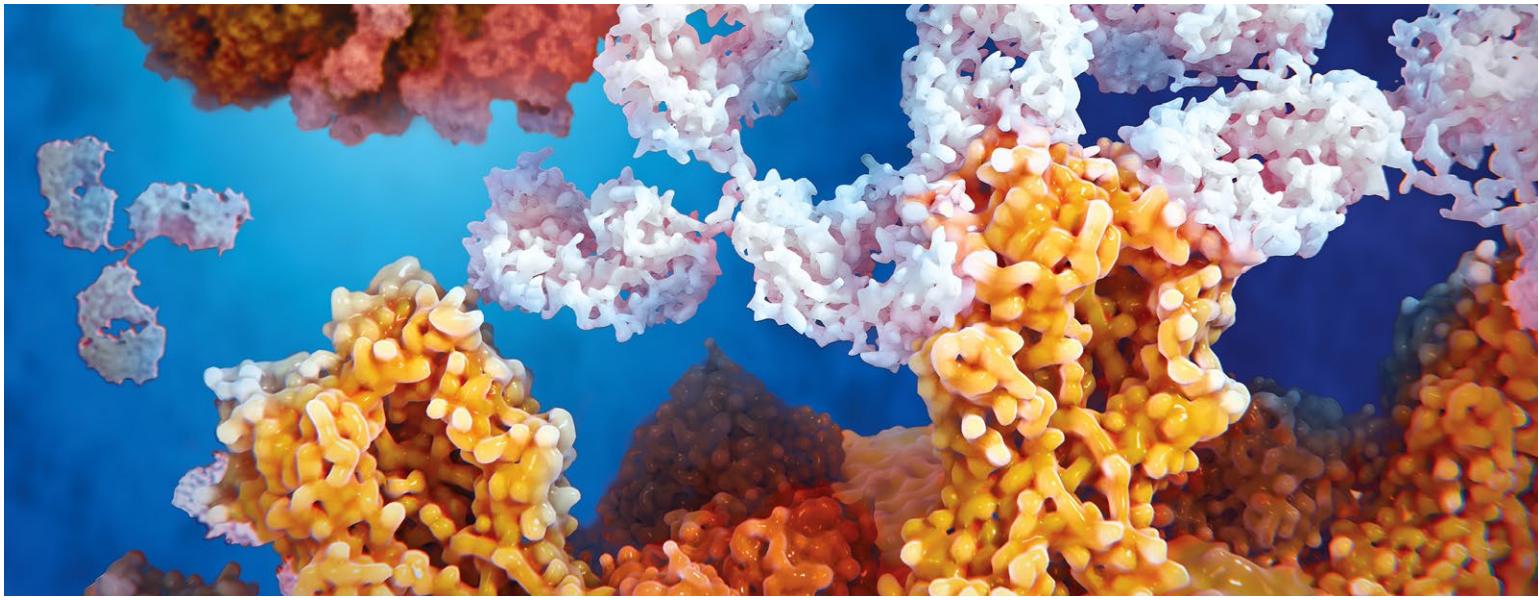
sartorius

Assays for Immunology Research
IncuCyte® S3 Live-Cell Analysis System

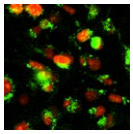


Immune system characterization of health and disease states requires quantitative data from dynamic experimental models of the cells that comprise both the innate and adaptive responses and their targets. The IncuCyte® S3 Live-Cell Analysis System enables real-time, automated analysis of the dynamic changes in immune cells and their interactions **inside your incubator**, providing deeper functional insight.

Key Advantages:

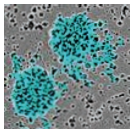
- Real-time, image-based measurements in a physiologically-relevant environment.
- Optimized protocols in 96- and 384-well assay formats.
- Applications, reagents and consumables for non-invasive, non-disruptive analysis of cell health, phenotype and function.
- Minimal manipulation and cell loss with mix-and-read reagents.

Protein Expression

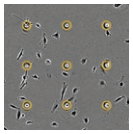


Live-cell Immuno-cytochemistry
Analysis of cell surface protein dynamics and distribution.

Immune System Mobilization

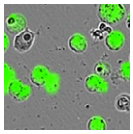


Immune cell activation & proliferation
Monitor immune cell activation in real time.

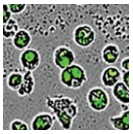


Chemotaxis and TEM
Measure immune cell migration toward chemokines.

Immune Response



NETosis
Quantify neutrophil extracellular traps in real time.



Phagocytosis
Real-time analysis of internalization and clearance using pH sensitive probes.

IncuCyte®
A SARTORIUS BRAND

IncuCyte® Live-Cell Immunocytochemistry

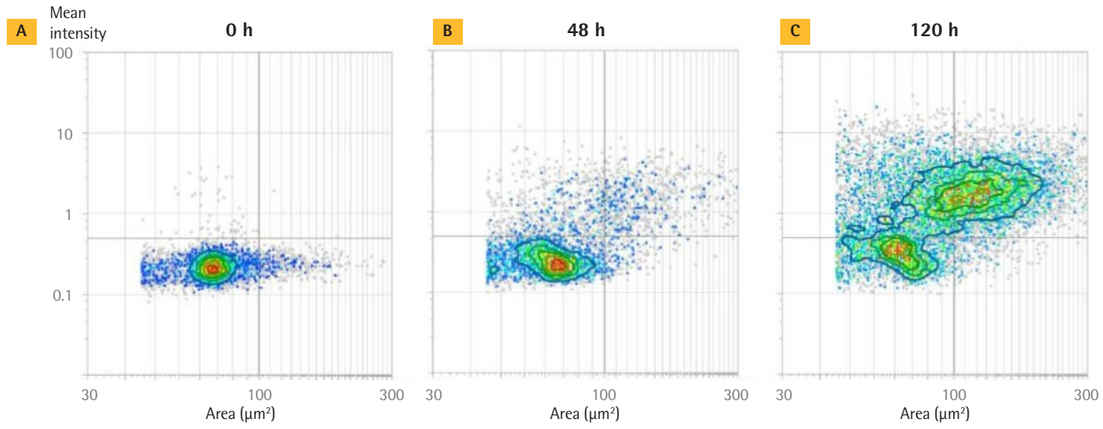
Gain insight into immune cell response to the external environment with continuous imaging and analysis of proteins in living cells. IncuCyte® Live-Cell Immunocytochemistry offers a powerful solution for long-term tracking and quantification of cell surface protein markers that can be linked to cell function and morphology, generating greater insight into cellular processes.

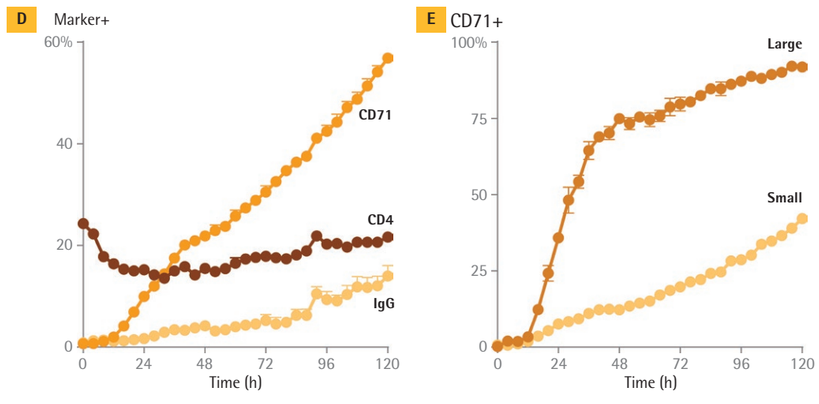
Perform kinetic immunophenotyping to identify subpopulations

Label cell surface markers in living cells using IncuCyte FabFluor-488 Antibody Labeling Reagents and then analyze using IncuCyte S3 Cell-by-Cell Analysis Software Module - temporally classify cells into subsets based on CD antigens without cell lifting or fix/wash protocols.

Key Advantages:

- Reveal protein dynamics in a physiological context using non-perturbing IncuCyte® FabFluor-488 Antibody Labeling Reagents.
- **NEW!** Leverage IncuCyte® S3 Cell-by-Cell Analysis Software Module for kinetic analysis of heterogeneous population changes - eliminating the need for cell lifting or fix/wash protocols.
- Associate changes in surface protein expression with cell function and morphology to reveal informative, temporal changes in cell behavior.

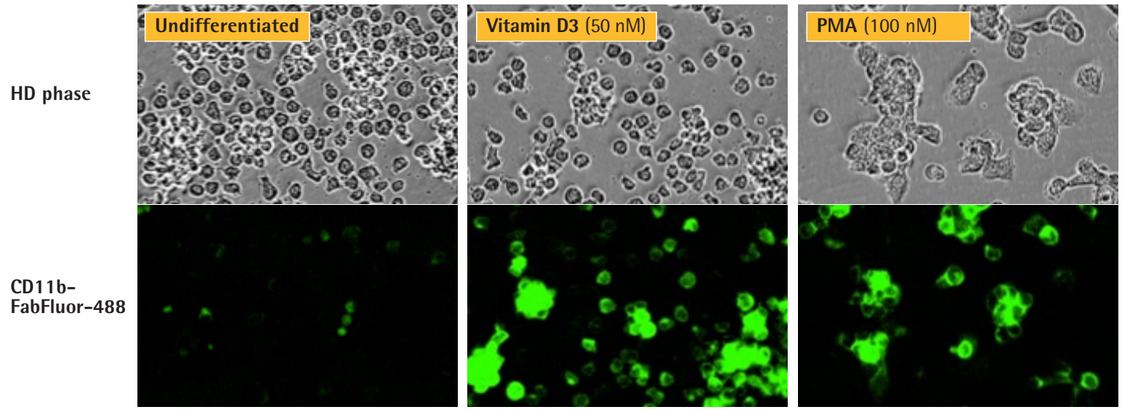


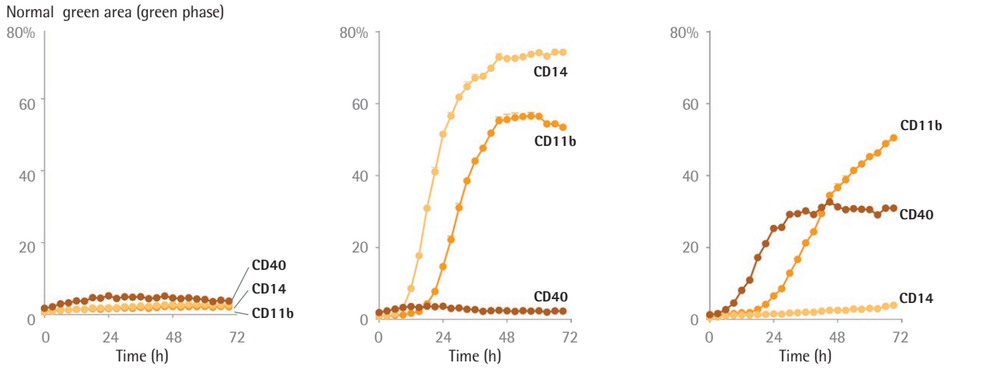


Subset analysis of activated T-cells: CD71 upregulation.
(A–C) Cell-by-cell density plots showing increase in CD71-fluorescence as a function of time and cell area (phase) in activated T-cells. **(D)** Dynamic changes in the proportion of CD71+, but not CD4+ cells, following activation. **(E)** Subset analysis showing preferential upregulation of CD71 expression in large (>110 µm²) vs small (<110 µm²) cells.

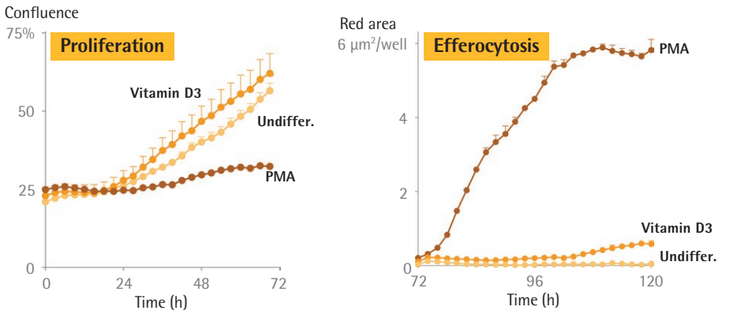
Associate changes in surface protein expression with cell function and morphology

Couple the dynamic changes in protein expression with cell function and morphology in real time.





Multiplex cell surface marker, phagocytic activity, and proliferation measurements plus visualize morphology to study differentiation.
THP-1 monocytes exposed to various treatments in the presence of IncuCyte FabFluor-488 Antibody Labeling Reagent complexed to CD11b, CD14 or CD40. PMA showed a marked change in cell morphology (HD-phase contrast images) compared to media alone or vitamin D3-treated cells. Kinetic graphs highlight differential and time-dependent surface protein expression in response to various treatments. PMA, but not media or vitamin D3, yields a decrease in cell proliferation (confluence) and concordant increase in phagocytic potential as measured by efferocytosis of apoptotic Jurkat cells labeled with IncuCyte pHrodo® Red Cell Labeling Kit.



IncuCyte® Activation, Proliferation & Cell Health Assays

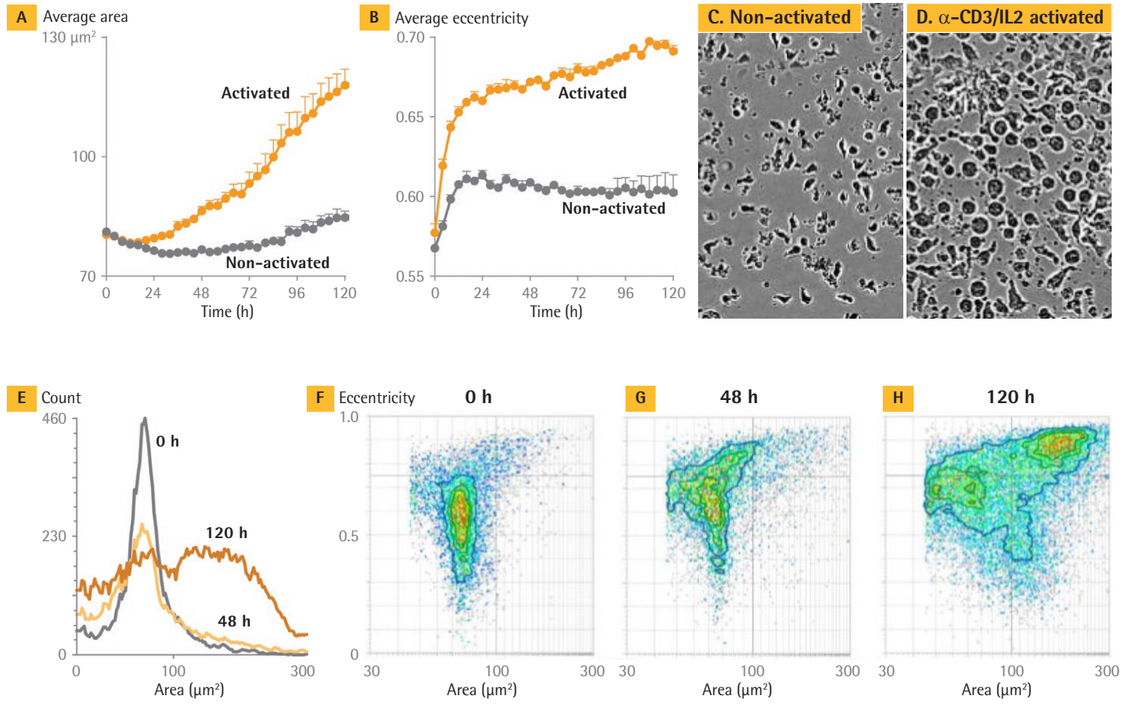
Immune cell activation and proliferation are fundamental for regulating immune responses and the extent to which they occur. IncuCyte® Immune Cell Activation, Proliferation and Cell Health Assays are an integrated solution for real-time visualization and automated analysis of immune cell proliferation, activation and cell health.

Key Advantages:

- **NEW!** Automatically generate label-free total cell counts using the IncuCyte S3 Cell-by-Cell Analysis Software Module – from over 2,000 assay wells in parallel!
- Automatically detect and quantify dynamic changes in cell morphology upon activation.
- Multiplex with IncuCyte cell health reagents for real-time analysis of apoptosis and cell death.

Observe and quantify dynamic changes in cell morphology

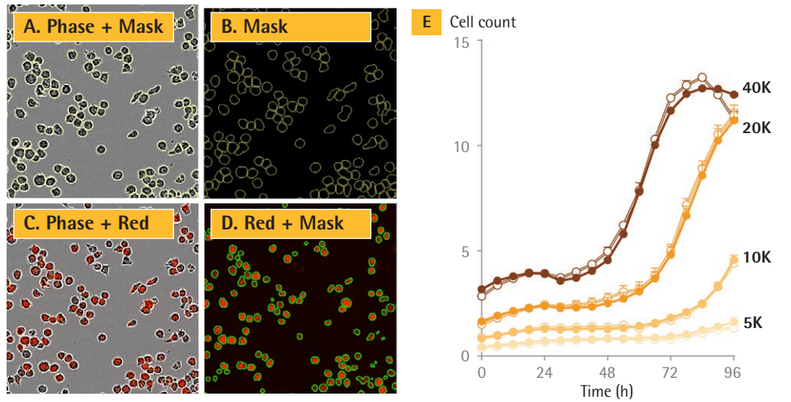
Use the power of live-cell analysis to quantify morphology changes associated with immune-cell activation using the IncuCyte® S3 Cell-By-Cell Analysis Software Module.



Enlargement and morphological change in activated T-cells.
PBMCs were treated with anti-CD3 and IL-2, or vehicle control, and monitored over time with the IncuCyte® S3 system. **(A-D)** Activation induced a time-dependent increase in average area and eccentricity (all cells). Note the change in eccentricity preceded the increase in area. The cell-by-cell area distribution **(E)** and density plots **(F-H)** highlight the increased heterogeneity over time following activation, and the appearance of a population of large cells with high eccentricity. Values shown are the mean ± SD of 4 wells.

Quantify immune cell proliferation in real-time

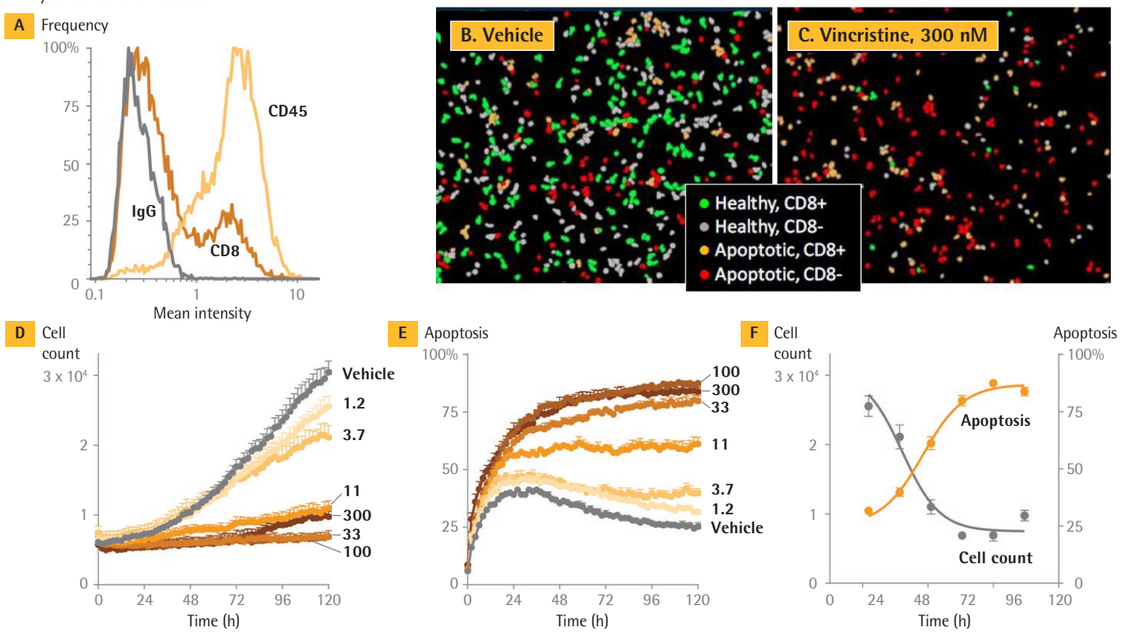
Automated label-free analysis of cell proliferation all within a physiologically relevant environment!



Label-free cell counting.
IncuCyte S3 Live-cell Analysis System (20x) images of NucLight Red-labeled Jurkat cells: **(A)** HD Phase + Phase Mask **(B)** Phase Mask only **(C)** Phase + Red Fluorescence **(D)** Phase Mask + Red Fluorescence. Scale bar = 50µM. **(E)** Time-course analysis of label free cell count (open symbols) and nuclear count (closed symbols) at different initial cell plating densities (5-40K, 96-well plate). Note the close similarity of values obtained by the phase object and nuclear label counting methods.

Uncover cell-specific cytotoxic treatment effects on immune cell health

Label a population of interest and multiplex with readouts of cell health using non-perturbing reagents and the IncuCyte® S3 Cell-By-Cell Analysis Software Module.



Measure cell health in sub populations of cells.
Human CD8+ T-lymphocytes are susceptible to vincristine-induced apoptotic cell death. **(A)** Frequency histograms of IncuCyte® FabFluor-488 conjugated to either -CD8, -CD45 and -IgG-labeled hPBMCs. **(B and C)** IncuCyte images showing color-coded subsets of healthy and apoptotic (IncuCyte® Annexin V+) CD8+ or CD8- cells (4 groups) following treatment with vincristine (300 nM) or vehicle (48 h). Vincristine induced a concentration- and time-dependent reduction in the proliferation of CD8+ cells **(D)** and a concomitant increase in apoptosis **(E)**. Concentration-response curves yielded IC50 or EC50 values of 4 nM for anti-proliferation and 8 nM for induction of apoptosis **(F)**.

IncuCyte® Chemotaxis Assays

Chemotaxis is the directional movement of cells in response to a chemical stimulus and is an essential component of immune responses, tumor metastasis, wound healing, and blood vessel formation. IncuCyte® Chemotaxis Migration and TEM Assays are an integrated solution for real-time visualization and automated analysis of chemotactic migration.

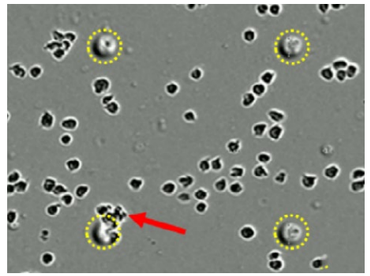
Visualize every cell throughout your experiment in real time

Gain deep phenotypic insight into cell movement and morphology with proprietary IncuCyte® ClearView Chemotaxis plates.

IncuCyte® ClearView 96-well Chemotaxis Plates. Combine the optical clarity of microfluidics devices with the throughput of transmembrane assays. Each well of an IncuCyte ClearView plate provides an optically clear surface for label-free imaging and analysis of chemotactic cell migration. 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.

Key Advantages:

- Visually monitor every cell in your experiment, assess morphology and gain phenotypic insight from images and movies.
- Measure label-free or labeled cell migration without fixing, staining or cell scraping steps.
- Investigate cell migration on biologically relevant surfaces or across an endothelial monolayer.



Analyze sensitive cells using a 96-well approach with high reproducibility

Capture cell migration or transendothelial migration in real time all from within your incubator.

SDF-1α mediated chemotaxis of human T cells.
(A) CD3/CD28 activated T-cells were seeded on an ICAM coated ClearView plate and migration toward SDF-1α was quantified by measuring the loss of cell area on the top of the membrane.
(B) Activated T-cells were also monitored for their ability to migrate through a HUVEC monolayer in response to SDF-1α chemoatrractant gradient. Optically clear IncuCyte ClearView plates allowed for the visualization of endothelial monolayer integrity and leukocyte diapedesis throughout the time course of the assay.

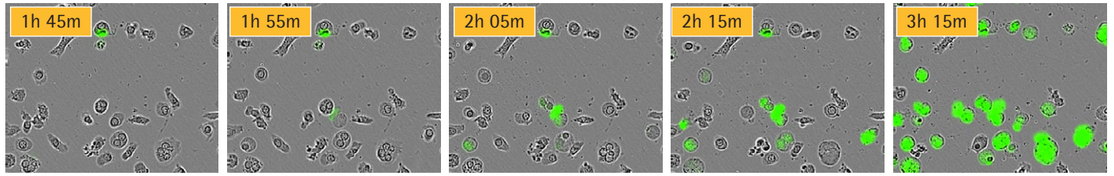
IncuCyte® NETosis Assay

Neutrophils are the first line of defense at the site of an infection and play an essential role in the innate immune system, employing multiple strategies to degrade and kill microbes, including the release of neutrophil extracellular traps (NETs). Automatically quantify neutrophils undergoing NETosis in real time with the IncuCyte® S3 Live-Cell Analysis System and IncuCyte® Cytotox Green Reagent.

Key Advantages:

- Simple mix-and-read protocols suitable for pharmacological screening – no washing, no fixing, no lifting.
- Automated analysis over the entire assay time course without removing sensitive cells from the incubator.
- Visualize the distinct morphological changes associated with NETosis.

HD Phase + Fluorescence



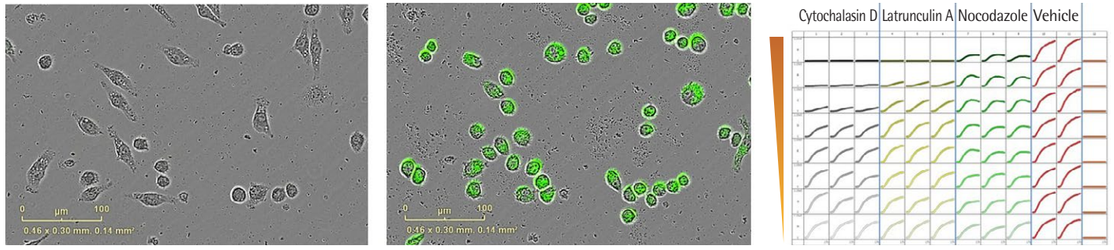
Validate NETosis with IncuCyte® HD Phase images.
IncuCyte images of differentiated HL-60 cells stimulated using PMA in the presence of IncuCyte® CytoTox Green reagent. As nuclear contents are moved to the plasma membrane and released, the network of extracellular fibers composed of DNA is bound by the Cytotox Green reagent, as seen by fluorescent enhancement overtime.

IncuCyte® Phagocytosis Assays

Scavenging and engulfing micro-organisms, apoptotic cells and tumor cells is a central role in host defense. The IncuCyte® pHrodo® Reagents and IncuCyte Live-Cell Analysis System enable real-time, automated analysis of phagocytosis inside your cell culture incubator.

Key Advantages:

- Visualize and validate dynamic phagocytic clearance by immune cells 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.



Real-time analysis and visualization of phagocytosis.
J774A.1 mouse macro- phages visualized engulfing IncuCyte® pHrodo® Green E. coli Bioparticles® which, on entering the acidic environment of the phagosome, increase in fluorescence. Images and movies enable confirmation of signal and provide insight into the dynamic morphological changes (e.g. formation of phagocytic cups) associated with phagocytosis. The signal was ablated by inhibitors of phagocytosis including cytochalasin D and nocodazole. 96-well plate view illustrates the time courses of phagocytosis and assay consistency.

Ordering information

Product	Description	Cat. No.
Immunocytochemistry		
IncuCyte® S3 Cell-By-Cell Analysis Software Module	1 each	9600-0031
IncuCyte® Mouse IgG2a FabFluor-488 Antibody Labeling Reagent	1 vial, 50 µg	4743
IncuCyte® Mouse IgG2b FabFluor-488 Antibody Labeling Reagent	1 vial, 50 µg	4744
IncuCyte® Mouse IgG1 FabFluor-488 Antibody Labeling Reagent	1 vial, 50 µg	4745
Chemotaxis		
IncuCyte® ClearView 96-Well Chemotaxis Plate	1 plate	4582
IncuCyte® ClearView 96-well Chemotaxis Plates	Case of 10 Plates	4648
IncuCyte® Chemotaxis Analysis Software Module	1 each	9600-0015
Immune Cell Activation, Proliferation, and Cell Health		
IncuCyte® S3 Cell-By-Cell Analysis Software Module	1 each	9600-0031
IncuCyte® Caspase-3/7 Green Apoptosis Assay Reagent	20 µL	4440
IncuCyte® Caspase-3/7 Red Apoptosis Assay Reagent	20 µL	4704
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® Cytotox Red Reagent for Counting Dead Cells	5 µL x 5	4632
IncuCyte® Cytotox Green Reagent for Counting Dead Cells	5 µL x 5	4633
IncuCyte® Mouse IgG2a FabFluor-488 Antibody Labeling Reagent	1 vial, 50 µg	4744
IncuCyte® Mouse IgG2b FabFluor-488 Antibody Labeling Reagent	1 vial, 50 µg	4745
IncuCyte® Mouse IgG1 FabFluor-488 Antibody Labeling Reagent	1 vial, 50 µg	4745
NETosis		
IncuCyte® Cytotox Green Reagent for Counting Dead Cells	5 µL x 5	4633
Phagocytosis		
IncuCyte® pHrodo® Red E. coli Bioparticles® for Phagocytosis	1 vial, 2 mg	4615
IncuCyte® pHrodo® Green E. coli Bioparticles® for Phagocytosis	1 vial, 2 mg	4616
IncuCyte® pHrodo® Red Zymosan Bioparticles for phagocytosis	1 vial, 1 mg	4617
IncuCyte® pHrodo® Green Zymosan Bioparticles® for Phagocytosis	1 vial, 1 mg	4618
IncuCyte® pHrodo® Red S. aureus Bioparticles® for Phagocytosis	1 vial, 2 mg	4619
IncuCyte® pHrodo® Green S. aureus Bioparticles® for Phagocytosis	1 vial, 2 mg	4620
IncuCyte® pHrodo® Red Cell Labeling Kit	1 kit	4649

Further Reading

See more exciting data, movies, application notes and scientific posters by visiting **www.essenbioscience.com/immunology**

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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**

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