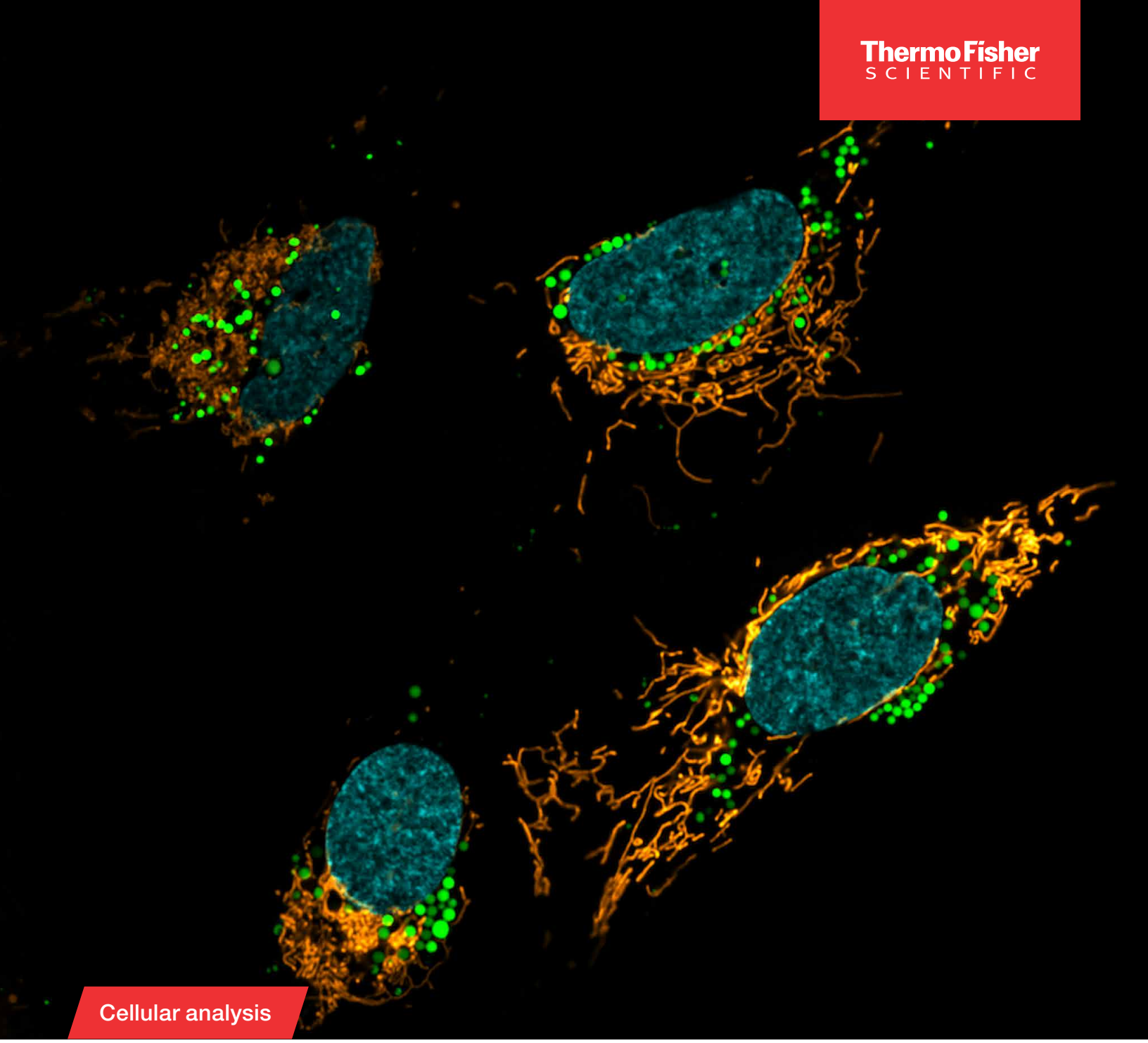
ThermoFisher SCIENTIFIC



Cellular analysis

Antibody-drug conjugate selection and characterization

Simple and effective tools to optimize your ADC discovery

invitrogen

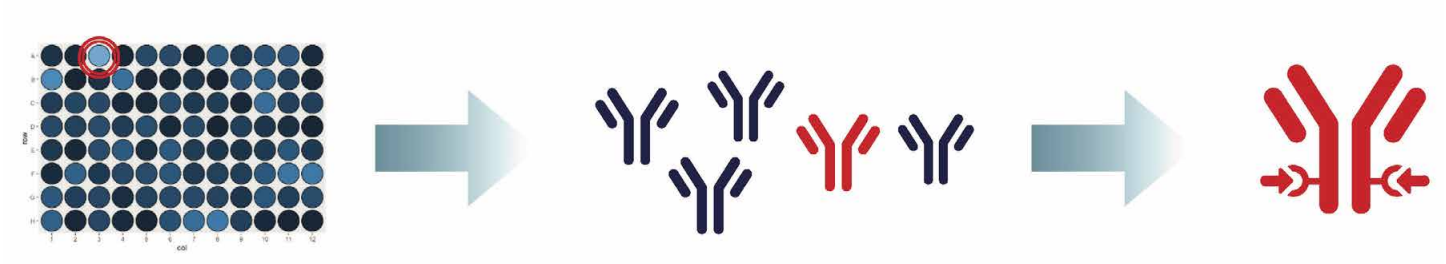
Introduction: Why study antibody-drug conjugates (ADCs)?

Antibody-drug conjugates (ADCs) are transforming the treatment landscape for cancer and other complex diseases by combining the specificity of monoclonal antibodies with the potency of cytotoxic drugs. These targeted therapies offer the ability to selectively deliver payloads to diseased cells while minimizing off-target effects.

Modern ADC development depends on identifying internalizing antibodies that efficiently traffic to the lysosome, where cleavable linkers release cytotoxic payloads. Emerging modalities like targeted protein degraders—including LYTACs and MabTACs—leverage similar trafficking mechanisms to route extracellular proteins for lysosomal degradation. In both cases, accurate and kinetic visualization of intracellular trafficking is essential for optimizing therapeutic design.

This brochure outlines tools and techniques to guide the journey from a wide pool of antibody candidates to a singular, well-characterized ADC. It covers each critical step along the way—starting with binding assays, moving through internalization characterization, evaluating lysosomal degradation, and optimizing antibody labeling through site-specific conjugation with Invitrogen™ SiteClick™ reagents. Together, these tools support the development of consistent, functional, and high-quality ADCs for therapeutic applications.

As ADCs and related modalities continue to evolve, the demand for sensitive, scalable methods to assess internalization and intracellular routing grows. The solutions featured in this brochure empower researchers to select, validate, and refine next-generation therapeutics with clarity and confidence.



Antibody discovery:
Zenon reagents

ADC development:
pHrodo and LysoLight reagents

Preclinical:
SiteClick reagents

Figure 1. Key stages of the ADC workflow. Starting from a broad panel of antibodies (left), researchers can use tools like the Invitrogen™ Zenon™ Alexa Fluor™ Plus or Zenon™ pHrodo™ reagents to rapidly identify candidates for binding or internalization. Promising candidates can undergo further characterization with Invitrogen™ pHrodo™ amine-reactive reagents or our innovative Invitrogen™ LysoLight™ reagents for lysosomal degradation studies. Finally, site-specific conjugation using SiteClick reagents enables generation of a singular, well-characterized ADC for preclinical evaluation. This streamlined pipeline supports efficient candidate selection and optimization for therapeutic success.

Tools to assess antibody binding

Why start with antibody binding?

One of the initial steps in ADC development involves the identification of antibody binding to help ensure that the antibody specifically recognizes the target antigen on the cell surface. This is crucial in narrowing down the candidate pool, because the effectiveness of an ADC relies on its ability to selectively bind to diseased cells while sparing healthy cells.

Rapidly identify antibody binding with Zenon Alexa Fluor Plus reagents

Zenon Alexa Fluor Plus labeling reagents are Fab fragments conjugated to fluorophores, allowing for rapid and efficient identification of antibody binding. These reagents are designed to bind specifically to the Fc region of antibodies, providing a robust signal that can be easily detected using standard imaging, high content screening, or flow cytometry platforms. The Zenon Alexa Fluor Plus IgG labeling reagents combine the rapid and scalable Zenon technology with the exceptional Alexa Fluor Plus dyes, making them excellent for identifying candidates that have a high specificity and affinity for the target antigen—an essential first step in narrowing the pipeline of candidate therapeutics.

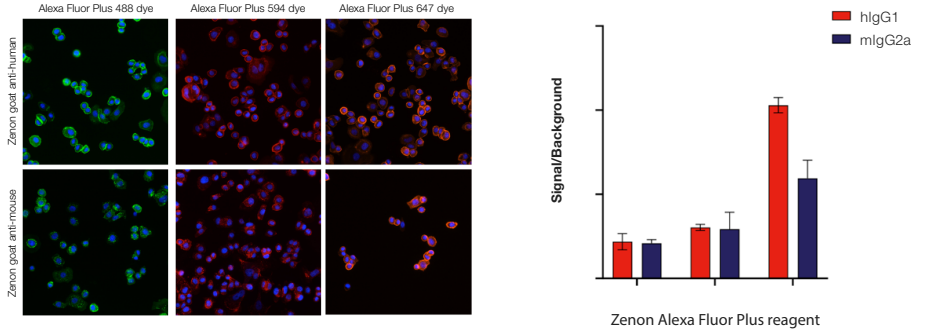


Figure 2. Zenon Alexa Fluor Plus reagents enable a sensitive indication of antibody binding regardless of dye color or species. Her-2 positive SKBR3 cells were treated with trastuzumab labeled with Zenon Alexa Fluor Plus labeling reagents for 30 minutes at 37°C and 5% CO_2 and imaged with the Thermo Scientific™ CellInsight™ CX7 LZR Pro High Content Screening (HCS) Platform. Background was determined by incubating cells with Zenon reagents without trastuzumab.

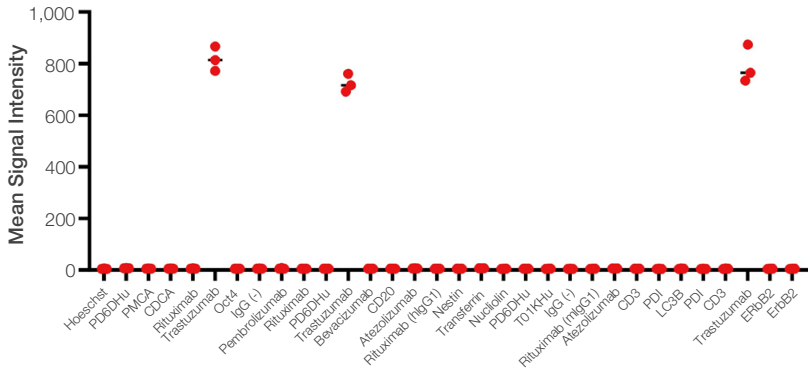


Figure 3. Zenon Alexa Fluor labeling reagents are well suited for high-throughput screening workflows to identify antibodies with high-affinity for target cells. Triplicate samples of 32 humanized or fully human antibodies were labeled with Zenon Alexa Fluor Plus 647 IgG labeling reagents for 5 minutes before addition to SKBR3 cells. After a 30-minute incubation, cells were co-stained with Invitrogen™ Hoechst 33342 Ready Flow™ reagent and imaged with the CellInsight CX7 LZR Pro HCS Platform. Bright signal indicates those clones with highest affinity for HER2.

Zenon Alexa Fluor Plus reagents

Excitation/Emission	Product	Labeling Scale	Cat. No.
Mouse IgG			
494/519	Zenon Alexa Fluor Plus 488 Mouse IgG Labeling Reagent	4 x 96-well plates	Z25619
590/617	Zenon Alexa Fluor Plus 594 Mouse IgG Labeling Reagent	4 x 96-well plates	Z25620
650/671	Zenon Alexa Fluor Plus 647 Mouse IgG Labeling Reagent	4 x 96-well plates	Z25621
Human IgG			
494/519	Zenon Alexa Fluor Plus 488 Human IgG Labeling Reagent	4 x 96-well plates	Z25615
590/617	Zenon Alexa Fluor Plus 594 Human IgG Labeling Reagent	4 x 96-well plates	Z25616
650/671	Zenon Alexa Fluor Plus 647 Human IgG Labeling Reagent	4 x 96-well plates	Z25617

Tools for identifying and characterizing ADC internalization

Why monitor ADC internalization?

Once antibodies have been identified for binding affinity, the next critical step is to identify antibodies with excellent internalization kinetics. Since ADCs rely on endocytosis to deliver their cytotoxic payload, selecting antibodies that efficiently enter target cells is essential for therapeutic efficacy.

Rapidly identify ADC internalization using Zenon pHrodo dyes

Zenon pHrodo reagents combine the rapid and scalable Zenon technology with the pH-sensitive pHrodo dye technology to allow researchers to study endocytosis at scale with high specificity. pHrodo dyes fluoresce only upon internalization into the acidic environment of the endosome or lysosome and remain non-fluorescent at neutral pH of the extracellular environment. pHrodo dyes are available in red, green, and deep red – which has a lower pKA than the red and green dyes, meaning it will not fluoresce until later in the endocytic pathway. This delayed fluorescence allows for the monitoring of different stages within the endocytic pathway, providing valuable insights into the internalization process. Zenon pHrodo–labeled antibodies can be used for various applications, including live-cell imaging and flow cytometry.

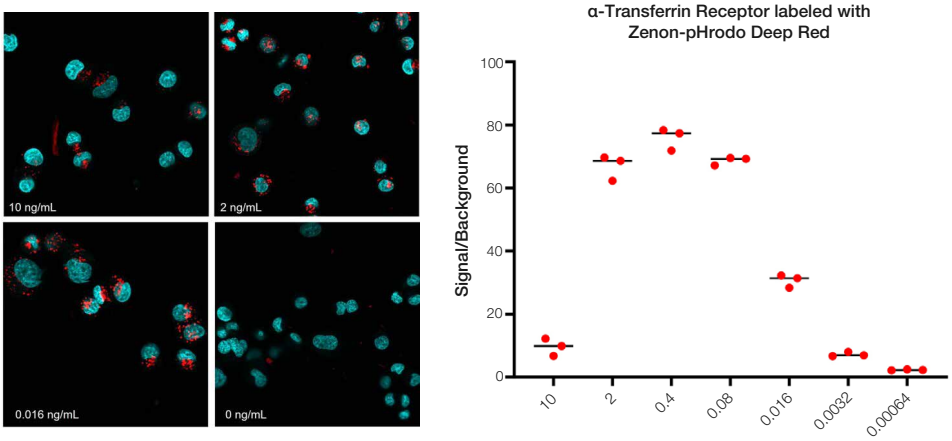


Figure 4. Zenon pHrodo Deep Red IgG labeling reagents are sensitive, allowing antibody screens for positive internalization over a wide range of antibody concentration. Five-fold serial dilutions of anti-transferrin receptor were prepared 10 ng/mL to 0.64 pg/mL while keeping Zenon pHrodo Deep Red Mouse IgG labeling reagent constant at a final assay concentration of 200 nM. After 5-minute labeling, SKBR3 cells were treated with labeled antibodies. Following 16-hour internalization at 37°C and 5% CO_2, cells were imaged and quantified on the CellInsight CX7 LZR Pro HCS system.

Zenon pHrodo labeling reagents

Excitation/Emission	Product	Labeling Scale	Cat. No.
Mouse IgG			
505/525	Zenon pHrodo Green Mouse IgG Labeling Reagent	4 x 96-well plates	Z25609
		20 x 96-well plates	Z25625
560/585	Zenon pHrodo Red Mouse IgG Labeling Reagent	4 x 96-well plates	Z25610
		20 x 96-well plates	Z25626
640/655	Zenon pHrodo Deep Red Mouse IgG Labeling Reagent	4 x 96-well plates	Z25622
		20 x 96-well plates	Z25624
Human IgG			
505/525	Zenon pHrodo Green Human IgG Labeling Reagent	4 x 96-well plates	Z25611
		20 x 96-well plates	Z25613
560/585	Zenon pHrodo Red Human IgG Labeling Reagent	4 x 96-well plates	Z25610
		20 x 96-well plates	Z25614
640/655	Zenon pHrodo Deep Red Human IgG Labeling Reagent	4 x 96-well plates	Z25618
		20 x 96-well plates	Z25623

Precisely characterize internalization using amine-reactive pHrodo labeling reagents

Following the selection of a smaller pool of candidate antibodies, internalization can be more precisely characterized through covalent labeling with amine-reactive pHrodo reagents. These pH-sensitive dyes enable confident tracking of antibody uptake, kinetics, and intracellular localization in live-cell models. The pHrodo antibody labeling kit simplifies conjugation with more soluble dyes and ready-to-use spin columns for efficient and reproducible results.

Similar to the pHrodo Zenon reagents, pHrodo amine-reactive dyes are available in green and red formats that fluoresce in the early endosome, while the deep red variant is activated only in the more acidic environment of late endosomes or lysosomes. This range allows for differentiation between trafficking stages, with the deep red dye offering reduced background fluorescence and improved signal specificity in low-pH compartments.

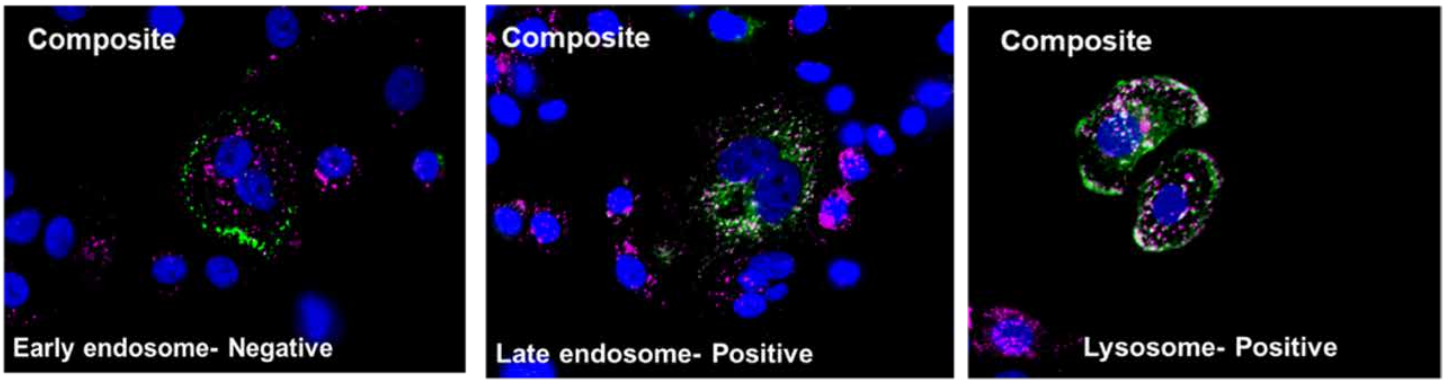


Figure 5. pHrodo Deep Red remains non-fluorescent until it reaches the late endosome, allowing for differentiation in internalization and trafficking stages. SKBR3 cells treated with pHrodo Deep Red-labeled Herceptin showed fluorescence in these organelles, but not in early endosomes, after incubation and imaging. This confirms that pHrodo Deep Red activates in acidic conditions.

pHrodo reactive dyes and kits

Excitation/Emission	Product	Labeling Scale	Cat. No.
505/525	pHrodo Green Amine-Reactive Dye	3 x 100 µg	P36013
		1 mg	P36012
	pHrodo Green Antibody Labeling Kit	3 x 20 µg	P36015
		3 x 100 µg	P36022
		3 x 1 mg	P36023
560/585	pHrodo Red Amine-Reactive Dye	3 x 100 µg	P36011
		1 mg	P36010
	pHrodo Red Antibody Labeling Kit	3 x 20 µg	P36014
		3 x 100 µg	P36020
		3 x 1 mg	P36021
640/655	pHrodo Deep Red Amine-Reactive Dye	3 x 100 µg	P35358
		1 mg	P35359
	pHrodo Deep Red Antibody Labeling Kit	3 x 100 µg	P35355
		3 x 1 mg	P35356

Tools for monitoring lysosomal degradation

Why monitor lysosomal degradation?

After ensuring that the antibody binds to the target antigen and is efficiently internalized, the next step is to monitor and characterize lysosomal degradation where the efficacy of ADCs is dependent on the release of cytotoxic drugs by lysosomal cathepsins. Monitoring lysosomal degradation confirms that the internalized ADCs are trafficked to the lysosome, where the drug is liberated to exert its therapeutic effect.

Confirm lysosomal degradation with high specificity using LysoLight reagents

LysoLight antibody labeling kits and reactive dyes are powerful tools for monitoring catabolism of ADCs within lysosomes. Unlike our pHrodo technology which relies on pH gradients, LysoLight dye–conjugated antibodies or proteins remain non-fluorescent even in the late endosome and only fluoresce upon cleavage by lysosomal cathepsins. This mechanism offers exceptional sensitivity, specificity, and photostability to understand the internalization and trafficking of a protein or ADC. Using LysoLight technology, researchers can also assess the propensity of lysosomally targeted protein degraders towards catabolic or recycling pathways. This can help researchers in the targeted protein degradation field fine-tune their candidates for maximum therapeutic potential. LysoLight kits and reagents are compatible with a wide range of platforms such as standard microscopy, flow cytometry, high content imaging, and incubator-based fluorescent imaging systems.

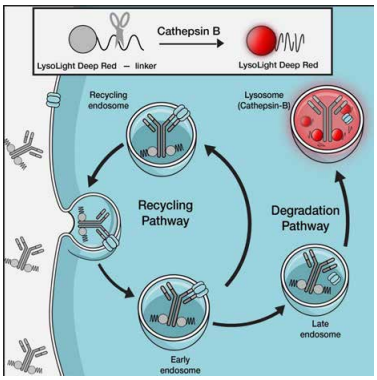


Figure 6. LysoLight dye mechanism of action. In its un-cleaved form, LysoLight Deep Red or LysoLight Green has no background fluorescence. Only upon cleavage by lysosomal cathepsins does the dye become fluorescent, leading to bright fluorescence in the Cy5 or GPF/FITC channel, respectively.

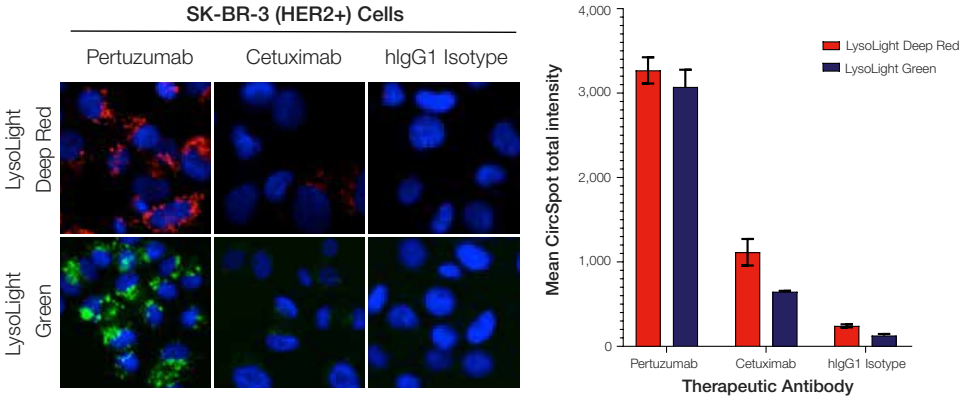


Figure 7. LysoLight catabolism results in strong lysosomal-specific signal. SKBR3 cells were treated with 1 ug/1mL trastuzumab, cetuximab, or hIgG1 isotope control labeled with LysoLight Green or Deep Red for 16 hours in McCoy's complete medium with 10% FBS. Cells were imaged and quantified on the CellInsight CX7 LZR Pro HCS Platform for mean circ spot average intensity.

LysoLight dyes and kits

Excitation/Emission	Product	Labeling Scale	Cat. No.
488/525 nm	LysoLight Green Amine Reactive Dye	3 x 100 µg of dye	L36007
		500 µg of dye	L36008
	LysoLight Green Antibody Labeling Kit	3 x 100 µg of IgG	L36005
		1 mg of IgG	L36009
650/668 nm	LysoLight Deep Red Amine Reactive Dye	3 x 100 µg of dye	L36003
		500 µg of dye	L36004
	LysoLight Deep Red Antibody Labeling Kit	3 x 100 µg of IgG	L36001
		1 mg of IgG	L36002

Optimizing your ADC with site-specific labeling

Why optimize with site-specific labeling?

After confirming binding, internalization, and lysosomal degradation, the final step is to optimize the antibody labeling process to help ensure consistent and reproducible results. Optimization involves fine-tuning the labeling conditions to achieve the desired degree of labeling (DOL) while preserving the antigen-binding site and maintaining antibody functionality. This step is crucial for producing high-quality ADCs that can be used in development of therapeutic applications.

SiteClick reagents for reproducible, site-specific labeling

SiteClick antibody-labeling technology allows for site-specific conjugation of fluorescent dyes or toxins, helping ensure consistent labeling on the Fc portion of the antibody. The Invitrogen™ SiteClick™ Antibody Azido Modification Kit uses enzymes to specifically modify the carbohydrate domains of the antibody with azide moieties. A variety of sDIBO or DBCO alkyne labels are available to conjugate with a simple incubation step, using copper-free click chemistry. For example, pHrodo Red sDIBO was clicked onto to azido modified trastuzumab to evaluate internalization in SKBR3 spheroids (Figure 8).

SiteClick assay mechanism

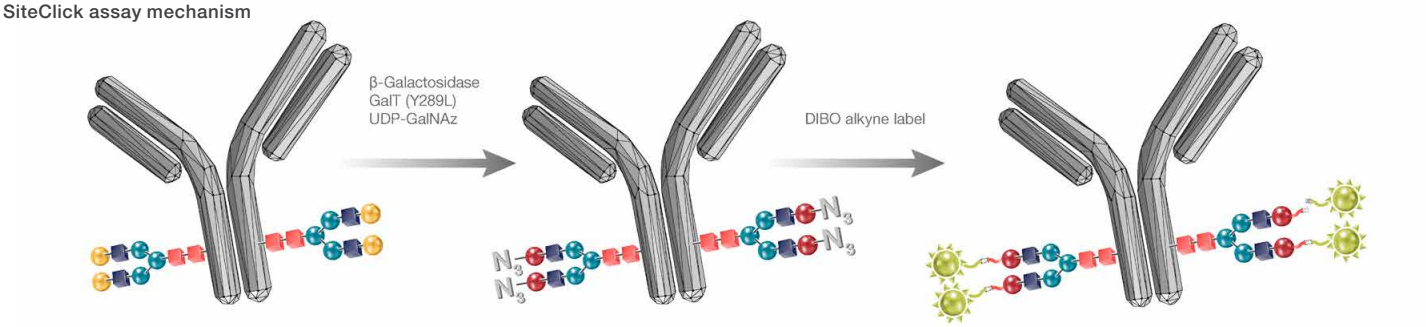
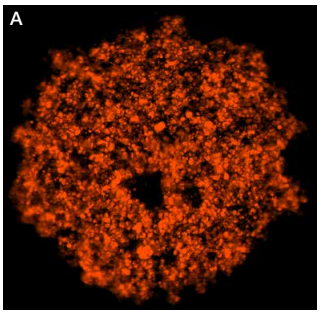


Figure 8. The site-specific method employed in the SiteClick modification kit uses the enzymes β-galactosidase and β-1,4-galactosyltransferase to modify the carbohydrate domain and then attach an azide-modified sugar on the heavy chains of an IgG antibody. Because the modification takes place only on the Fc portion of the heavy chains, the location and degree of labeling is very consistent, and the antigen-binding domains remain unaltered.



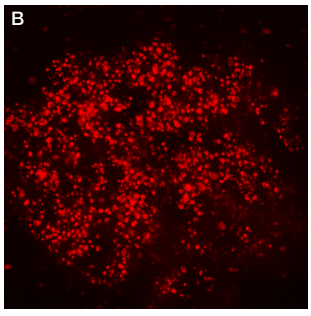


Figure 9. SiteClick label does not affect antigen binding to get accurate assessment of internalization in 3D models. Two spheroids were labeled with pHrodo red-conjugated anti-HER2 antibody trastuzumab. **(A)** All of the cells have expressed Her-2, so they all internalized the antibody, showing bright red signal. **(B)** A spheroid made of mixture of SKBR3 and MDA-231 cells, shows that only half of the cells express Her-2, showing decreased amount of fluorescent signal. The cells were incubated for 24 hours and imaged on the Invitrogen™ EVOS™ M7000 Imaging System.

SiteClick kits and reagents

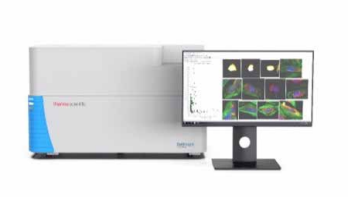
Excitation/Emission	Product	Labeling Scale	Cat. No.
n/a (sDIBO label not included)	SiteClick Antibody Azido Modification Kit	250 µg	S20026
		1 mg	S10900
		5 mg	S10901
560/585	SiteClick pHrodo Red sDIBO Alkyne	250 µg	C20034
		1 mg	S10903
		5 mg	S10908
640/655	SiteClick pHrodo Deep Red sDIBO Alkyne	250 µg	S10914
		1 mg	S10915

Thermo Fisher instrument platforms: Versatile tools to visualize and quantify internalization

Understanding how therapeutic antibodies internalize is key to advancing antibody-drug conjugate development. Thermo Fisher Scientific offers a range of platforms to support this, from dynamic live-cell imaging to high-throughput quantification.

The Thermo Scientific™ CellInsight™ CX7 LZR Pro HCS Platform or the Invitrogen™ EVOS™ M7000 Imaging System, paired with an Invitrogen™ EVOS™ Onstage Incubator, maintains optimal conditions for live-cell experiments, enabling real-time visualization of antibody internalization and trafficking using tools like pHrodo dyes and LysoLight probes. For higher throughput needs, the Thermo Scientific™ Varioskan™ LUX multimode reader allows rapid, plate-based quantification across time points and conditions. The Invitrogen™ Attune™ flow cytometer family accelerates discovery with acoustic focusing technology and models tailored for every need—from the reliable Invitrogen™ Attune™ NxT Flow Cytometer, to the imaging-enabled Invitrogen™ CytPix™ Flow Cytometer with AI-powered analysis, to the flexible, high-parameter Invitrogen™ Attune™ Xenith™ Flow Cytometer with spectral and conventional capabilities.

Together, these platforms help support complementary insights—qualitative and quantitative—to support every stage of the ADC development workflow.



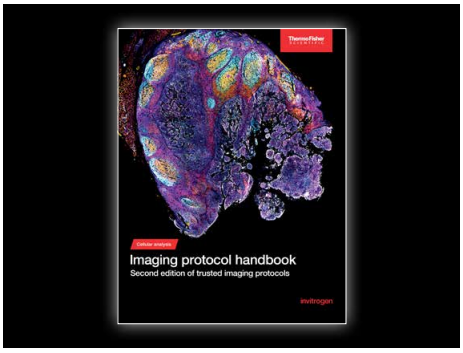
CellInsight™ CX7 LZR Pro High Content Screening Platform

EVOS™ Cell Imaging Systems

VarioSkan™ Microplate Readers

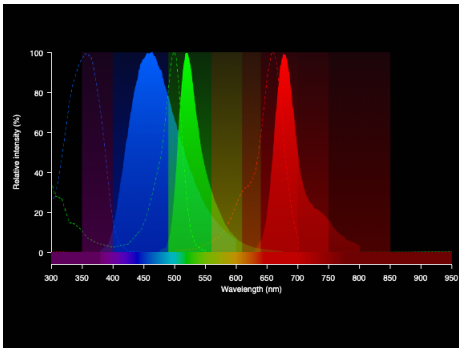
Attune™ Flow Cytometers

Additional Resources:



Imaging Protocol Handbook

Custom Conjugation Services



Fluorescence SpectraViewer

Learn more at **thermofisher.com/adcdiscovery**

invitrogen