

# Incucyte®

Reagents, Consumables and Software

Simplifying Progress



# Incucyte® Reagents, Consumables and Software

Sartorius offers a range of reagents, consumables and software specifically designed around your application needs to enable long-term, kinetic live-cell imaging and analysis. Obtain specific and robust measurements

of cell health, movement and function with optimized, turnkey solutions allowing you to connect phenotype and function with pathological processes. Getting biologically relevant information has never been easier!

#### Find Your Solution to Meet Your Application Needs:

#### Cell Health

Proliferation

Viability

Cell Cycle

**Apoptosis** 

Cytotoxicity

Mitochondrial Membrane Potential

ATP Metabolism

#### **Cell Function**

Immune Cell Killing

Antibody Internalization

Live-Cell Immunocytochemistry

Phagocytosis

**NETosis** 

Neuronal Activity

#### Cell Movement and Morphology

Chemotaxis Migration and Invasion

Scratch Wound Migration and Invasion

Immune Cell Activation and Proliferation

Neurite Outgrowth

#### Assays for 3D Models

Spheroid Growth

Spheroid Invasion

Spheroid Immune Cell Killing

Organoid Assay

#### Cell Monitoring and Workflows

Cell Culture QC

Organoid QC

**EV** Uptake

## Key Advantages

- Derive meaningful data with sensitive, non-perturbing reagents for long term, live-cell studies.
- Unlock your productivity with lab-tested protocols and purpose-built, intuitive software.
- Generate data rich information within every sample with fluorescent reagent combinations.
- Support visualization and automation of cell movement studies with Incucyte® consumables.

Representation Find out more: sartorius.com/incucyte

# Incucyte® Reagents At-a-Glance

Application	Reagent	Reagent Consumable Software Modu		Compatible Instrument			
				CX3	SX5	S3	SX1
Cell Health							
Proliferation:							
Label-free, Confluence							
Label-free, Cell Counting							
Fluorescent Labeling, Cell Counting							
Viability							
Cell Cycle							
Apoptosis							
Cytotoxicity							
Mitochondrial Membrane Potential							
ATP Metabolism			•				
Advanced Label-Free Classification Analysis				*			
Cell Function							
Immune Cell Killing							
Antibody Internalization							
Live-Cell Immunocytochemistry							
Phagocytosis							
NETosis							
Neuronal Activity				*			
Cell Movement and Morphology							
Chemotaxis Migration and Invasion	-			*			
Scratch Wound Migration and Invasion				*			
Immune Cell Activation and Proliferation							
Neurite Outgrowth				*			
Advanced Label-Free Classification Analysis				*			
Assays for 3D Models							
Spheroid Growth (Single and Multi-Spheroid)	-		-				
Spheroid Invasion (Single Spheroid)							
Spheroid Immune Cell Killing	-		•				
Organoid Assay			•	*			
Cell Monitoring and Workflows							
Cell Culture QC							
Organoid Culture QC			•	*			
EV Uptake							



# Proliferation, Cell Counting, Viability and Cell Cycle

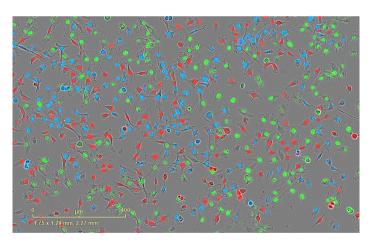
Incucyte® live-cell labeling reagents and purposebuilt software enable long-term (> 48 h) analysis of cell proliferation, viability and cell cycle state.

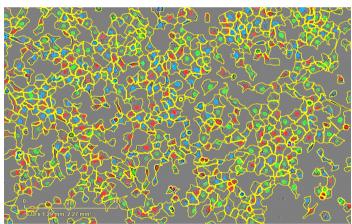
- Minimize experimental artifacts using non-perturbing, live-cell reagents—preservative free, sterile, or concentrated formulations to protect cell health
- Quantify cell proliferation kinetically using label or labelfree strategies in adherent or non-adherent cell cultures
- Investigate a range of cell models (mono-, co- or tri-culture) to answer relevant scientific questions
- Maximize each sample with multiplexed readouts of proliferation, cell cycle or cell health that are easily validated by eye

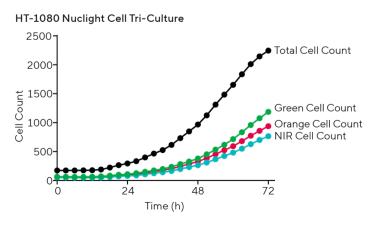
## Application Spotlight: Incucyte® Live-Cell Proliferation Assays

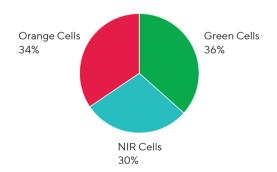
Perform long-term, kinetic measurements of proliferation, with or without labels. Incucyte® Nuclight Reagents homogenously label a variety of cell types for non-

perturbing, continuous analysis of the same population of cells. The Incucyte® Cell-by-Cell Analysis Software Module enables label-free cell identification and counting.









HT-1080 fibrosarcoma cells stably expressing Nuclight Green, Orange, or NIR were monitored for 72 hours. Representative images taken at 48 hours, with and without the label-free Cell-by-Cell Analysis mask, automatically identify the entire population of cells and quantify percentages of green, orange or NIR expressing cells.

	Product	Description	Cat. No.	Instrument Compatibility			
Software	Perform label-free cell counts and subsequent cell-by-cell classification based on shape, size or fluorescence intensity to quantify dynamic changes in cell subsets within heterogeneous living cell cultures with Cell-by-Cell Analysis Software Module. Perform counts and track changes in adherent cell morphology via label-free image segmentation and multivariate analysis of cell shape. Classifier is trained using control wells. Advanced Label-Free Classification Software Module an addon and requires Incucyte® Cell-by-Cell Analysis Software Module (Cat. No. 9600-0031).						
	Incucyte® Cell-by-Cell Analysis Software Module	1 module	9600-0031	SX5, S3, SX1, CX3			
	Incucyte® Advanced Label-Free Classification Analysis Software Module	1 module	BA-04867	SX5, S3, SX1, CX3			
	Incucyte® Al Nuclei Detection	1 module	BA-04897	SX5, S3, SX1, CX3			
Nuclear Dye abeling Reagents	Cell-permeable DNA stains that specifically labe quantification of cell proliferation and viability.	el nuclei in cells and	are ideally suite	d for mix-and-read, live-cell			
	Incucyte® Nuclight Rapid Red Dye	One vial: 50 μL	4717	SX5 (configured with Green/Red Optical Module), S3, SX1			
	Incucyte® Nuclight Rapid NIR Dye	One vial: 50 μL	4804	SX5			
Nuclear Lentivirus abeling Reagents	Lentivirus reagents provide homogenous expression of a nuclear-restricted fluorescent protein without altering cell function for live-cell quantification of cell proliferation and viability.						
	Incucyte® Nuclight Red Lentivirus 2.0 (puro)	One vial: 0.6 mL	BA-04887	SX5 (configured with Green/Red Optical Module), S3, SX1			
	Incucyte® Nuclight Green Lentivirus 2.0 (puro)	One vial: 0.6 mL	BA-04888	SX5, S3, SX1, CX3			
	Incucyte® Nuclight Red Lentivirus 2.0 (bleo)	One vial: 0.6 mL	BA-04889	SX5 (configured with Green/Red Optical Module), S3, SX1			
	Incucyte® Nuclight Green Lentivirus 2.0 (bleo)	One vial: 0.6 mL	BA-04890	SX5, S3, SX1, CX3			
	Incucyte® Nuclight Orange Lentivirus 2.0 (puro)	One vial: 0.6 mL	BA-04891	SX5, CX3			
	Incucyte® Nuclight NIR Lentivirus (puro)	One vial: 0.2 mL	4805	SX5, CX3			
Cell Cycle Reagents	Cell Cycle Lentivirus Reagents are fluorescent ul homogeneous expression of two fluorescent pro enable the visualization of cell cycle progression.	teins to distinguish					
	Incucyte®Cell Cycle Green/Red Lentivirus 2.0	One vial: 0.2 mL	BA-04892	SX5 (configured with Green/Red Optical Module), S3, SX1			
	Incucyte®Cell Cycle Green/Orange Lentivirus 2.0	One vial: 0.2 mL	BA-04893	SX5. CX3			

# Apoptosis, Cytotoxicity, Mitochondrial Membrane Potential and ATP Metabolism

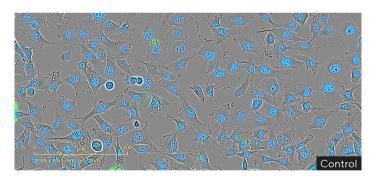
Incucyte® non-perturbing cell health reagents allow for kinetic readouts over multiple days for the evaluation of time-dependent and cell-specific responses to treatments.

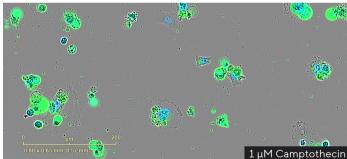
- Preserve cell health using non-perturbing, live-cell reagents to track true, artifact-free biological responses
- Reduce loss of precious or compromised cells with mixand-read or novel genetically encoded reagents and optimized protocols
- Perform multi-parametric analysis using cell health reagent combinations within a single well to generate data rich information
- Validate results and confirm changes in cell morphology with high density (HD) phase images at every time point

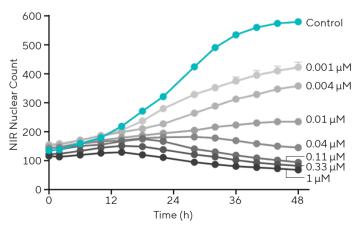
# Application Spotlight: Incucyte® Apoptosis Assay

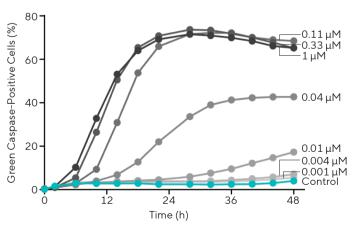
Evaluate cell death with two unique assays for apoptotic pathway analysis using specifically formulated reagents. Utilize Incucyte® Caspase-3/7 Dyes to allow direct detection of caspase-3/7 activation or Incucyte®

Annexin V Dyes to measure exposed phosphatidylserine in apoptotic cells. Enhance your insight with multiplexed measurements of nuclear counts of cell death.









HT-1080 fibrosarcoma cells stably expressing Nuclight NIR Lentivirus (pseudo-colored blue) were treated with decreasing concentrations of camptothecin in the presence of Incucyte® Caspase-3/7 Green Dye (pseudo-colored green). Representative images validate kinetic data of both cell viability and apoptotic death.

	Product	Description	Cat. No.	Instrument Compatibility				
Software	to quantify dynamic changes in cell subsets Module. Perform counts and track changes analysis of cell shape. Classifier is trained usi	Perform label-free cell counts and subsequent cell-by-cell classification based on shape, size or fluorescence intensity to quantify dynamic changes in cell subsets within heterogeneous living cell cultures with Cell-by-Cell Analysis Software Module. Perform counts and track changes in adherent cell morphology via label-free image segmentation and multivariate analysis of cell shape. Classifier is trained using control wells. Advanced Label-Free Classification Software Module an addon and requires Incucyte® Cell-by-Cell Analysis Software Module (Cat. No. 9600-0031).						
	Incucyte® Cell-by-Cell Analysis Software Module	1 module	9600-0031	SX5, S3, SX1 , CX3				
	Incucyte® Advanced Label-Free Classification Analysis Software Module	1 module	BA-04867	SX5, S3, SX1, CX3				
	Incucyte® Al Nuclei Detection	1 module	BA-04897	SX5, S3, SX1, CX3				
Caspase Activity Reagents	Inert, non-fluorescent (DEVD) substrates t caspase-3/7 to release a DNA-binding fluo		mbrane where	they can be cleaved by activated				
	Incucyte® Caspase-3/7 Green Dye	One vial: 20 µL (100-200 tests)	4440	SX5, S3, SX1, CX3				
	Incucyte® Caspase-3/7 Red Dye	One vial: 20 μL (100-200 tests)	4704	SX5 (configured with Green/Red Optical Module), S3, SX1				
	Incucyte® Caspase-3/7 Dye for Metabolism	One vial: 20 μL (100-200 tests)	4776	SX5 (configured with SX5 Metabolism Optical Module), CX3				
Apoptosis Plasma Membrane Integrity	Membrane impermeable, highly-selective phosphatidylserine (PS) cyanine fluorescent dyes label PS exposure on the extracellular surface of cells undergoing apoptosis.							
Reagents	Incucyte® Annexin V Green Dye	One vial: 100-200 tests	4642	SX5, S3, SX1, CX3				
	Incucyte® Annexin V Red Dye	One vial: 100-200 tests	4641	SX5 (configured with Green/Red Optical Module), S3, SX1				
	Incucyte® Annexin V Orange Dye	One vial: 100-200 tests	4759	SX5,CX3				
	Incucyte® Annexin V NIR Dye	One vial: 100-200 tests	4768	SX5,CX3				
Cytotoxicity Reagents	Highly sensitive cyanine-based dyes that do not enter living cells. When the cell membrane is compromised, it enters the cell, binds to DNA, and becomes fluorescent. Dye does not enter cells with intact cell membranes.							
	Incucyte® Cytotox Green Dye	Five vials: 5 μL (100 tests each)	4633	SX5, S3, SX1 , CX3				
	Incucyte® Cytotox Red Dye	Five vials: 5 μL (100 tests each)	4632	SX5 (configured with Green/Red Optical Module), S3, SX1				
	Incucyte® Cytotox NIR Dye	One vial: 100 uL (500-100 tests)	4846	SX5 , CX3				
Mitochondrial Membrane Potential (MMP)	Fluorescent dye that diffuses across the intermembrane space and accumulates in proportion to the MMP. Shifts in fluorescent intensity denote mitochondrial membrane potential state. Requires Incucyte® Cell-by-Cell Analysis Software Module for analysis.							
	Incucyte® MMP Orange Reagent Kit:	One kit	4775	SX5,CX3				
	■ MMP Orange Dye	One vial: 30 µL (200 tests)	_					
	• FCCP	One vial: 10 µL (16 tests)	_					
	■ Oligomycin A	One vial: 10 µL (16 tests)						
ATP Metabolism								
Software	Enables analysis of ATP dynamics by captu cell morphology in each well of a 96- or 38-		ile qualitatively	monitoring associated changes in				
	Incucyte® ATP Analysis Software Module	1 module	9600-0033	SX5 (configured with SX5 Metabolism Optical Module)				
Metabolism	Genetically-encoded fluorescent ATP ind	icator for direct measurem	ents of cytosol	lic ATP in living cells.				
Reagent	Incucyte® CytoATP Lentivirus	One vial: 0.2 mL	4772	SX5 (configured with SX5				

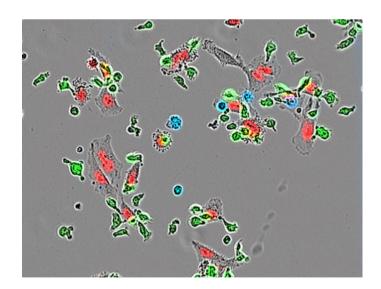
# Immune Cell Activation, Killing and NETosis

Incucyte® Immune Cell Assays are an integrated solution for real-time visualization and automated analysis of a range of immune cell functions from T cell activation and killing to programmed neutrophil cell death.

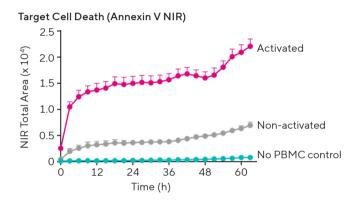
- Derive meaningful data with sensitive, non-perturbing reagents and HD phase images
- Make multiplexed measurements of death, viability and proliferation in 2D or 3D in vitro assay models
- Visualize and quantify dynamic cell interactions overtime in complex co-cultures

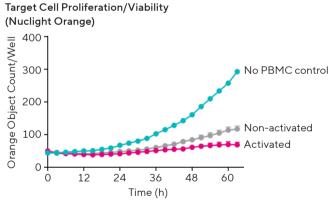
# Application Spotlight: Incucyte® Immune Cell Killing

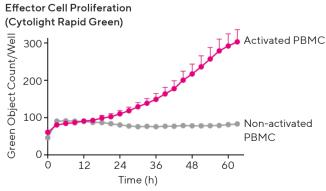
Capture, visualize and automatically quantify dynamic biological changes and cellular interactions of immune cell-mediated killing of tumor cells. With Incucyte® cell health and proliferation reagents you can make multiplexed measurements of tumor cell death (Annexin V NIR Dye), tumor cell proliferation/viability (Incucyte® Nuclight Orange Lentivirus) and immune cell health (Incucyte® Cytolight Rapid Green Dye) in the same population of cells over time.



MDA-MB-231 adenocarcinoma cells transduced with Incucyte® Nuclight Orange co-cultured with Incucyte® Cytolight Rapid Green labeled activated or non-activated PBMCs in the presence of Incucyte® Annexin V NIR. Quantification of NIR (pseudo-colored blue) fluorescence area indicates target cell death and object count of orange (pseudo-colored red) fluorescence, target cell proliferation/viability (pseudo-colored green). Effector cell proliferation was quantified based on green object count over the course of the experiment.







	Product	Description	Cat. No.	Instrument Compatibility			
Software	Perform label-free cell counts and subsequent cell surface protein expression for eval subsets of cells in complex immune-cell kill track changes in adherent cell morphology Classifier is trained using control wells. Adv	uation of immune cell activi ling models with Cell-by-Ce via label-free image segme vanced Label-Free Classific	ation and prolife ell Analysis Softv entation and mu ation Software N	ration in mono-cultures or track ware Module. Perform counts and Itivariate analysis of cell shape.			
	Incucyte® Cell-by-Cell Analysis Software Module	1 module	9600-0031	SX5, S3, SX1, CX3			
	Incucyte® Advanced Label-Free Classification Analysis Software Module	1 module	BA-04867	SX5, S3, SX1, CX3			
luclear Lentivirus abeling Reagents	Lentivirus reagents provide homogenous e cell function for live-cell quantification of c			nt protein without altering			
	Incucyte® Nuclight Red Lentivirus 2.0 (puro)	One vial: 0.6 mL	BA-04887	SX1, S3, SX5 (Green/Red Optical Module)			
	Incucyte® Nuclight Green Lentivirus 2.0 (puro)	One vial: 0.6 mL	BA-04888	SX1, S3, SX5 , CX3			
	Incucyte® Nuclight Red Lentivirus 2.0 (bleo)	One vial: 0.6 mL	BA-04889	SX1, S3, SX5 (Green/Red Optical Module)			
	Incucyte® Nuclight Green Lentivirus 2.0 (bleo)	One vial: 0.6 mL	BA-04890	SX1, S3, SX5 , CX3			
	Incucyte® Nuclight Orange Lentivirus 2.0 (puro)	One vial: 0.6 mL	BA-04891	SX5,CX3			
	Incucyte® Nuclight NIR Lentivirus (puro)	One vial: 0.2 mL	4805	SX5,CX3			
Cytoplasmic Dye Labeling Reagents	Live-cell cytoplasmic labeling dyes that fre into a cell membrane-impermeant form, proreffector cells.  Incucyte® Cytolight Rapid Green Dye						
	Incucyte® Cytolight Rapid Red Dye	Five vials: 50 µg	4706	SX5 (configured with Green/Red Optical Module), S3, SX1			
	Incucyte® Cytolight Rapid Orange Dye	One vial: 1 mg	4839	SX5,CX3			
aspase Activity eagents	Inert, non-fluorescent (DEVD) substrates that freely cross the cell membrane where they can be cleaved by activated caspase-3/7 to release a DNA-binding fluorescent label. Recommended for quantifying apoptosis in adherent target cells.						
	Incucyte® Caspase-3/7 Green Dye	One vial: 20 μL (100-200 tests)	4440	SX5, S3, SX1, CX3			
	Incucyte® Caspase-3/7 Red Dye	One vial: 20 μL (100-200 tests)	4704	SX5 (configured with Green/Red Optical Module), S3, SX1			
poptosis Plasma 1embrane Integrity	Membrane impermeable, highly-selective phosphatidylserine (PS) cyanine fluorescent dyes label PS exposed on the extracellular surface of cells undergoing apoptosis. Recommended for quantifying apoptosis in non-adherent target cells.						
eagents	Incucyte® Annexin V Green Dye	One vial: 100-200 tests	4642	SX5, S3, SX1, CX3			
	Incucyte® Annexin V Red Dye	One vial: 100-200 tests	4641	SX5 (configured with Green/Red Optical Module), S3, SX1			
	Incucyte® Annexin V Orange Dye	One vial: 100-200 tests	4759	SX5,CX3			
	Incucyte® Annexin V NIR Dye	One vial: 100-200 tests	4768	SX5,CX3			
ytotoxicity eagents	Highly sensitive cyanine-based dyes enter binding to DNA. Dye does not enter cells w of NETosis as extracellular DNA is released	vith intact cell membranes. A	Allows for rapid	visualization and quantification			
	Incucyte® Cytotox Green Dye	Five vials: 5 μL (100 tests each)	4633	SX5, S3, SX1 , CX3			
	Incucyte® Cytotox Red Dye	Five vials: 5 μL (100 tests each)	4632	SX5 (configured with Green/Red Optical Module), S3, SX1			
	Incucyte® Cytotox NIR Dye	One vial: 100 uL (500-100 tests)	4846	SX5,CX3			

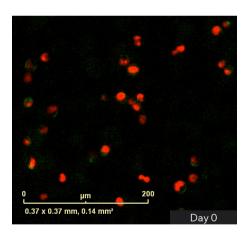
# Antibody Internalization and Immunocytochemistry

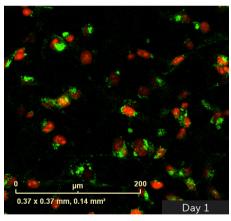
Incucyte® antibody labeling reagents are novel fluorescently labeled Fabs that can be mixed with Fc-containing antibodies and applied directly to living cells for long-term monitoring of spatial and temporal protein dynamics.

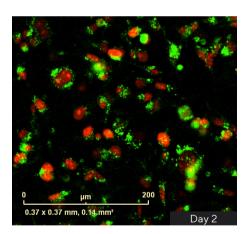
- Increase productivity with rapid single-step labeling paired with mix-and-read protocols for efficient testing of antibody panels
- Associate changes in surface protein expression or antibody internalization with cell function and morphology over time
- Combine sensitive, kinetic fluorescent measurement of protein dynamics with images and movies for visual confirmation of biology in every well

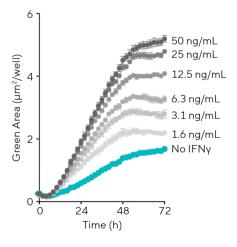
# Application Spotlight: Monitoring Dynamic Cell Surface Protein Expression

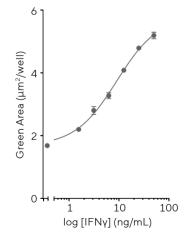
Quantify cell surface protein expression and distribution in live cells to study long-term protein dynamics and their relationship to function and morphology using Incucyte® Fabfluor-488, Fabfluor-555 or Fabfluor-594 Antibody Labeling Reagents.









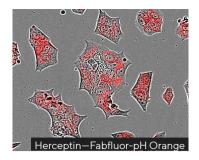


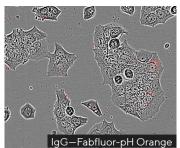
Incucyte® Fabfluor-488 was conjugated to anti-PD-L1 Ab (BioLegend) and added to Nuclight Red MDA-MB-231 breast cancer cells in the absence and presence of IFNy (+ Incucyte® Opti-Green background suppressor). Quantification of the green fluorescent area shows that IFNy induces a time- and concentration-dependent increase in PD-L1 expression.

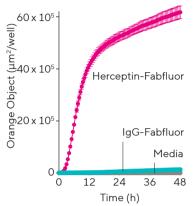
# Application Spotlight: Antibody Internalization

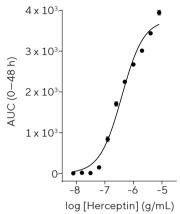
Efficiently evaluate the full time course of antibody internalization for real-time analysis of internalization rates under physiological conditions using Incucyte® Fabfluor-pH Antibody Labeling Reagents.

HD phase and orange fluorescence images (10X) show HER-2 positive BT-474 cells treated with Incucyte® Fabfluor-pH Orange labeled Herceptin display orange (pseudo-colored red), cytosolic fluorescence while cells treated with an isotype control display no cellular fluorescence. Time-course data shows a rapid increase in orange object area over time in cells treated with labeled Herceptin, but not with IgG1 isotype control.









	Product	Description	Cat. No.	Instrument Compatibility				
Software	Perform label-free cell counts and quantify dyr	namic changes in c	ell subsets withir	n heterogeneous living cell cultures.				
	Incucyte® Cell-by-Cell Analysis Software Module	1 module	9600-0031	SX5, S3, SX1, CX3				
abfluor-pH	Novel pH-sensitive Fc-targeting antibody fragment labels antibody of choice for analysis of antibody internalization.							
Antibody Labeling Reagents	Incucyte® Human Fabfluor-pH Orange Antibody Labeling Dye	One vial: 50 μg	4812	SX5, CX3				
	Incucyte® Human Fabfluor-pH Red Antibody Labeling Dye	One vial: 50 μg	4722	SX5 (configured with Green/Red Optical Module), S3, SX1				
	Incucyte® Mouse IgG1 Fabfluor-pH Red Antibody Labeling Dye	One vial: 50 μg	4723	SX5 (configured with Green/Red Optical Module), S3, SX1				
	Incucyte® Mouse IgG1 Fabfluor-pH Orange Antibody Labeling Dye	One vial: 50 μg	BA-04885	SX5, CX3				
	Incucyte® Mouse IgG2a Fabfluor-pH Red Antibody Labeling Dye	One vial: 50 μg	4750	SX5 (configured with Green/Red Optical Module), S3, SX1				
	Incucyte® Mouse IgG2a Fabfluor-pH Orange Antibody Labeling Dye	One vial: 50 μg	BA-04886	SX5, CX3				
	Incucyte® Mouse IgG2b Fabfluor-pH Red Antibody Labeling Dye	One vial: 50 μg	4751	SX5 (configured with Green/Red Optical Module), S3, SX1				
	Incucyte® Rat Fabfluor-pH Red Antibody Labeling Dye	One vial: 50 μg	4737	SX5 (configured with Green/Red Optical Module), S3, SX1				
abfluor Live-Cell	Novel fluorescently tagged Fc-targeting Fab fragments label your antibody of choice for cell surface protein expression							
mmunocytochemistry Labeling Reagents	Incucyte® Mouse IgG2a Fabfluor-488 Antibody Labeling Dye	One vial: 50 μg	4743	SX5, S3, SX1, CX3				
	Incucyte® Mouse IgG2b Fabfluor-488 Antibody Labeling Dye	One vial: 50 μg	4744	SX5, S3, SX1, CX3				
	Incucyte® Mouse IgG1 Fabfluor-488 Antibody Labeling Dye	One vial: 50 μg	4745	SX5, S3, SX1, CX3				
	Incucyte® Mouse IgG1 Fabfluor-555 Antibody Labeling Dye	One vial: 50 μg	BA-04873	SX5,CX3				
	Incucyte® Mouse IgG1 Fabfluor-594 Antibody Labeling Dye	One vial: 50 μg	4844	SX5 (configured with a Green/Red Optical Module), S3, SX1				
	Incucyte® Mouse IgG2a Fabfluor-594 Antibody Labeling Dye	One vial: 50 μg	BA-04863	SX5 (configured with a Green/Red Optical Module), S3, SX1				

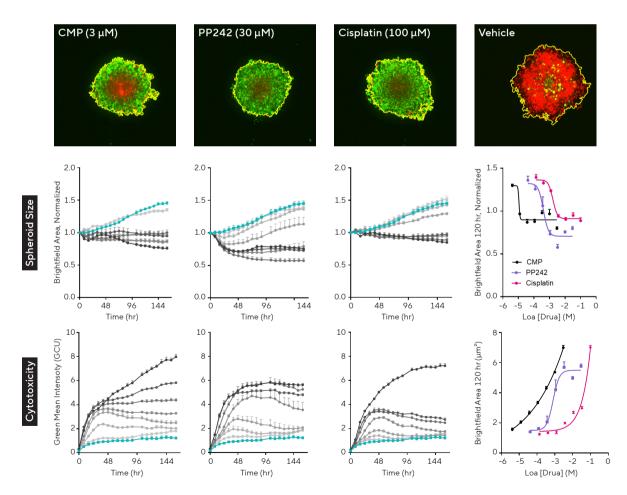
# Spheroid Growth, Invasion and Immune Cell Killing

The Incucyte® Spheroid Analysis Software Module is designed to acquire and analyze label or label-free spheroids to automatically monitor and quantify the formation, growth, shrinkage and invasive properties of advanced cell models in real time inside your tissue culture incubator.

- Flexible acquisition mode to enable studies of both single and multi-spheroid assays
- Enable long-term imaging with enhanced depth of focus brightfield (DF® Brightfield) image acquisition, HD phase, widefield fluorescence, or NEW! spinning disck confocal
- Analyze relevant spheroid metrics using intuitive Incucyte® processing definitions

## Application Spotlight: Spheroid Cell Health

Quantify growth and investigate morphology of solid tumors in real-time using non-perturbing reagents and purpose-built, integrated Incucyte® Spheroid Analysis Software Module.



Compare brightfield and fluorescent readouts. Confocal images show fluorescence within masked BF area of Incucyte® Nuclight Orange A549 spheroids 4 days post-treatment with cytotoxic compounds. Time course profiles of Brightfield Area show cytotoxic response to drugs at three-fold dilutions, with spheroid growth being more inhibited as drug concentrations increase. Mean Green Intensity measured within Brightfield Boundary (bottom row) shows a differential response to compounds.

	Product	Description	Cat. No.	Instrument Compatibility				
Spheroid Software	Analyze growth, viability and invasion of single spheroids in round-bottom multi-well formats or measure multiple spheroids in flat bottom plates to detect changes in growth and viability.							
	Incucyte® Spheroid Analysis Software Module	1 module	9600-0019	SX5, S3, SX1, CX3				
Nuclear Lentivirus Labeling Reagents	Lentivirus reagents provide homogenous e function for live-cell quantification of cell p			rescent protein without altering cell				
	Incucyte® Nuclight Red Lentivirus 2.0 (puro)	One vial: 0.6 mL	BA-04887	SX1, S3, SX5 (Green/Red Optical Module)				
	Incucyte® Nuclight Green Lentivirus 2.0 (puro)	One vial: 0.6 mL	BA-04888	SX1, S3, SX5, CX3				
	Incucyte® Nuclight Red Lentivirus 2.0 (bleo)	One vial: 0.6 mL	BA-04889	SX1, S3, SX5 (Green/Red Optical Module)				
	Incucyte® Nuclight Green Lentivirus 2.0 (bleo)	One vial: 0.6 mL	BA-04890	SX1, S3, SX5, CX3				
	Incucyte® Nuclight Orange Lentivirus 2.0 (puro)	One vial: 0.6 mL	BA-04891	SX5, CX3				
	Incucyte® Nuclight NIR Lentivirus Reagent (puro)	One vial: 0.2 mL	4805	SX5, CX3				
Apoptosis Plasma Membrane Integrity	Membrane impermeable, highly-selective phosphatidylserine (PS) cyanine fluorescent dyes label PS exposed on the extracellular surface of cells undergoing apoptosis.							
Reagents	Incucyte® Annexin V Green Dye	One vial: 100- 200 tests	4642	SX5, S3, SX1, CX3				
	Incucyte® Annexin V Red Dye	One vial: 100- 200 tests	4641	SX5 (configured with Green/Red Optical Module), S3, SX1				
	Incucyte® Annexin V Orange Dye	One vial: 100- 200 tests	4759	SX5,CX3				
	Incucyte® Annexin V NIR Dye	One vial: 100- 200 tests	4768	SX5,CX3				
Cytoplasmic Dye Labeling Reagents	Live-cell cytoplasmic labeling dyes that free into a cell membrane-impermeant form, pro							
	Incucyte® Cytolight Rapid Green Dye	One vial: 15 µg	4705	SX5, S3, SX1, CX3				
	Incucyte® Cytolight Rapid Red Dye	Five vials: 50 µg	4706	SX5 (configured with Green/Red Optical Module), S3, SX1				

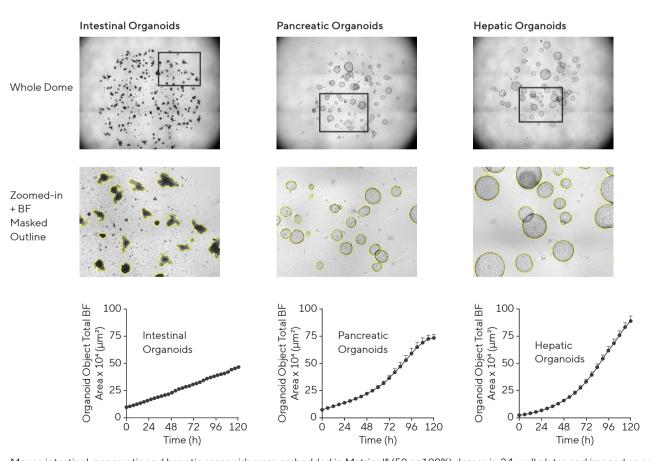
# Organoid Culture QC and Organoid Assay

The Incucyte® Organoid Analysis Software Module enables you to standardize your entire organoid workflow, from generation, maintenance (Organoid Culture QC) or access treatment effect on organoid growth (Organoid Assay) and analysis, using image-based, label-free measurements.

- Locate and analyze Matrigel® embedded organoids automatically
- Perform continuous label-free analysis in physiologically relevant conditions
- Use quantitative data to support and document passaging decisions or probe the effects of treatments through unbiased assessment of size, count and morphology
- Improve efficiency with simple, high-throughput formation and evaluation of organoids

# Application Spotlight: Organoid Culture QC

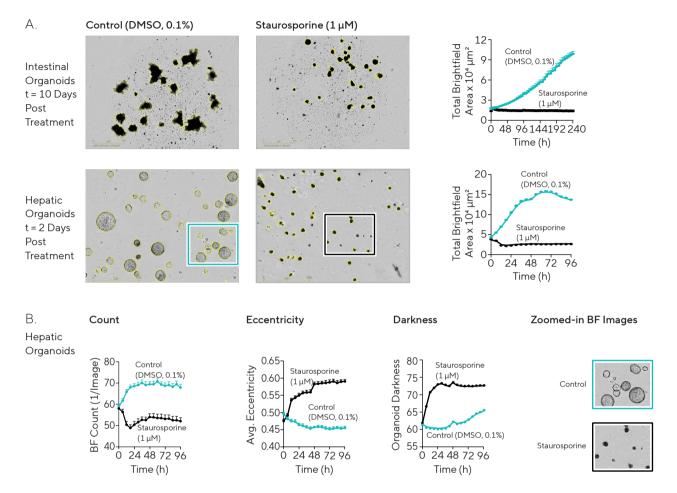
Evaluate multiple organoid types using image-based, kinetic analysis and characterize differentiation and maturation of organoids in Matrigel® domes in 24- or 48-well plates.



Mouse intestinal, pancreatic and hepatic organoids were embedded in Matrigel® (50 or 100%) domes in 24-well plates and imaged on an Incucyte® Organoids were automatically located, imaged and over 6-8 days using the Incucyte® Organoid Analysis Software Module. Time course profiles and brightfield images show cell type-specific organoid differentiation, morphology and growth.

## Application Spotlight: Organoid Assay

Track and standardize the analysis of organoids embedded in Matrigel® to generate real-time, physiologically relevant analysis of organoids to better predict therapeutic outcomes using the Incucyte® Organoid Analysis Software Module.



Brightfield images (A) show mouse intestinal and hepatic organoids (1K cells/well) were embedded in Matrigel\* (50%) in 96-well plates and allowed to form organoids for 3 days prior to treatment (vehicle or staurosporine; SSP). Corresponding time-courses of BF area demonstrate the continued growth of vehicle-treated organoids and the inhibitory effects of SSP across both cell types. SSP-treated organoids lose distinctive rounded phenotype (increased eccentricity) and increase in darkness over time (B).

	Product	Description	Cat. No.	Instrument Compatibility		
Organoid Software	Characterize the differentiation and maturation of organoid cultures in 24- or 48-well plates and assess treatment effects on organoid growth in 96-well microplates.					
	Incucyte® Organoid Analysis Software Module	1 module	9600-0034	SX5, S3, SX1, CX3*		

 $<sup>^{\</sup>star}$ Coming soon with future software iterations

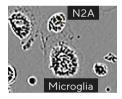
# Efferocytosis

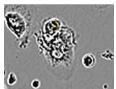
pHrodo® Cell Labeling Kits for Incucyte® are formulated for long-term and sensitive analysis of phagocytosis of dead, apoptotic or antibody-targeted cells over time.

- Study the model of your choice over time with specific and sensitive reagents paired with simple mix-and-read 96 / 384-well protocols
- Quantify antibody-dependent cellular phagocytosis (ADCP) with the addition of antibodies (e.g., anti-CD-47)
- Confirm phagocytosis signals using HD phase-contrast and fluorescent images

# Application Spotlight: Efferocytosis Assay

Observe and measure efferocytosis over the entire assay time course using your choice of target cells labeled with pHrodo® Orange Cell Labeling Dye for Incucyte®.





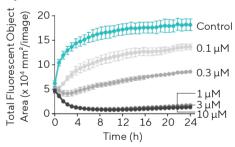


Time-lapse visualization of iPSC-derived microglia (Axol BioSciences) engulfing pHrodo® Orange-labeled apoptotic Neuro-2A (N2A) cells. Images verify the entry of an apoptotic target cell into the cytoplasm of the microglia. Inhibition of this engulfment by cytochalasin D was quantified based on orange fluorescence object area over the course of the experiment.

# Cytochalasin D IC<sub>50</sub> Area (x 10<sup>4</sup> mm<sup>2</sup>/image) To 10<sup>-</sup> To 10

Inhibition of Efferocytosis With Cytochalasin D

log (M)



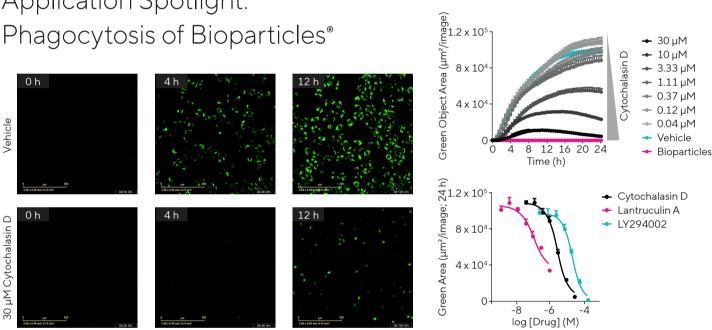
	Product	Description	Cat. No.	Instrument Compatibility		
pHrodo® Cell Labeling Kits	Fluorescent dyes label whole cells with a pH-sensitive fluorophore for real-time analysis of efferocytosis and antibody mediated cellular phagocytosis.					
	pHrodo® Orange Cell Labeling Kit for Incucyte®	One kit	4766	SX5, CX3		
	pHrodo® Red Cell Labeling Kit for Incucyte®	One kit	4649	SX5 (configured with Green/Red Optical Module), S3, SX1 , CX3		

# Phagocytosis

pHrodo® Bioparticles® for Incucyte® are pH-sensitive conjugated probes specifically formulated for analysis of the internalization of bacterial Gram-positive, Gramnegative or yeast-derived bioparticles by immune cells.

- Monitor phagocytosis in real-time with simple mix-and-read 96- and 384-well protocols
- Validate phagocytosis with time-lapse images and movies
- Study the effect of treatments on full time course phagocytosis profiles

## Application Spotlight: Phagocytosis of Bioparticles®



J774A.1 murine macrophages phagocytosing pHrodo® Green E. coli Bioparticles® for Incucyte® in the presence of inhibitors. Time-lapse visualization of cytochalasin D inhibition of Bioparticle® phagocytosis compared to vehicle over 12 h. Time course also shown for cytochalasin D inhibition. Concentration-response curves at 24 h compare inhibition of phagocytosis by cytochalasin D, lantruculin A, and LY294002.

	Product	Description	Cat. No.	Instrument Compatibility
pHrodo®	Sterile fluorogenic reagents ideally suited to a simple	mix-and-read, real	-time live cell qu	antification of phagocytosis.
Bioparticles <sup>®</sup>	pHrodo® Red <i>E. coli</i> Bioparticles® for Incucyte®	One vial: 2 mg	4615	SX5 (configured with Green/Red Optical Module), S3, SX1
	pHrodo® Green <i>E. coli</i> Bioparticles® for Incucyte®	One vial: 2 mg	4616	SX5, S3, SX1, CX3

# Neurite Dynamics and Neuronal Activity

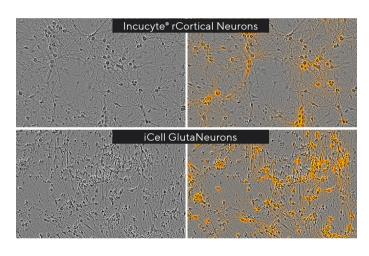
Incucyte® novel, live-cell labeling reagents and purposebuilt software quantify long-term changes in neurite dynamics and neuronal activity that enable continuous analysis of sensitive neuronal cell models.

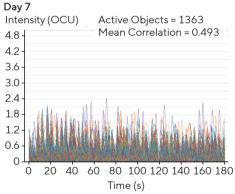
- Conduct long-term studies of neuronal function with novel, non-perturbing fluorescent reagents
- Capture transient events in your choice of cell model with non-invasive, repeated measurements of the same neuronal culture in physiologically relevant conditions
- Analyze relevant morphological and functional metrics using intuitive, purpose-built Incucyte® software

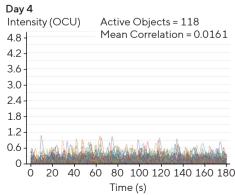
# Application Spotlight: Neuronal Activity Assay

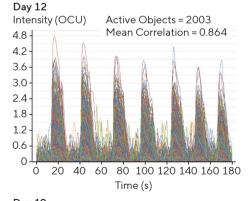
Access complex, neuronal activity and connectivity measurements from thousands of cells chronically to gain unprecedented functional insight into neuronal cell models using our novel Incucyte® Neuroburst Orange Lentivirus and Incucyte® Neuronal Activity Analysis Software Module.

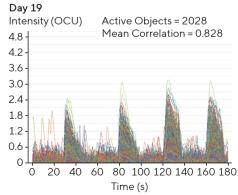
Incucyte® rCortical Neurons and iCell GlutaNeurons (Cellular Dynamics International) express the Incucyte® Neuroburst Orange Lentivirus, without perturbing the health and morphology of the cells.

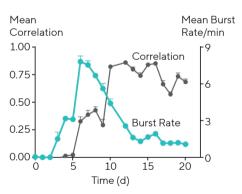












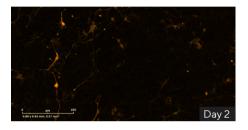
Kinetic quantification (graph above) of longitudinal, dynamic changes in neuronal activity (mean burst rate and mean correlation) of iCellGluta Neurons expressing Neuroburst Orange Lentivirus, showing changes over time during neuronal network maturation. Active object traces (corner traces) provide detailed insight into the dynamic changes in neuronal activity and connectivity for every acquired time point.

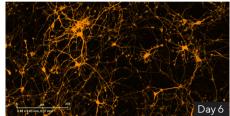
## Application Spotlight: Neurite Dynamics Assay

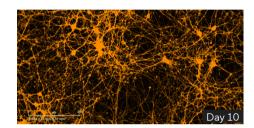
Generate kinetic, image-based and automated measurements using Incucyte® Neurotrack Analysis Software Module for continuous analysis of neurite outgrowth and stability—inside your incubator.

Incucyte® rCortical Neurons transduced with Incucyte® Neurolight Orange Lentivirus labeled cells cultured in the presence of Incucyte® rAstrocytes were treated with glutamate at Day 10. Time course analysis of orange fluorescence neurite length reveals concentration-dependent treatment effects.

# Neurite Length in Response to Glutamate 250 200 200 300 MM 3 MM 3 MM 3 MM 300 MM 50 6 8 10 12 14 Time (d)







<u> </u>						
	Product	Description	Cat. No.	Instrument Compatibility		
Neuronal Activity	Record activity from over a thousand cells t	o study changes in	neuronal networl	k activity and connectivity.		
Software	Purpose-built acquisition and analysis softw	ware for the detection	on of calcium osc	illations in 96-well plates.		
	Incucyte® Neuronal Activity Analysis Software Module	1 module	9600-0032	SX5 , CX3*		
Neuronal Activity	Fluorescently detect changes in activity usi	ng a novel genetica	ally-encoded fluo	rescent calcium indicator.		
Reagents	Incucyte® Neuroburst Orange Lentivirus	One vial: 2 mL	4736	SX5, CX3*		
	Incucyte® Neuroactive Orange Kit	One kit	4761	SX5, CX3*		
Neurite Dynamics	Characterize neurite dynamics over time in Annexin V Reagents)	mono- or co-cultur	re models while a	ssessing cell viability (refer to page 7,		
Software	Enables label-free or fluorescent analysis of 384-well plate.	f neurite outgrowth	, maturation and	disruption in each well of a 96- or		
	Incucyte® Neurotrack Analysis Software Module	1 module	9600-0010	SX5, S3, SX1 . CX3*		
Neuronal Cells	Ready-to-use cryopreserved cells from the cortex of Sprague Dawley rats at Day 18 of gestation.					
	Incucyte® rCortical Neurons	One vial: 2 x 10 <sup>6</sup> cells	4753	Assay dependent		
	Incucyte® rAstrocytes	One vial: 2 x 10 <sup>6</sup> cells	4586	Assay dependent		

<sup>\*</sup>Coming soon with future software iterations

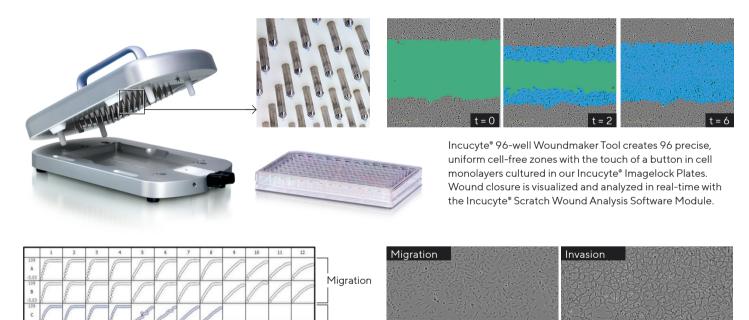
# Invasion and Migration Assays

Incucyte® Scratch Wound and Chemotaxis Assays allow you to continuously monitor and analyze migration and invasion using purpose-built consumables and automated software.

- Unlock your productivity with optimized protocols, peripherals and automated analysis
- Confirm cell movement and morphology with images and movies—all from inside your incubator
- Perform reproducible 96-well kinetic migration or invasion assays using the cell model of your choice adherent or non-adherent

## Application Spotlight: Scratch Wound Migration and Invasion Assay

Make label-free, quantitative measurements of migration and invasion while visualizing morphological changes due to treatment effects, using the integrated Incucyte® Scratch Wound Analysis Software Module.



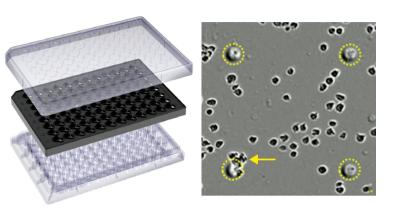
Microplate graph showing progression of differential cell migration and invasion of three different cell types using the Relative Wound Density metric. Representative images of HT-1080 fibrosarcoma cells highlight differences in morphology of cells during migration versus invasion.

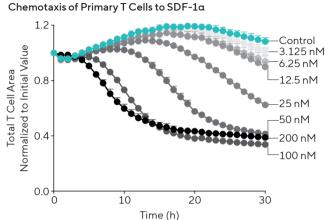
Invasion

MCF-7

## Application Spotlight: Incucyte® Chemotaxis Assay

Maximize your productivity and insight into dynamic cell movement with automated, label-free imaging and analysis using optically clear Incucyte® Clearview 96-well Chemotaxis Plates and Incucyte® Chemotaxis Analysis Software Module.





Incucyte® Clearview 96-well Chemotaxis Plates provide an optically clear surface for label-free imaging and analysis of chemotactic cell migration or invasion. Cells are added to the upper chamber and chemoattractant to the lower reservoir plate. Chemotactic transmembrane migration is automatically quantified as the cells migrate through laser etched pores (yellow circles) toward chemoattractant. Example data of concentration-dependent SDF-1 $\alpha$  mediated chemotaxis of CD3/CD28 activated human T cells.

	Product	Description	Cat. No.	Instrument Compatibility		
Scratch Wound	Analyze 96-well Incucyte® Scratch Woo	und Assay images to analyze ce	ell migration and	invasion with or without labels		
Cell Migration and Invasion	Incucyte® Scratch Wound Analysis Software Module	1 module	9600-0012	SX5, S3, SX1, CX3*		
	Incucyte® 96-well Woundmaker Tool	1 wounder	4563	SX5, S3, SX1, CX3*		
	Incucyte® Cell Migration Kit	Woundmaker Tool 2 Rinse boat assemblies 15 Imagelock Plates 1 Incucyte® Microplate Tray Alconox	BA-04858	SX5, S3, SX1, CX3*		
	Incucyte® Imagelock 96-well Plate	Pack of 10 plates	BA-04856	SX5, S3, SX1, CX3*		
		Pack of 50 plates	BA-04857	SX5, S3, SX1, CX3*		
Chemotaxis Cell	Track and quantify label-free and fluorescently labeled chemotaxis cell migration and invasion in microplate format.					
Migration and Invasion	Incucyte® Chemotaxis Analysis Software Module	1 module	9600-0015	SX5, S3, SX1, CX3*		
	Incucyte® Clearview 96-well	1 plate	4582	SX5, S3, SX1, CX3*		
	Chemotaxis Plates	Pack of 10 plates	4648	SX5, S3, SX1, CX3*		
	Incucyte® Clearview 96-well	10 plates	4600	SX1, S3, SX5, CX3*		
	Reservoir Plates	100 plates	4601			

 $<sup>^{\</sup>star}$ Coming soon with future software iterations

# **EV Uptake Assay**

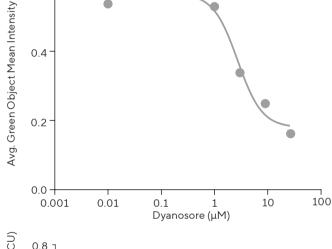
The Incucyte® EV Uptake Assay enables kinetic measurements of intracellular EV uptake in the same population of cells, utilizing a simple protocol that prevents the loss of precious EVs and maintains their biological functionality.

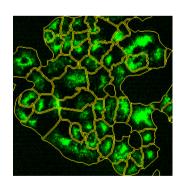
- Efficiently label EVs regardless of extraction method from a variety of source cells
- Gather reliable, qualitative measurements of EV uptake with kit that eliminates aggregation, free dye, and is nonperturbing to cell health
- Supports functional uptake of EVs and homogenous labeling for kinetic measurements or downstream analysis

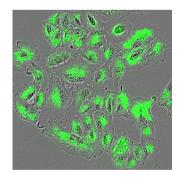
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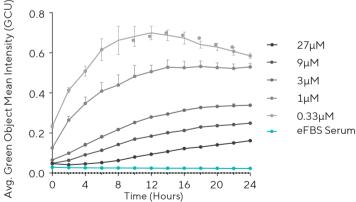
## Application Spotlight: EV Uptake and Endocytosis Inhibition

Observe and evaluate changes in intracellular EV uptake or morphological changes in host cells over time on a population level with Incucyte® Exofluor Green EV Labeling Kit. Gather real-time, kinetic measurements for valuable insights into mechanisms of EV uptake and drive decisions on potential therapeutics.









A549 Cells were seeded overnight and then treated with the endocytosis inhibitor Dynasore. Immediately following Dynasore treatment, Hansa A549 EVs labeled with Incucyte® Exofluor Green Dye were added. A concentration-dependent inhibition of EV uptake was reflected in the intensity of green fluorescence.

	Product	Description	Cat. No.	Instrument Compatibility
EV	Fluorescently label extracellular vesicles (EVs	s) (e.g., exosomes) in order to investigate upta	ake in live-cel	l assays
Labeling Dye	Incucyte® Exofluor Green EV Labeling Kit	One kit	BA-04877	SX5, S3, SX1, CX3

# Incucyte® Software Modules

Incucyte® software makes the process of acquiring, viewing, analyzing and sharing images of living cells easier than ever before. Using the base software's purpose-built analysis tools and comprehensive software modules that enable powerful phenotypic cellular analysis, research teams are able to convert images into insight.

Software Module	Description	Cat. No.
Incucyte® AI Cell Health Analysis Software Module	Simplify the complexity of classifying live versus dead cells using artificial intelligence (AI)-driven image analysis without the use of labels. Requires a GPU co-processor installed as a drop-in hardware upgrade to the controller and Incucyte® Software v2022B or newer software version.	BA-04871
Incucyte® AI Confluence Analysis (update for Incucyte® Base Analysis Software)	Al-driven analysis provides a simple workflow for highly accurate segmentation of cells in phase-contrast images, adapting to a wide range of cell types and morphologies with minimal user input. Incucyte® Al Confluence Analysis is available with Incucyte® Software v2022A or newer software version.	Part of Incucyte® Base software
Incucyte® AI Nuclei Detection Software Module	Accelerate your image-based research with an Al-powered solution for label-free segmentation of adherent cell nuclei from HD phase-contrast images.	BA-04897
Incucyte® Advanced Label-Free Classification Analysis Software Module	Perform counts and track changes in adherent cell morphology via label-free image segmentation and multivariate analysis of cell shape. Classifier is trained using control wells. The Incucyte® Advanced Label-Free Classification Software Module is an add-on and requires Incucyte® Cell-by-Cell Analysis Software Module (Cat. No. 9600-0031).	BA-04867
Incucyte® Cell-by-Cell Analysis Software Module	Perform label-free cell counts and subsequent cell-by-cell classification based on shape, size or fluorescence intensity to quantify dynamic changes in cell subsets within heterogeneous living cell cultures.	9600-0031
Incucyte® Organoid Analysis Software Module	Characterize the differentiation and maturation of organoid cultures in 24- or 48-well plates and assess treatment effects on organoid growth in 96-well microplates.	9600-0034
Incucyte® Spheroid Analysis Software Module	Analyze growth, viability and invasion of single spheroids in round-bottom multi-well formats or measure multiple spheroids in flat-bottom plates to detect changes in growth and viability.	9600-0019
Incucyte® Neuronal Activity Analysis Software Module	Purpose-built acquisition and analysis software for the detection of calcium oscillations in 96-well plates. For use with the Incucyte® SX5 configured with the Green/Orange/NIR Optical Module.	9600-0032
Incucyte® Neurotrack Analysis Software Module	Enables label-free or fluorescent analysis of neurite outgrowth, maturation and disruption in each well of a 96- or 384-well plate.	9600-0010
Incucyte® Chemotaxis Analysis Software Module	Track and quantify label-free and fluorescently-labeled chemotactic cell migration and invasion in microplate format.	9600-0015
Incucyte® Scratch Wound Analysis Software Module	Automated detection and quantification of wounds from each well of a 96-well plate in order to analyze cell migration and invasion with or without labels.	9600-0012
Incucyte® ATP Analysis Software Module	Enables analysis of ATP dynamics by capturing fluorescent images while qualitatively monitoring associated changes in cell morphology in each well of a 96- or 384-well plate. For use with the Incucyte® SX5 configured with the Incucyte® SX5 Metabolism Optical Module.	9600-0033
Incucyte® Angiogenesis Analysis Software Module	Identify and analyze the formation and disruption of endothelial vascular networks over time in each well of a 96-well plate.	9600-0011
Incucyte® 21 CFR Part 11 Software Module	Support your compliance needs while still keeping Incucyte's software intuitive and easy to use. Configure full system access with user permissions, experiment visibility, and adherence to templates	BA-04876

Whether its the Incucyte® S3, Incucyte® SX1, Incucyte® SX5, or our newest Incucyte® CX3 with spinning disk confocal for 3D biology, we deliver real-time insights to simplify progress for labs of any size.

Analyze your cells for days, weeks or even months as they sit stationary in the stable environment of your tissue culture incubator.

With the Incucyte® user-friendly interface and robust instrument portfolio, any cell biologist can gain dynamic insights into the health, morphology, movement and function of their cell models.



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