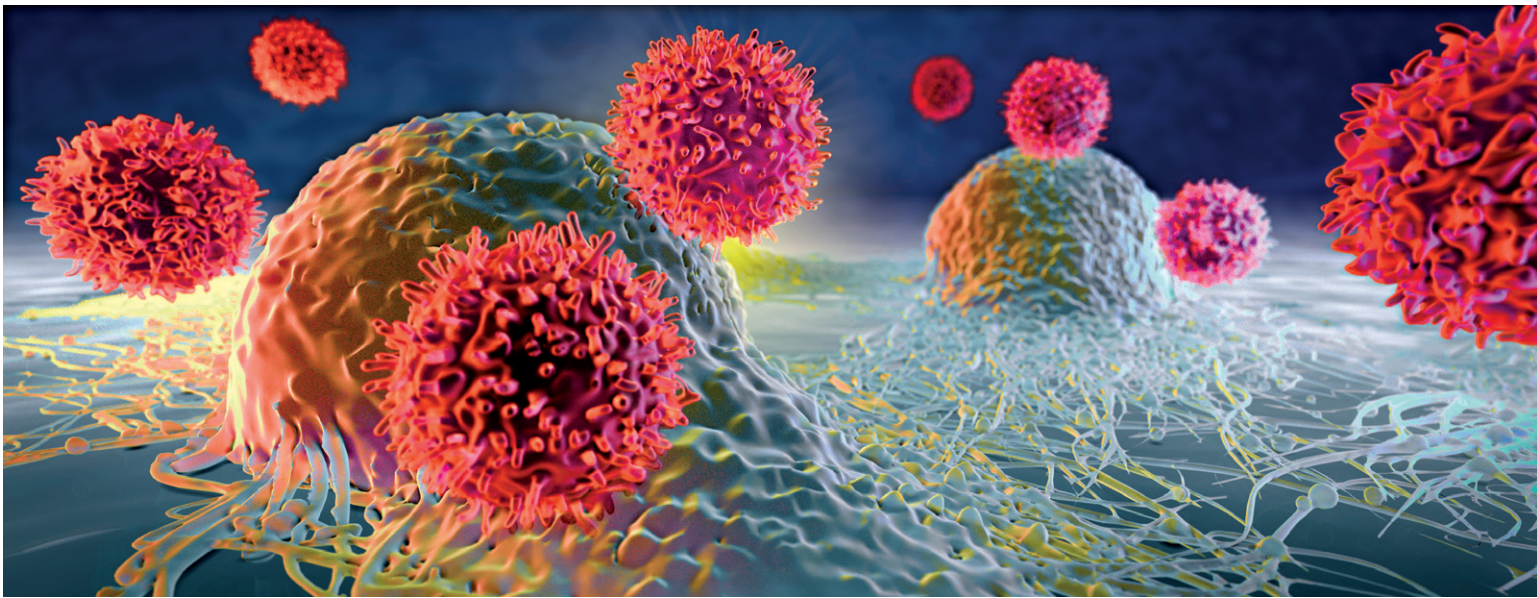


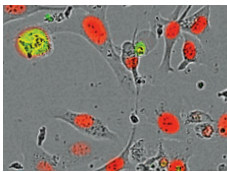
Assays for Immuno-Oncology Research

IncuCyte® S3 Live-Cell Analysis System



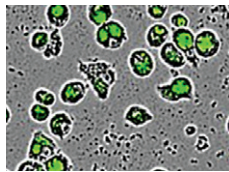
Developing the next generation of immune therapies for cancer relies on advanced technologies that provide detailed morphological and functional insight into living cells. The IncuCyte® Live-Cell Analysis System enables real-time, automated measurements of the dynamic interplay between immune and cancer cells, directly from the incubator.

Immune System Mobilization



Immune cell killing assay

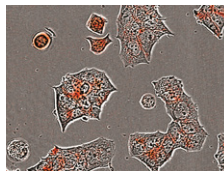
Visualize and automatically analyze immune cell killing of tumor cells.



Phagocytosis assay

Analyze tumor cell internalization in real time using pH-sensitive probes.

Drug Delivery



Antibody internalization assay

Quantify antibody internalization in 96- or 384-well formats.

Key Advantages:

- Real-time, image-based measurements in a physiologically-relevant environment.
- Optimized protocols in 96- and 384-well assay formats.
- Non-invasive study of morphology, health and dynamic interactions.
- Minimal manipulation and cell loss with mix-and-read reagents.

IncuCyte®

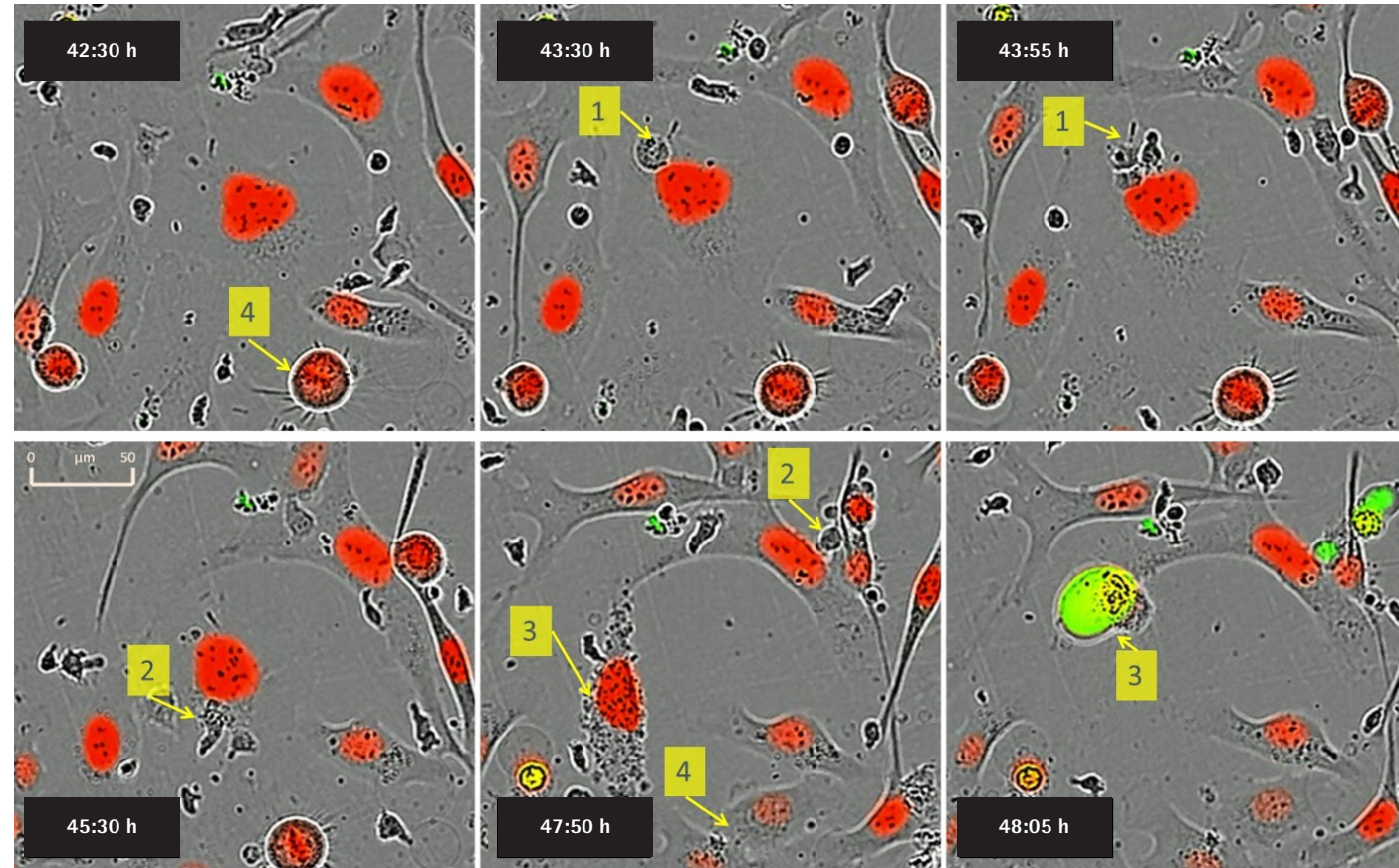
A SARTORIUS BRAND

Immune Cell Killing Assays

Immune-cell recognition and killing of tumor cells is a critical step in the human host defense response. IncuCyte® Immune Cell Killing Assays are an integrated solution for real-time visualization and automated analysis of immune cell-mediated killing of tumor cells.

Visualize immune and tumor cell interactions in real time

Live movies and images help reveal phenotypic insight into the dynamic interaction between immune cells and adherent or suspension tumor cells, without the need to lift cells or use labels.



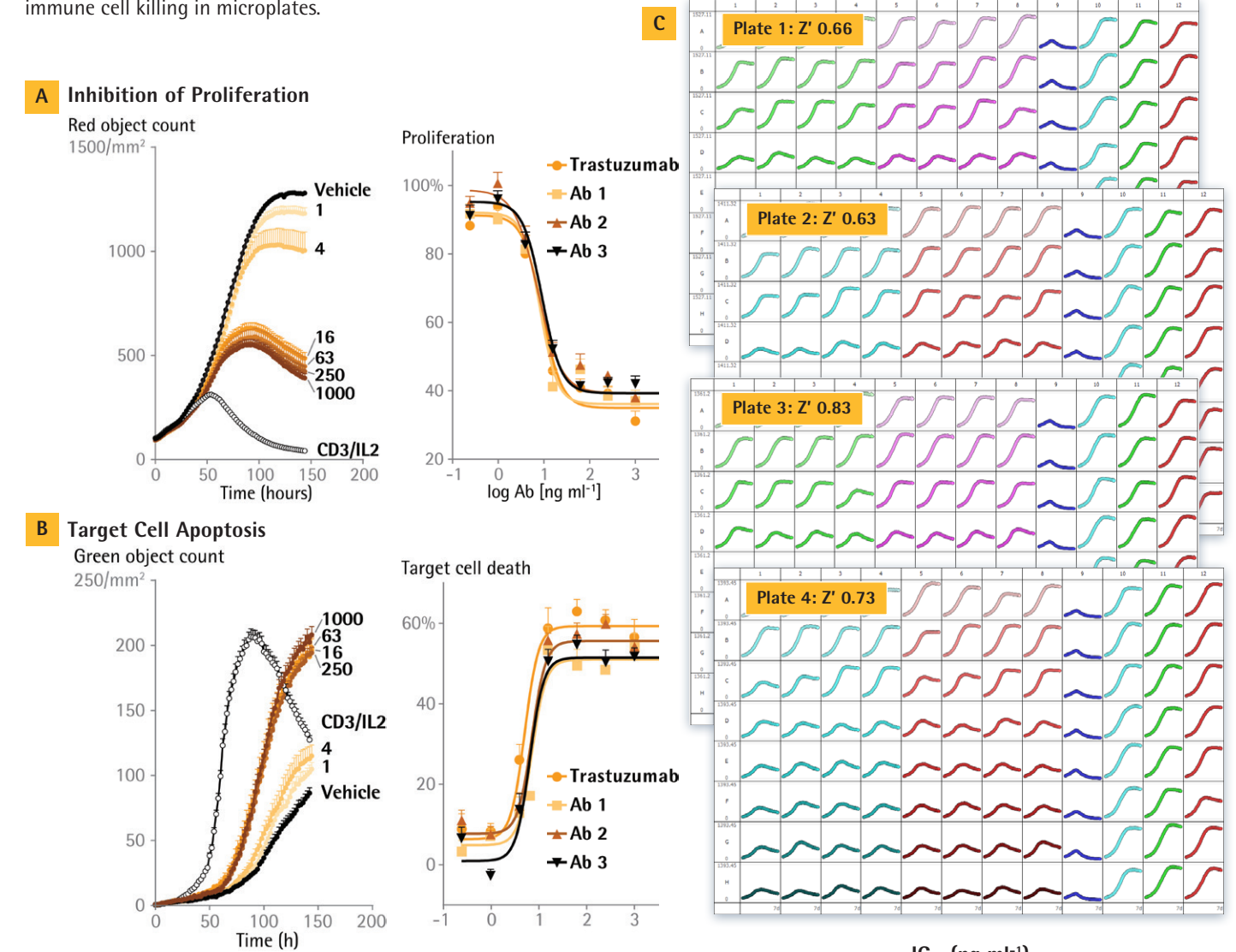
Sequential images of immune-cell killing (PBMCs) of SKOV-3 ovarian cancer cells. SKOV3 NuLight Red™ cells (2K/well) seeded with PBMC's (20K/well) with activation by anti-CD3 and IL-2 in the presence of caspase 3/7 reagent. Images were captured every 5 mins using a 20X objective. (1), (2) engagement of cancer cell by immune cells; (3) tumor cell death, granulation and green caspase 3/7 signal; (4) tumor cell division.

Key Advantages:

- Protocols for PBMCs, cytotoxic T lymphocytes, or NK cells co-cultured with adherent or suspension tumor cells in 2D and 3D models.
- Real time, image-based analysis of T cell-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC).
- Dynamic analysis of cell surface markers, including checkpoint inhibitors, during immune-cell interaction.

Quantify inhibition of proliferation and tumor cell death over time

Combine the power of automated image acquisition and analysis with non-perturbing detection reagents to measure the full time course of immune cell killing in microplates.



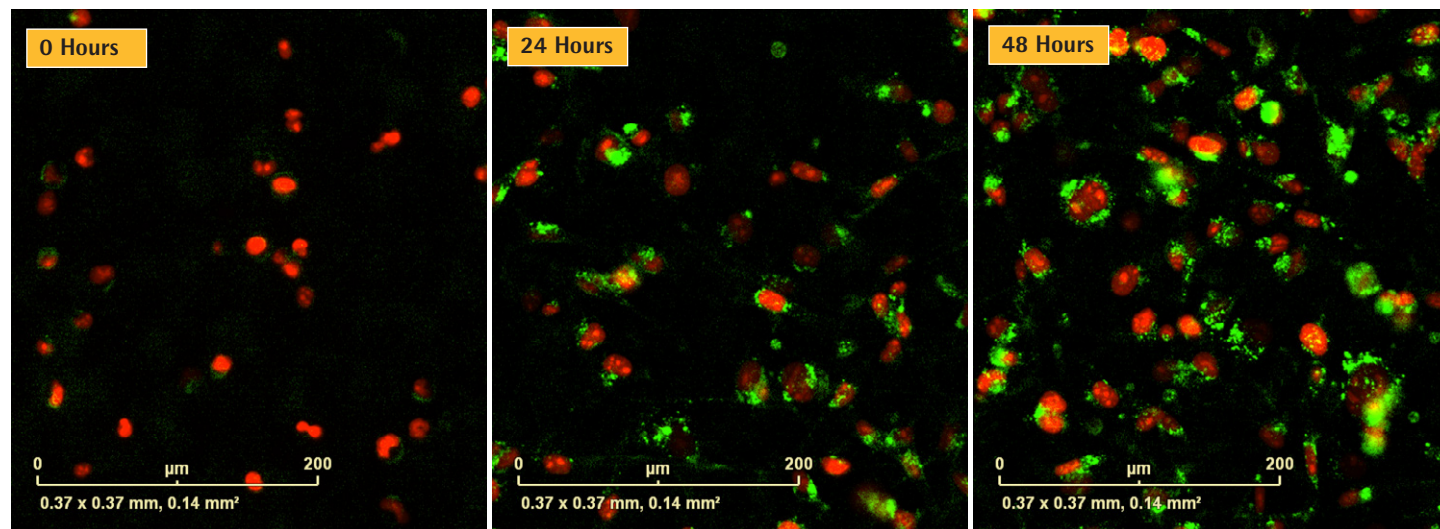
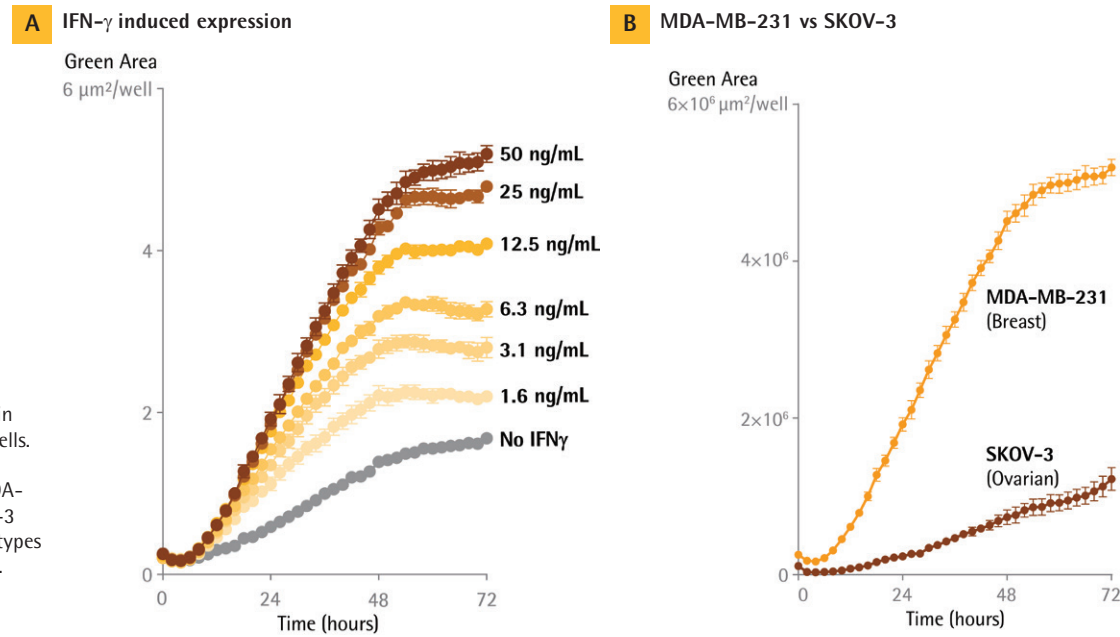
Directly measure tumor cell death and viability. Quantification of trastuzumab (Herceptin) and biosimilar antibodies for ADCC. Her2-positive SKOV3 NuLight Red™ cells were combined with PBMCs in the presence of trastuzumab and three biosimilars. Time course and concentration-response curves for tumor cell number (A) and apoptotic target cell death measured using the IncuCyte Caspase-3/7 Green Reagent (B). No response was seen in Her2 negative cells (A549; lung carcinoma). Microplate views for four 96-well plates provide rapid visualization of experimental results and derived Z' values (C). IC50's can be calculated for a time point of interest (D).

Sample	IC ₅₀ (ng ml ⁻¹)	
	Proliferation	Apoptosis
Trastuzumab	8.1	4.6
Ab 1	6.9	6.0
Ab 2	8.1	6.8
Ab 3	8.9	5.5

Visualize and quantify cell surface proteins of interest

Study the dynamic expression of checkpoint-inhibitors or identify specific cell subtypes and immune cell-tumor cell interactions using IncuCyte® FabFluor-488 reagent for cell surface marker analysis.

Quantify dynamic changes in checkpoint inhibitors.
(A) IFN- γ induces a time- and concentration-dependent increase in PD-L1 expression in MDA-MB-231 cells.
(B) Time-course profile showing differential PD-L1 expression in MDA-MB-231 (high expresser) and SKOV-3 (medium expresser) cells. Both cell types were treated with IFN- γ (50 ng/mL).



Visualize dynamic changes in checkpoint inhibitors.
 Upregulation of PD-L1 expression by IFN- γ shown in MDA-MB-231 breast adenocarcinoma cells, treated with IFN- γ (50 ng/mL) and labeled with B7-H1/PD-L1 antibody conjugated to IncuCyte® FabFluor-488 (cell nuclei labeled with IncuCyte® NuLight Red). Images reveal a time-dependent increase in PD-L1 expression.

Tumor Phagocytosis Assays

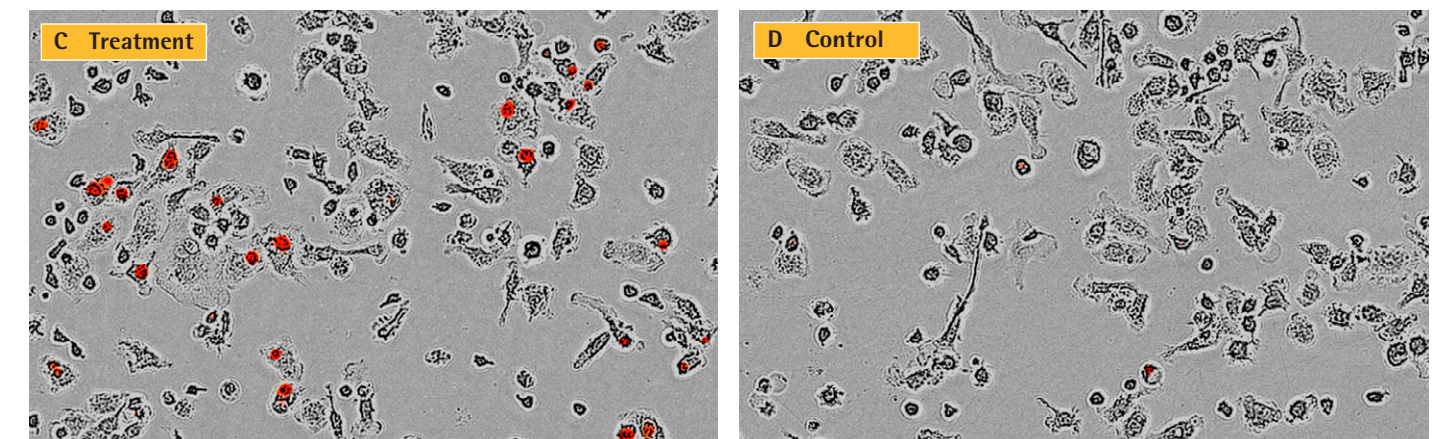
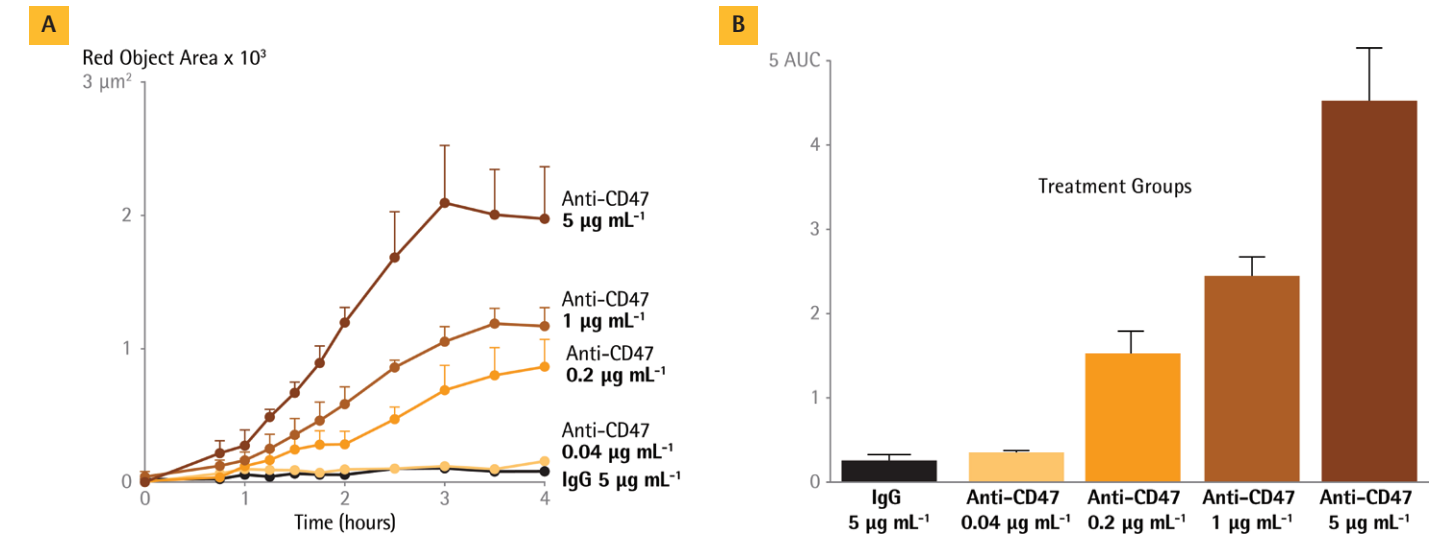
Targeting tumor cells with agents that promote phagocytic uptake and clearance is one therapeutic strategy to treat cancer. The IncuCyte® pHrodo® Red Cell Labeling Kit and IncuCyte Live-Cell Analysis System enable real-time, automated analysis of tumor phagocytosis inside your cell culture incubator.

Visualize and automatically quantify phagocytic clearance of tumor cells

Measure phagocytosis of diseased, dead or dying cells with your phagocytic and target cells of interest. Investigate antibody-mediated cellular phagocytosis (e.g., anti-CD47 antibody mediated phagocytosis / ADCP) or efferocytosis (phagocytosis of apoptotic cells).

Key Advantages:

- Visualize and validate phagocytic tumor cell clearance dynamics with images and movies.
- Generate quantitative, reproducible and specific measurements of engulfed cells.
- Efficiently study the model of your choice in either 96- or 384-well plate format.



Validate phagocytosis with images and movies using your choice of target and effector cells.
 IncuCyte® phagocytosis assay demonstrates anti-CD47 antibody-mediated cellular engulfment. Addition of anti-CD47 binds to the "don't eat me" signal on CCRF-CEM cells labelled using the IncuCyte pHrodo Red Cell Labeling Kit to promote phagocytosis by human bone-marrow derived macrophages in a concentration dependent manner (A, B). Images (C, treatment and D, control) show uptake of CCRF-CEM cells.

Antibody Uptake Assays

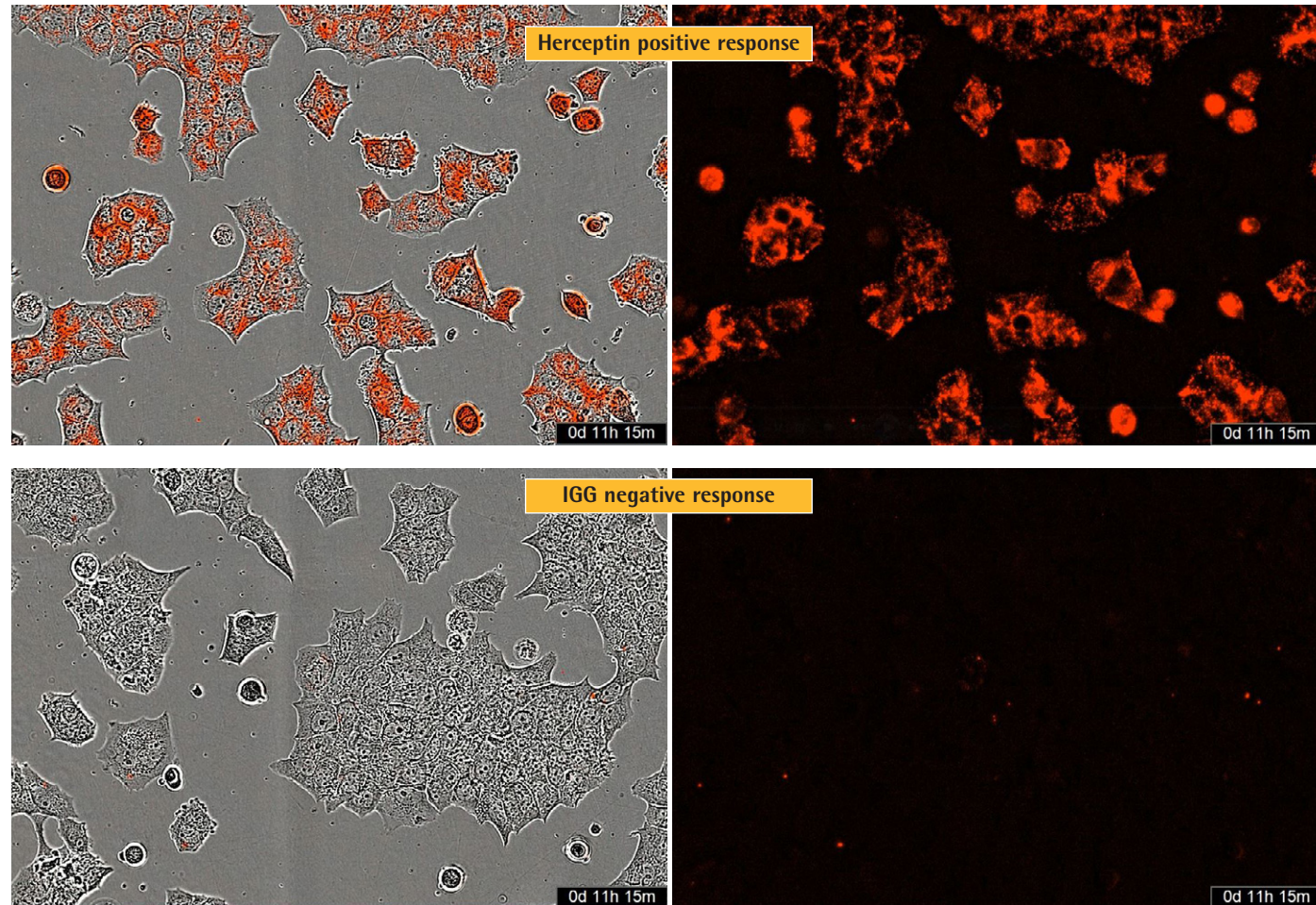
An important property of monoclonal antibody (mAb) and antibody-drug conjugate (ADC) cancer therapies is the extent, location and rate of internalization into cells. The IncuCyte® Antibody Internalization Assays allow you to automatically visualize and quantify antibody internalization, in real time and in 96- or 384-well formats.

Visualize and automatically quantify antibody uptake over time

IncuCyte® FabFluor pH Red Reagent and the IncuCyte® Live-Cell Analysis system provide kinetic fluorescent measurement of internalization with images and movies for visual confirmation.

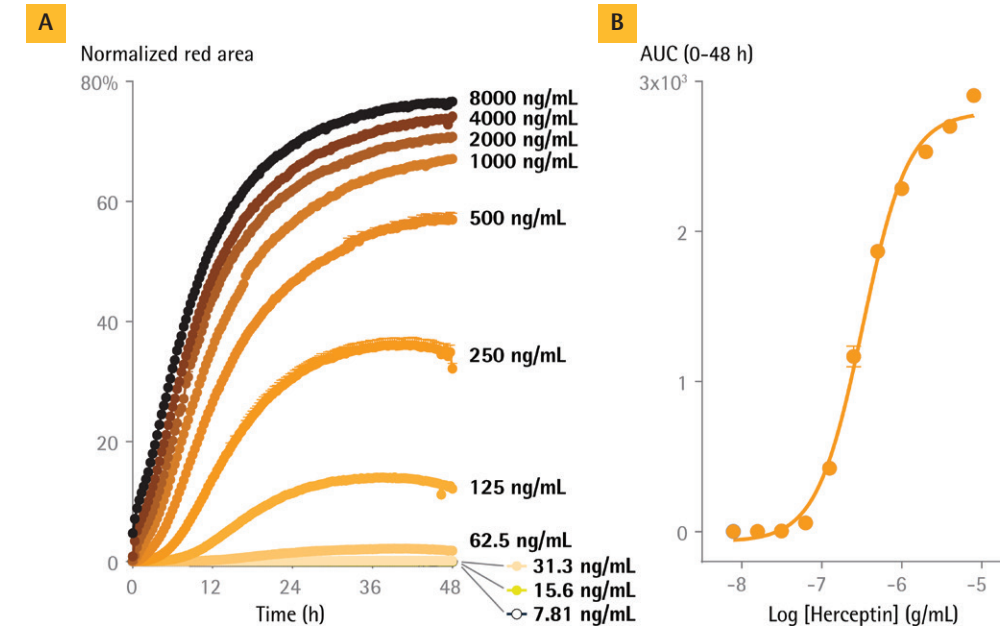
Key Advantages:

- Combine sensitive, kinetic fluorescent measurements with images and movies for visual confirmation.
- Efficiently test antibody panels with a simple protocol that pairs rapid, single-step antibody-labeling with a mix-and-read protocol.
- Unlock your antibody discovery potential with a quantitative, reproducible assay that provides crucial functional insight.



Acquire fluorescent images and visualize antibody internalization.

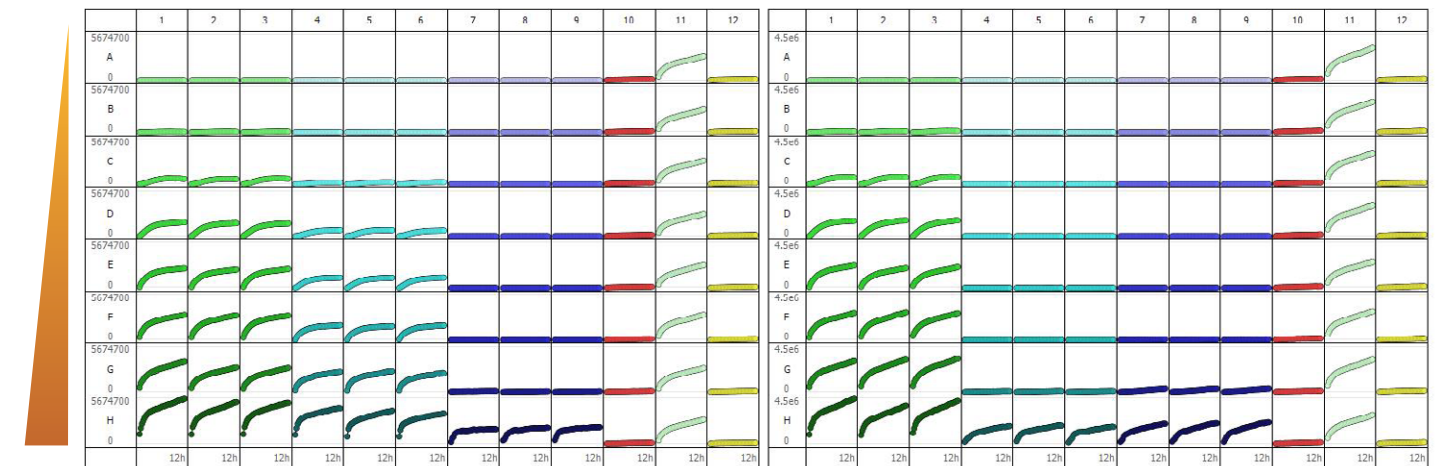
Images of BT-474 Her2-positive cells treated with IncuCyte® FabFluor pH Red-labelled Herceptin (top row) or IgG1 isotype control (second row) provide visual verification of internalization over time. FabFluor pH Red-labelled antibodies produce increased red fluorescence upon acidification, indicating internalization.



Create dose response curves and perform pharmacological analyses. Generate concentration response curves that can be used to calculate EC50 values. BT-474 Her2-positive cells were treated with increasing concentrations of IncuCyte FabFluor pH Red-labelled Herceptin and isotype control. The time course graph displays an increase normalized red area over time with increasing Herceptin concentrations (A). AUC analysis shows a clear concentration dependence with an EC50 of 323 ng/mL (B).

High-throughput, reproducible antibody screening

Screen large numbers of antibodies in 96- or 384-well format to generate consistent, cell-specific internalization response data for full characterization of test antibodies.



Easily perform functional profiling of multiple test antibodies for internalization.

Head to head testing of a panel of six different CD71 antibodies, including one clone from two different suppliers (clone 1a and 1b), in HT1080 cells reveal a range of kinetic responses over 12h (visualized in IncuCyte plate view).

Further Reading

See more exciting data, movies, application notes and scientific posters by visiting www.essenbioscience.com/immuno-oncology

Scientific Posters:

- 96-well live-cell assays for immune cell killing of 3D tumor spheroids.
- Kinetic live cell imaging and analysis enables direct, long-term measurement of t-cell mediated tumor cell killing.
- Validation of novel continuous live-cell assays for immune cell activation and killing of blood cell cancers.
- CD47 antibody-induced engulfment of human leukaemic T-cells by bone-marrow derived macrophages.
- Fluorescent Fab/Ab complexes and IncuCyte live-cell analysis to track live cell surface markers and cell populations in mixed cultures.
- Antibody internalization assays for cancer drug discovery.

Ordering information

Product	Description	Cat. No.
Immune Cell Killing		
IncuCyte® Caspase-3/7 Green Apoptosis Assay Reagent	20 µL	4440
IncuCyte® Annexin V Red Reagent for apoptosis	1 vial, 100 tests	4641
IncuCyte® Annexin V Green Reagent for apoptosis	1 vial, 100 tests	4642
IncuCyte® CytoLight Rapid Red Reagent for cell labeling	5 vials, 100-1,000 tests	4706
IncuCyte® CytoLight Rapid Green Reagent for cell labeling	5 vials, 100-1,000 tests	4717
IncuCyte® Mouse IgG2a FabFluor-488 Antibody Labeling Reagent	One vial: 50 µg	4743
IncuCyte® Mouse IgG2b FabFluor-488 Antibody Labeling Reagent	One vial: 50 µg	4744
IncuCyte® Mouse IgG1 FabFluor-488 Antibody Labeling Reagent	One vial: 50 µg	4745
Phagocytosis		
IncuCyte® pHrodo® Red Cell Labeling Kit	1 kit	4649
Antibody Internalization		
IncuCyte® Human FabFluor pH Red Reagent for Antibody Internalization	50 µg	4722
IncuCyte® Mouse IgG1 FabFluor Red Antibody Labeling Reagent	50 µg	4723
IncuCyte® Rat FabFluor Red Antibody Labeling Reagent	50 µg	4737

Harness the power of live-cell analysis with a full range of IncuCyte reagents and consumables to revolutionize the way you quantify cell behavior. To view a complete listing of our reagents and consumables visit: essenbioscience.com/reagents

To place an order or request additional information

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