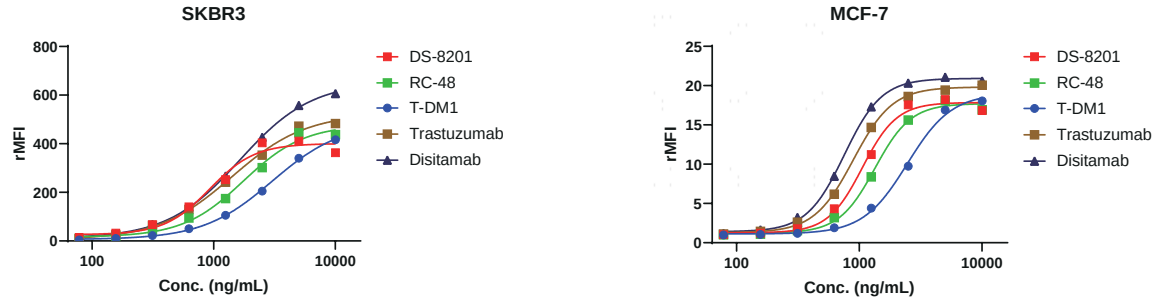


Figure 7. Cell binding activity of Trastuzumab and Disitamab in different cancer cell lines with different HER2 expression levels.



	T-DM1	DS-8201	RC-48	Trastuzumab	Disitamab
EC50	3020	923.4	1754	1413	1630

	T-DM1	DS-8201	RC-48	Trastuzumab	Disitamab
EC50	2458	1057	1338	892.1	752.4

Figure 8. Cellular affinity comparison of ADCs and corresponding antibodies in SKBR3 (left) and MCF-7 (right) cell lines. Tested samples include T-DM1, DS-8201, RC-48, Trastuzumab, and Disitamab.

Comprehensive Internalization Assays

Our Internalization Assay Service utilizes Incucyte live-cell imaging, flow cytometry, and high-content analysis to provide detailed, real-time analysis of ADC internalization into target cancer cells. By combining these advanced technologies, we offer precise, quantitative insights into the uptake and intracellular trafficking of fluorescently labeled ADCs, ensuring optimal design and efficacy of your ADC therapies.

Incucyte provides real-time, continuous monitoring of ADC internalization in live cells. This system captures dynamic processes, allowing visualization of ADC internalization and intracellular trafficking over extended periods, which helps in understanding the temporal aspects of ADC behavior.

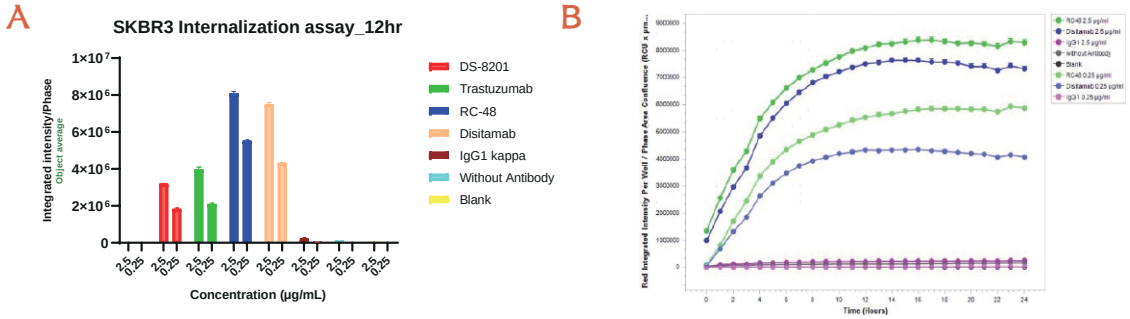


Figure 9. (A) Internalization activity of ADCs and the corresponding antibodies in SKBR3 cell line with 12 hours incubation, detected by IncuCyte. Tested samples include DS-8201, RC-48, Trastuzumab, and Disitamab. (B) Internalization activity of Disitamab and RC-48 in SKBR3 cell line detected by IncuCyte. Internalization was monitored from 1 to 24 hrs.

Temperature shift-based internalization assay is a classical method to evaluate the antibody internalization rate, by calculating MFI of the surface antibody in two groups at 37°C and 4°C respectively.

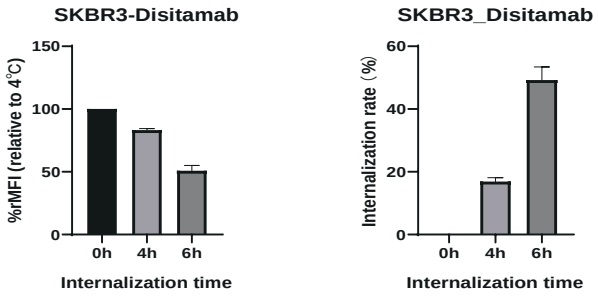


Figure 10. Internalization rate of Disitamab in SKBR3 cell line was measured at different incubation time points.

The internalization efficiency of the antibody can be assessed by measuring the cell killing effect, by using **Toxin-based** cytotoxicity assay.

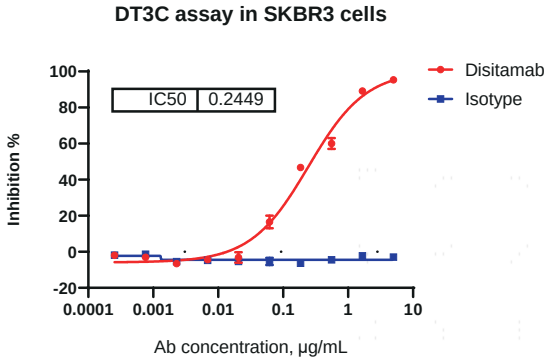


Figure 11. Internalization activity of Disitamab in SKBR3 cell line was evaluated by DT3C assay.

The **pH-indicator based** internalization flow cytometry assay provides a specific and accurate method for measuring ADC internalization. By utilizing pH-sensitive dyes, this technique ensures that only ADCs internalized into the acidic compartments of cells are detected, offering precise insights into the efficiency and kinetics of ADC internalization and trafficking.

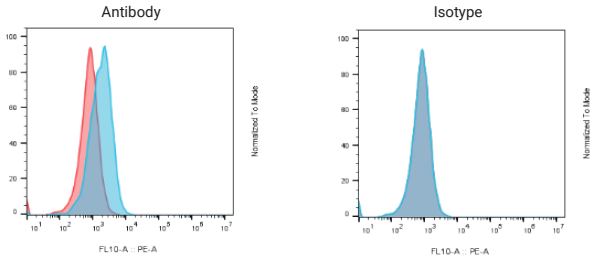


Figure 12. Internalization activity of Trastuzumab in SKBR3 cell line detected by flow cytometry.

High-Content Analysis combines automated microscopy and image analysis to provide detailed, multiparametric data on ADC internalization at the single-cell level. It allows for in-depth analysis of intracellular localization, colocalization with organelles, and morphological changes, offering comprehensive insights into the mechanisms of ADC internalization.

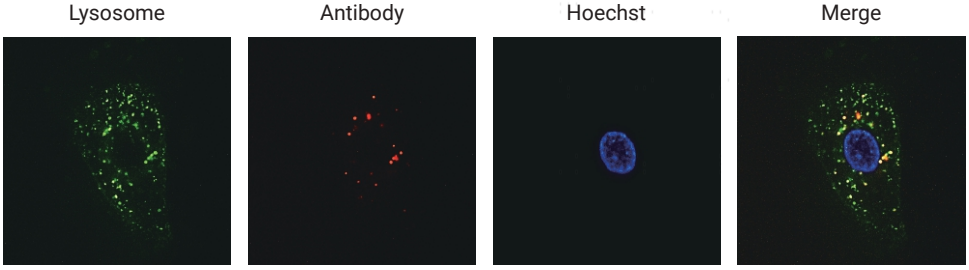


Figure 13. High-Content Analysis of ADC Internalization in cells. The images show cells treated with a fluorescently labeled antibody (red) and the lysosome marker (green). Nuclei are stained with Hoechst dye (blue). The merged image demonstrates the internalization and colocalization of the Antibody within the cells, highlighting its intracellular trafficking and accumulation in specific cellular compartments.

Cytotoxicity Assays

Our live cell imaging-based cytotoxicity assay service provides real-time, kinetic analysis of cell viability and cytotoxic effects of therapeutic agents, including ADCs. Using the Incucyte live-cell imaging system, we offer a detailed and dynamic view of how your ADC affect cancer cell populations over time.

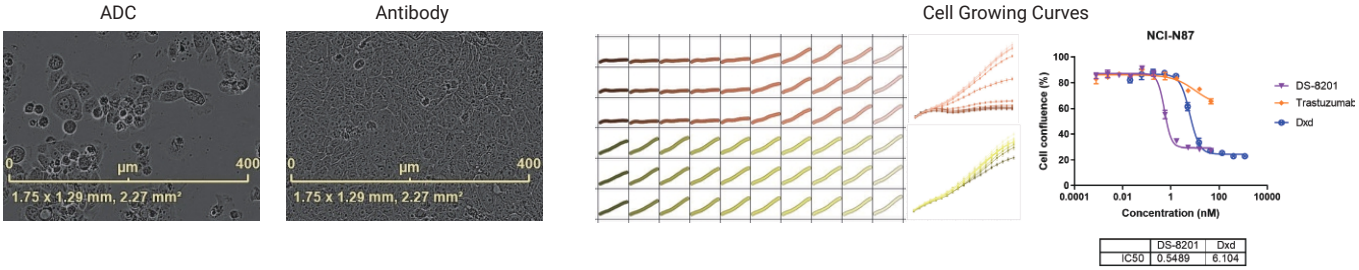
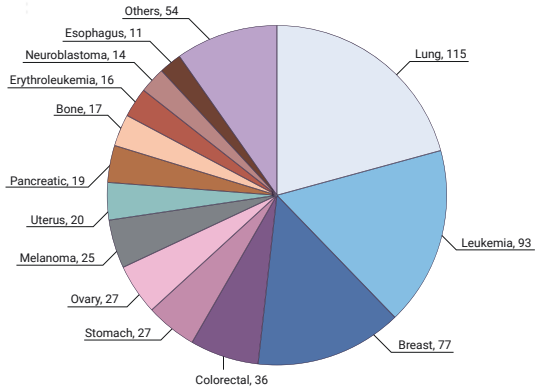


Figure 14. Cytotoxicity of DS-8201 and DXd in the NCI-N87 cell line. The IC_{50} value was approximately 10 times lower after conjugation to the antibody.

ADC Cytotoxicity Cell Panel Screening

ADC Cytotoxicity Cell Panel is specifically designed for evaluating the efficacy of ADCs for specific targets using cell viability assays. For different ADC targets, we may supply tumor cell lines of different caner types with different expression levels.

Human Cancer Cell bank in ICE



Frequently Used Cell Lines for HER2-Targeted ADC

Cell Line	Cancer Type	HER2 Expression
SKBR3	Breast	High
HCC1954	Breast	High
NCI-N87	Gastric	High
SKOV-3	Ovarian	High/Medium
JIMT-1	Breast	Medium
ZR-75-1	Breast	Low
MCF-7	Breast	Low
MDA-MB-468-GFP	Breast	None

In Vitro Bystander Effect Assays

Our Bystander Effect Assay Service evaluates the indirect cytotoxic effects of ADCs on neighboring cancer cells that do not express the target antigen. Utilizing advanced cell culture techniques and real-time imaging, this assay provides critical insights into the ability of ADCs to release cytotoxic agents that diffuse and kill nearby antigen-negative cells, thereby enhancing overall therapeutic efficacy.

Bystander Effect Assays	Co-culture of Ag^+ and Ag^- Cells with GFP and RFP Fluorescence		Co-culture of Ag^+ Cells w/wo GFP/RFP Fluorescence and Ag^- Cells with Luciferase		Co-culture of Ag^+ and Ag^- cells without Tag	
Analyzer	IncuCyte/High-Content Analysis	Flow Cytometer	IncuCyte + Microplate Reader	Microplate Reader	Flow Cytometer	Microplate Reader
Assay Feature	Could be real-time; Specific	Specific	Specific	Specific	Could be specific	Cost-effective; Not specific
Testing Options	• ~40 tag-expression reporter cell lines • Different project stages • Different tumor types • Different Ag expression levels			• Different detection methods • Different co-culture combinations • Different co-culture ratios • Different concentrations or IC_{50} of Ag^- cells		

IncuCyte-Based Bystander Effect Assays

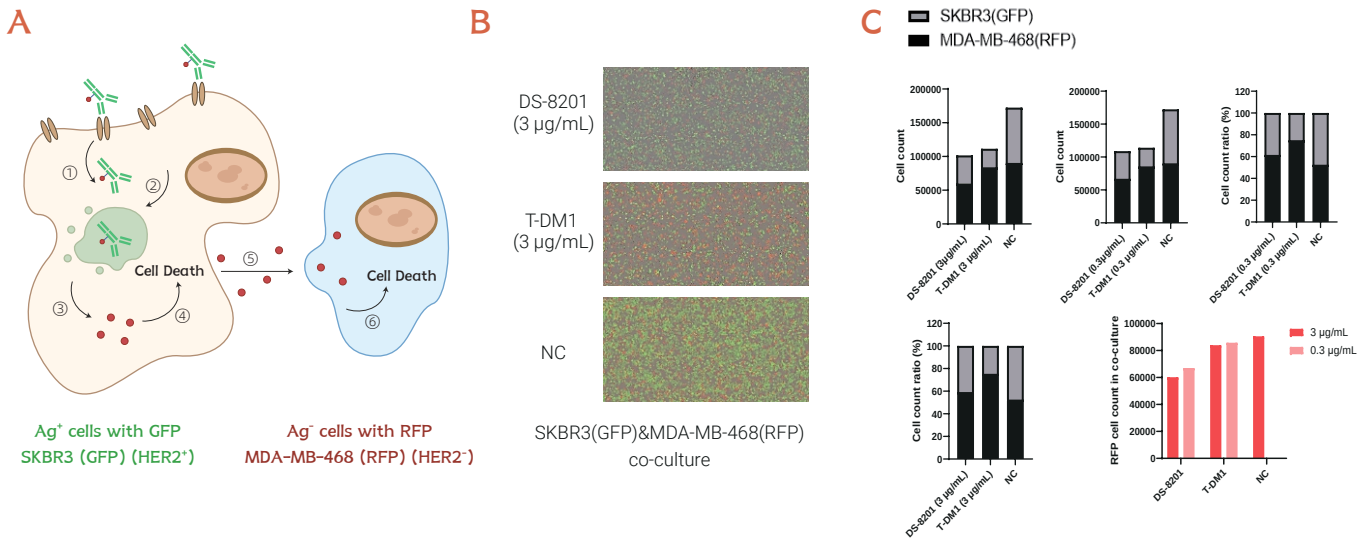


Figure 15. Bystander Effect Assay in Co-Cultured SKBR3 (GFP) and MDA-MB-468 (RFP) Cell Lines by Incucyte. (A) Engineered breast cancer cell lines SKBR3 (GFP) (HER2+) and MDA-MB-468 (RFP) (HER2-) were co-cultured to evaluate the bystander effect of an ADC. T-DM1 is negative control ADC for bystander effect assay. (B)The images, captured using the Incucyte live-cell imaging system, show the interaction and cytotoxic impact on both cell types after treatment for 3 days. (C) Cell counts were performed to assess the extent of the bystander effect, indicated by the reduction in RFP-labeled MDA-MB-468 cells.

Flow Cytometry-Based Bystander Effect Assays

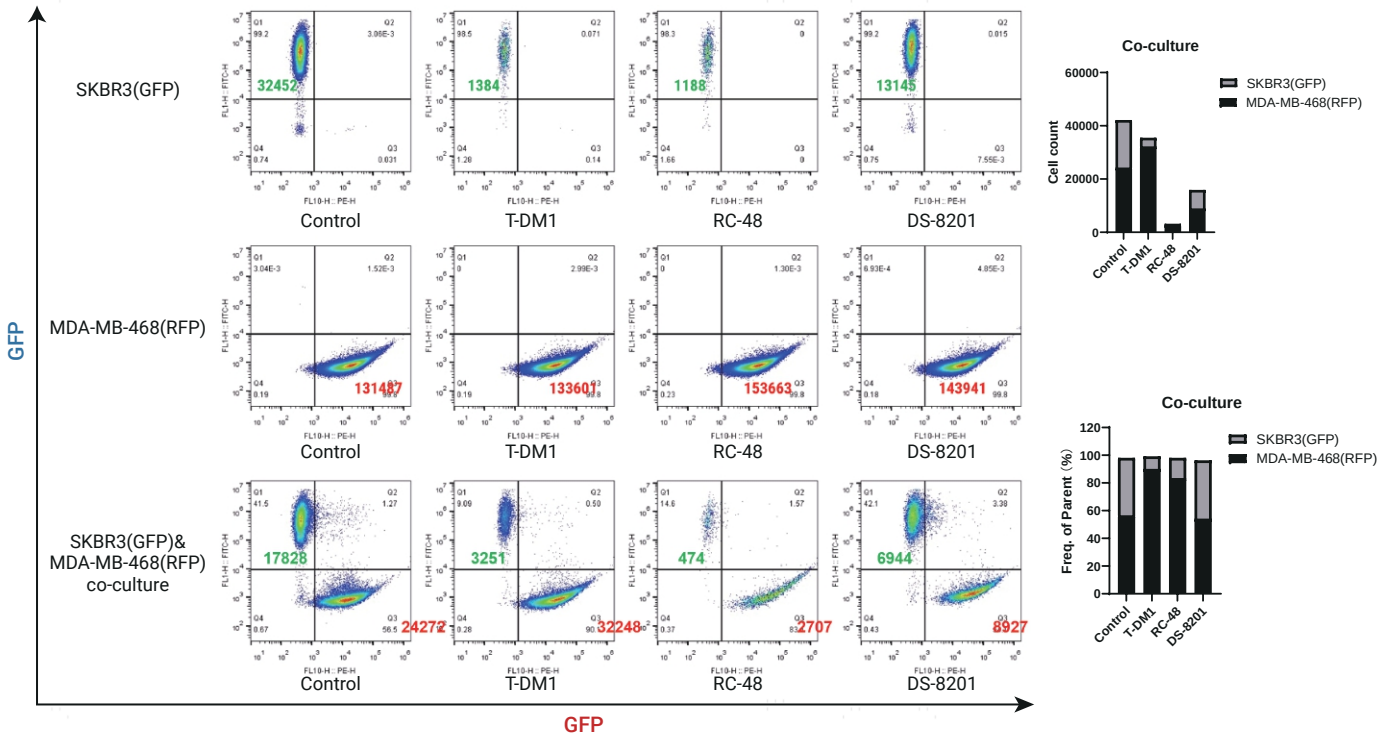


Figure 16. *In vitro* bystander effect assay of DS-8201 and RC-48 in engineered breast caner cell lines detected by flow cytometry. T-DM1 is negative control ADC for bystander effect assay. A. Flow cytometry results of SKBR3(GFP)&MDA-MB-468(RFP) co-culture after ADC treatment. B. GFP and RFP cell counts and cell count ratio in the co-culture system.

Ready-to-Use Tag-Expression Reporter Tumor Cell Lines

Our Tag-expression Reporter Tumor Cell Lines are engineered to express fluorescent or luminescent tags, enabling specifically monitoring of ADC interactions and effects. These cell lines simplify and accelerate ADC research by allowing precise tracking of efficacy in target and bystander cells. With these reporter lines, researchers can efficiently evaluate ADC bystander effect, optimize linker-payload design, and understand the mechanisms of action, enhancing the development of targeted cancer therapies.

Fluorescent Protein-Expression Tumor Cell Lines

Cancer Cell Type	GFP Cells	High Expression Targets
Breast cancer	SKBR3-GFP	HER2, TROP2...
Breast cancer	HCC1954-GFP	HER2, TROP2, MUC1, CDH3...
Breast cancer	HCC70-GFP	MUC1, B7-H4, CDH3...
Breast cancer	ZR-75-1-GFP	MUC1, LIV-1, B7-H4...
Ovarian cancer	SKOV-3-GFP	HER2, TROP2, Nectin4, B7-H4...
Ovarian cancer	OVCAR-3-GFP	MSLN, B7-H4, MUC16, CLDN6...
Gastric Cancer	NCI-N87-GFP	HER2, TROP2...
Gastric Cancer	NUGC-4-GFP	CLDN18.2, HER3...
Pancreatic cancer	BXPC-3-EGFP	MET, CEACAM6, CEACAM5, CDCP1, EPHA2...
...		

Note: these cells can also be used for other low expression targets.

Cancer Cell Type	RFP Cells	Low Expression Targets
Breast cancer	MDA-MB-468-RFP	HER2, Nectin4, EGFR, B7-H4, NOTCH3...
Breast cancer	MCF-7-RFP	FOLR1, Nectin4, EGFR, ROR1, PTK7...
Lung cancer	NCI-H1792-RFP	FOLR1, Nectin4, MUC1, ERBB3, CEACAM6...
Lung cancer	NCI_H226-RFP	DLL3, TACSTD2, Nectin-4, CEACAM6, ITGB6...
Colon cancer	SW620-RFP	TACSTD2, CEACAM5...
Pancreatic cancer	MIA-PaCa-2-RFP	CLDN18.2, ITGB6, 5T4...
...		

Note: these cells can also be used for other high expression targets.

Luciferase/Fluorescent Protein-Expression Tumor Cell Lines

Cancer Cell Type	Cell Lines
Breast cancer	MDA-MB-231-Luc
Breast cancer	MDA-MB-468-Luc
Breast cancer	MCF-7-Luc-mEGFP
Breast cancer	H1299-Luc-mEGFP
Brain Carcinoma	LN299-Luc-mEGFP
Brain Carcinoma	U251-Luc-mEGFP
Brain Carcinoma	CT2A-Luc-mEGFP
Colorectal Carcinoma	DLD-1-BRCA2-KO-Luc-mEGFP
Colorectal Carcinoma	HCT116-Luc-mEGFP
Lung cancer	NCI-H1975-Luc-mEGFP
Lung cancer	PC-9-Luc
Lung cancer	A549-Luc-mEGFP
Lung cancer	H1299-Luc-mEGFP
Liver Carcinoma	HepG2-Luc
Liver Carcinoma	HepG2-Luc-mEGFP
Hepatoma	HepG2-Luc
Pancreatic cancer	MIA-PaCa-2-Luc
Prostate Adenocarcinoma	PC-3-Luc
Prostate Adenocarcinoma	PANC-1-Luc-mEGFP
Prostate Adenocarcinoma	BXPC-3-Luc-mEGFP
Prostate Adenocarcinoma	MIA PaCa-2-Luc-mEGFP
Leukemia	Jurkat-Luc
Leukemia	K562-Luc
Lymphoma	Raji-Luc

In Vivo Bystander Effect and Luciferase Expressing CDX Models

The *in vivo* bystander effect refers to the phenomenon where cytotoxic agents released by targeted cancer cells (those directly affected by ADCs) can diffuse and induce cell death in neighboring non-targeted cancer cells within a living organism. By using tumor cell lines engineered to express luciferase, we can visualize and monitor the bystander effect using our IVIS *in vivo* imaging systems.

Using untagged Ag^+ cells and luciferase-tagged Ag^- cells in co-inoculation CDX models is an effective approach to evaluate the *in vivo* bystander effect. The luciferase tag on Ag^- cells allows for precise and specific monitoring of their response to the ADC treatment, ensuring that the effects observed are due to the bystander effect mediated by the Ag^+ cells. This method provides clear and valuable insights into the efficacy of ADCs in heterogeneous tumor environments.

Cancer Cell Type	Cell Lines (Ready)	CDX Model-Single Cell Line (Ready)
Breast cancer	4T-1-Luc-mEGFP	√
Colorectal Carcinoma	HT-29-Luc-mEGFP	√
Glioblastoma (GBM)	U-87 MG-Luc	√
Lung cancer	PC-9-GFP-Luc	√
Lung cancer	H460-Luc-mEGFP	√
Ovarian cancer	SKOV-3-Luc-mEGFP	√
Prostate Adenocarcinom	ASPC-1-Luc-mEGFP	√
Multiple myeloma	H929-Luc-mEGFP	√
Breast cancer	MDA-MB-231-Luc	√

Non-Clinical DMPK Services for ADC

	Lead Discovery and Optimization	Pre-Clinical Candidate Selection and Evaluation	IND-Enabling Studies and Mass Balance Studies
Biotransformation	• Mechanisms of Payload Release from ADCs in Liver S9, Lysosomes, and Target-Expression Cells • Plasma Metabolite profiling	• Metabolism of Payload in Hepatocytes/Microsomes from Different Species • Tissue Distribution Studies of RadiolabeledADCs in Rats and Tumor-Bearing Mice • Metabolic Enzyme Mapping Studies	• ADME Studies of Radiolabeled Payload in Animals • ADME Prediction in Humans • Definitive Metabolic Enzyme Phenotyping
***In vitro* ADME/DDI**	• Solubility and Permeability Assessment of New Payloads • Plasma Stability Studies of ADCs (Payload Release and DAR Value Changes) • Metabolic Stability Studies of Payloads • Protein Binding Analysis of Payloads • Payload hERG test	• Studies on CYP Inhibition and Induction by Payloads • Studies on Transporter Inhibition by Payloads • P-gp/BCRP Substrate Analysis of Payloads	• Transporter Substrate Assessment • Prediction of DDI Risk of ADC in Humans Using PBPK Models • Plasma Stability Studies of ADCs (Payload Release and DAR Value Changes)
Bioanalysis/PK	• PK Studies of Tab, ADC, and Payload in Pharmacological Models • PK Screening of ADC and Payload in Rodents • DAR analysis in PK Samples	• PK/TK Studies of Tab, ADC, Ac-payload and Payload in Animals • Immunogenicity Studies in Animals • Biomarker Exploration	• DAR analysis in PK Samples

Advantages

- √ **Identification of Unknown Small Molecules:** Expertise in using non-targeted LC-HRMS methods to identify unknown small molecules containing payloads released from ADCs and their metabolites.
- √ **Comprehensive ADC Stability Studies:** Ability to thoroughly investigate ADC stability, from lysosomal stability to payload release in biological matrices, ADC concentration changes, and DAR (Drug-to-Antibody Ratio) variations.
- √ **Our DMPK platform** offers exceptional flexibility and extensive experience across multiple modalities, including payloads, small molecules, peptides, biologics, and Antibody-Drug Conjugates (ADCs), providing tailored solutions to meet the diverse needs of drug development projects.
- √ **Complete Transporter Evaluation Platform**
- √ **Experience in Animal Radioisotope ADME Studies**
- √ **Experience in Bioanalytical Assay Development**

Case Study

LC-HRMS Analysis of Metabolites Containing Different Forms of Payload Released from Uncleavable ADC

The LC-HRMS analysis provided detailed insights into the metabolites formed from the uncleavable ADC. The identification of various metabolic forms of the payload, reveals the complexity of ADC metabolism and highlights the importance of thorough metabolic characterization in ADC development. This knowledge aids in the optimization of ADC design to improve stability, efficacy, and safety.

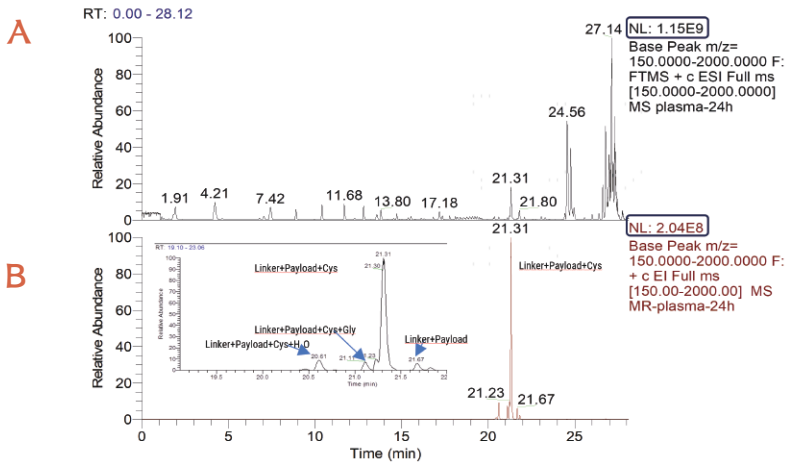


Figure 17. (A) The control chromatogram and (B) ADC treated plasma after 24 hours incubation.

Using LC-MS Platform for Monitoring DAR Changes and Plasma Stability of DS-8201 in Mice

For DS-8201, a HER2-targeting ADC, this evaluation helps in understanding how the Drug-to-Antibody Ratio (DAR) changes over time and how stable the payload and antibody components are in plasma. The findings highlight the importance of early stability assessments in ADC development, particularly for optimizing the linker and overall molecular stability. By leveraging our comprehensive stability study platform, including free small molecule toxins, conjugated small molecule toxins, conjugated antibodies, total antibodies, and DAR values, we can guide the optimization of ADC molecules to improve their therapeutic potential.

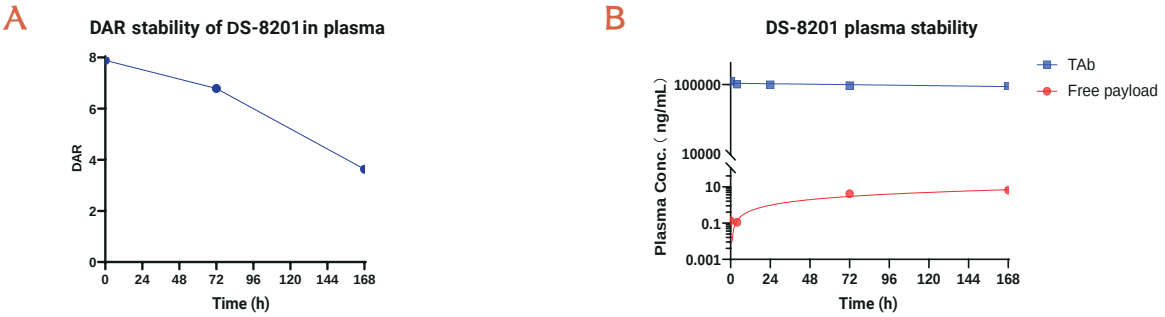


Figure 18. *In vitro* stability of DS-8201 in mouse plasma. (A) DAR stability of DS-8201 in plasma. (B) Concentration of free payload and total Antibody (TAb) in DS-8201 treated plasma. DS-8201 was incubated in mouse plasma at 37 C with different time points.

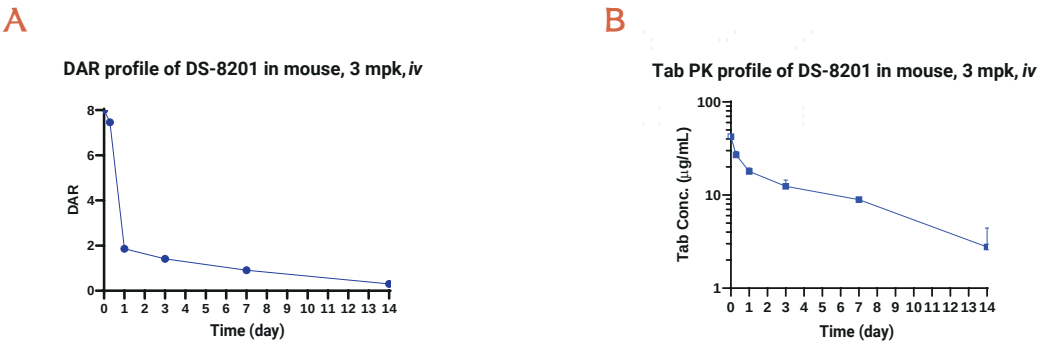
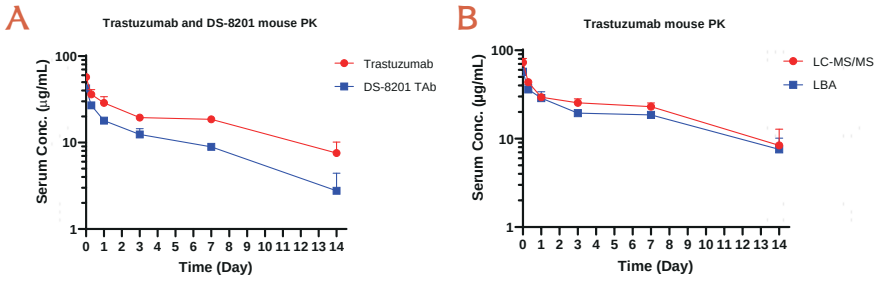


Figure 19. *In vivo* PK and DAR profiles of DS-8201 in mouse, detected by HR-MS. DS-8201 was intravenously administered to mouse at the dose of 3 mg/kg. (A) DAR profile of DS-8201 in mouse. (B) Total antibody (Tab) PK profile of DS-8201 in mouse.

Establishing Quantitative Methods for DS-8201 and Trastuzumab Using LC-MS Platform and Comparison with ELISA

We have developed a robust LC-MS-based quantitative method for analyzing DS-8201 (an Antibody-Drug Conjugate) and Trastuzumab (an antibody). Our LC-MS method provides results consistent with those obtained using ELISA, effectively meeting the diverse needs for biologics quantification in various scenarios. This comparison demonstrates the reliability and accuracy of our LC-MS method, making it a valuable tool for comprehensive ADC and antibody analysis.



PK parameter	Unit	ELISA Assay	LC-MS Assay
$t_{1/2}$	day	7.54±3.17	7.53±3.09
C_0	μg/mL	57.57±0.62	73.39±9.75
AUC_{last}	day*μg/mL	252.5±11.61	270.95±86.98
CL	mL/day/kg	8.97±1.71	7.48±2.17

Figure 20. (A) Pharmacokinetics of Trastuzumab and DS-8201 in mouse. DS-8201 was intravenously administered to mouse at the dose of 3 mg/kg. (B) Pharmacokinetics of Trastuzumab in mouse, detected by LC-MS/MS and ELISA. Trastuzumab was intravenously administered to mouse at the dose of 3 mg/kg.

ICE Bioscience

Innovative CRO+ Explorer

ICE Bioscience was founded in 2010 as an Innovative CRO+ Explorer company. We specialize in early drug discovery services, spanning from target validation to the identification of pre-clinical candidates. We stand out for our collaborative spirit and expertise in boldly exploring new therapeutic target research. Our commitment to drug discovery services, delivered with enthusiasm and professionalism, empowers clients to overcome challenges, address scientific puzzles, and fulfill our promises to clients, communities, the environment, and global health.

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