SARTORIUS

Product Guide

Incucyte® Cytotox Dyes

For Detection of Cell Membrane Integrity Disruption

Product Information

Presentation, Storage and Stability
Incucyte® Cytotox Dyes are supplied as solution in dimethyl sulfoxide (DMSO), providing sufficient quantity for performing 500–1000 tests (1 test = 1 well of 96-well microtiter plate).

Upon receipt, the solution should be stored at -20° C or 4° C according to the table below and protected from light. When stored as described, the stock solutions will be stable for 6–12 months.

Product Name	Cat. No.	Ex. Max	Em. Max	Amount	Concentration	Storage	Stability
Compatible with Incucyte® Live-Cell Analysis Systems configured with a Green | Orange | NIR or Green | Red Optical Module							
Incucyte® Cytotox Green Dye	4633	491 nm	509 nm	5 x 5 µL	1 mM	-20° C	6-12 months
Compatible with Incucyte® Live-Cell Analysis Systems configured with a Green | Red Optical Module							
Incucyte® Cytotox Red Dye	4632	612 nm	631 nm	5 x 5 µL	1 mM	-20° C	6-12 months
Compatible with Incucyte® Live-Cell Analysis Systems configured with a Green| Orange | NIR or Orange | NIR Optical Module							
Incucyte® Cytotox NIR Dye	4846	665 nm	695 nm	1 x 100 µL	0.6 mM	4° C	6-12 months

Safety data sheet (SDS) information can be found on our website at **www.sartorius.com**

Background

Incucyte® Cytotox Dyes are highly sensitive nucleic acid dyes ideally suited to a simple mix-and-read, real-time quantification of cell death. Addition of the Incucyte® Cytotox Dyes to normal healthy cells is non-perturbing to cell growth or morphology and yields little or no intrinsic fluorescent signal. Once cells become unhealthy, the plasma membrane integrity diminishes, allowing entry of the Incucyte® Cytotox Dye and yielding a 100- to 1000-fold increase in fluorescence upon binding to deoxyribonucleic acid (DNA). With the Incucyte® integrated analysis software, fluorescent objects can be quantified, and background fluorescence minimized. These dyes have been validated for use with the Incucyte® Live-Cell Analysis System and enable real-time evaluation of cell membrane integrity and cell death in response to pharmacological agents and/or genetic and environmental factors. Furthermore, the Incucyte® Cytotox Dyes can be combined with the Incucyte® confluence metric, Incucyte® Annexin V Dyes, Incucyte® Nuclight Labeling Reagents, or Incucyte® Caspase 3/7 Dyes for multiplexed measurements of proliferation and apoptosis alongside cytotoxicity in a single well.

Recommended Use

We recommend that each vial of Incucyte® Cytotox Green or Red Dye is diluted to a stock concentration of 100 µM in full media or PBS. This may then be diluted further in full media for direct addition to cells seeded in a 96-well plate to yield a final concentration of 250 nM (e.g., 1:4000 dilution of the original green or red dye). Incucyte® Cytotox NIR Dye should be diluted to a final concentration of 0.6 µM (1:000 dilution) in full culture media for use. When used in an Incucyte® Live-Cell Analysis System, we recommend data collection every 2–3 hours.

Example Data

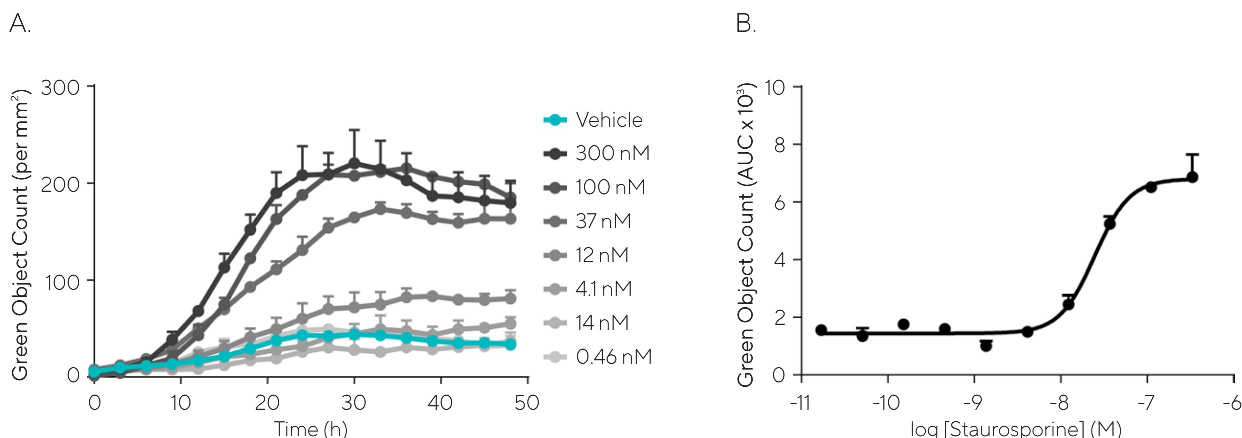


Figure 1: Concentration- and time-dependent increase of nucleic acid binding by Incucyte® Cytotox Green Dye following addition of Staurosporine to HT-1080 cells (adherent cells). (A) Time-course for the effect of Staurosporine on HT-1080 cell death as measured by Green Object Count. (B) Area under curve (AUC) analysis of the green fluorescent time-course data was used to generate a concentration response curve.

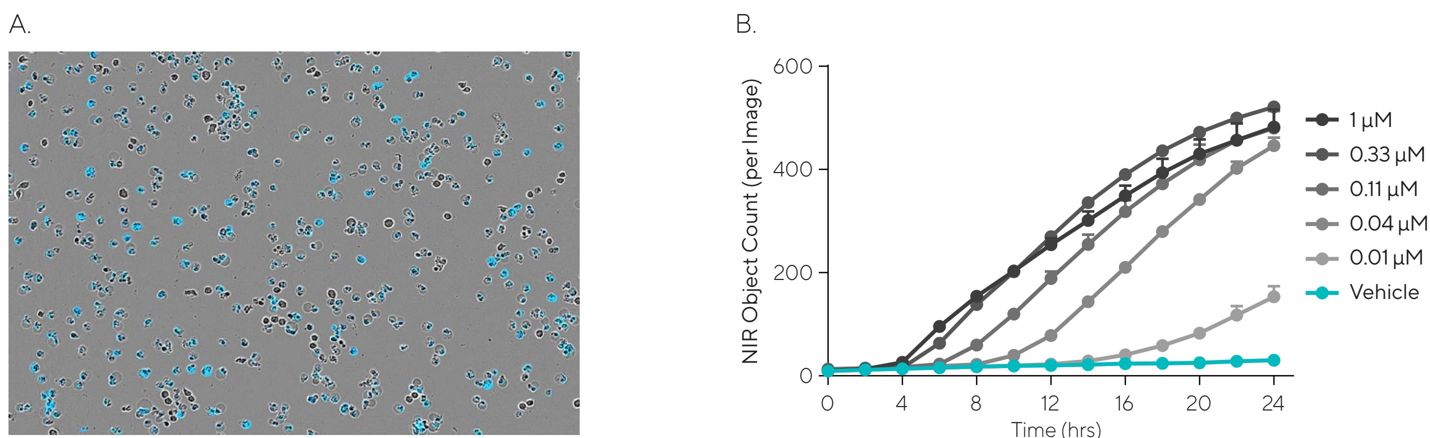
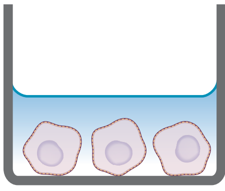


Figure 2: Evaluation of Camptothecin-induced Jurkat cell death (non-adherent cells) with Incucyte® Cytotox NIR Dye. (A) Representative image of Incucyte® Cytotox NIR stained dead Jurkat cells treated with 1 µM Camptothecin for 24 hours. (B) Time-course for the concentration-dependent effect of Camptothecin on Jurkat cell death as measured by NIR Object Count.

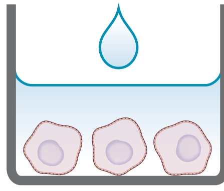
Adherent Cell Line Protocol Quick Guide

1. Seed cells



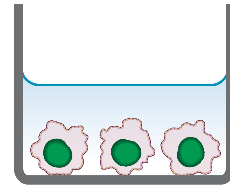
Seed adherent cells (100 µL/well) into a 96-well plate.

2. Prepare cytotoxicity reagent and treat cells



Prepare the desired treatments in medium containing Incucyte® Cytotox Dye and add treatment.

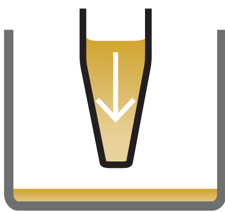
3. Live-cell imaging



Capture images every 2–3 hours (20X or 10X) in Incucyte® Live-Cell Analysis System.

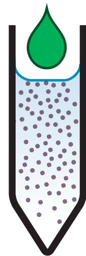
Non-Adherent Cell Line Protocol Quick Guide

1. Coat plate



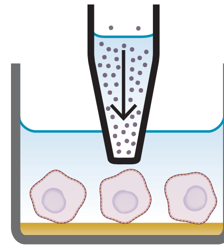
Coat plate with a 0.01% poly-L-ornithine solution.

2. Prepare cytotoxicity reagent and treatment



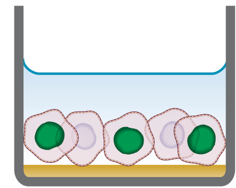
Dilute Incucyte® Cytotox Dye in media and prepare cell treatments.

3. Seed cells and add treatment



Seed cells (100 µL/well) into the coated 96-well plate. Immediately add Incucyte® Cytotox Dye ± treatments and triturate.

4. Live-cell imaging



Capture images every 2–3 hours (20X or 10X) in Incucyte® Live-Cell Analysis System.

Protocols and Procedures

Materials

- Incucyte® Cytotox Green Dye (Sartorius Cat. No. 4633) or
- Incucyte® Cytotox Red Dye (Sartorius Cat. No. 4632) or
- Incucyte® Cytotox NIR Dye (Sartorius Cat. No. 4846)
- Flat bottom tissue culture plate (e.g., Corning Cat. No. 3595, TPP Cat. No. 92096 for neuronal cell health)
- Complete cell culture media for cell line of choice
- Poly-L-ornithine (Sigma Cat. No. P4957)
  - Optional, for non-adherent cells

General Guidelines

- We recommend medium with low levels of riboflavin to reduce the green fluorescence background. EBM, F12-K, and Eagles MEM have low riboflavin (< 0.2 mg/L).
- Following cell seeding, place plates at ambient temperature for 30 minutes to ensure homogenous cell settling.
- Remove bubbles from all wells by gently squeezing a wash bottle (containing 70–100% ethanol with the inner straw removed) to blow vapor over the surface of each well.
- After placing the plate in the Incucyte® Live-Cell Analysis System, allow the plate to warm to 37° C for 30 minutes prior to scanning.
- If monitoring cytotoxicity in primary neuronal cultures, we recommend use of the Incucyte® Cytotox Red Dye to eliminate risk of green channel excitation issues in these sensitive cell types.
- When using Cytotox Dyes for single spheroids, please follow the Incucyte® Single Spheroid Assay protocol for optimal concentrations and procedure.

Adherent Cell Line Protocol

Seed Cells—Day 0

1. Seed your choice of cells (100 µL per well) at an appropriate density into a 96-well plate, such that by Day 1 the cell confluence is approximately 30%. The seeding density will need to be optimized for the cell line used; however, we have found that 1,000 to 5,000 cells per well (10,000–50,000 cells/mL seeding stock) are reasonable starting points.
   Note: For non-proliferating cell lines (e.g., rat forebrain neurons), we recommend seeding at 15,000 to 30,000 cells per well, and culturing for 14 days for the neural network to establish, prior to evaluating cytotoxicity.
2. Place the plate back to the incubator and culture overnight. You can also monitor cell growth using the Incucyte® Live-Cell Analysis System to capture phase contrast images every 2 hours.

Prepare Cytotox Dye and Treat Cells—Day 1

1. Dilute Cytotox Dye to 1X final concentration in desired medium formulation.
   Note: All test agents will be diluted in this dye containing medium, so make up a volume that will accommodate all treatment conditions. The volumes and dilutions added to cells may be varied; however, a volume of 100 µL per well is generally sufficient for the duration of the assay.
2. Prepare treatments with dye-containing medium from step 1 at desired 1X concentrations.
3. Remove the cell plate from the incubator and aspirate off growth medium.
4. Add treatments and controls (100 µL per well) to appropriate wells of the 96-well plate.
   Note: Alternatively, the dye and treatments can be prepared at 2X final concentrations, added directly to cells on top of the culture media, resulting in 200 µL volume per well.

Live-Cell Imaging

Place the cell plate into the Incucyte® Live-Cell Analysis System and allow the plate to warm to 37° C for 30 minutes prior to scanning.

1. Objective: 10X or 20X
2. Channel selection: Phase Contrast and appropriate fluorescence channel
3. Scan type: Standard (2–4 images per well)
   Scan interval: Typically, every 2 hours, until your experiment is complete
   Note: For neuronal cultures we recommend scanning every 6 to 12 hours to minimize risk of phototoxicity.

Non-Adherent Cell Line Protocol

Coat Plate

1. Add 50 µL of 0.01% poly-L-ornithine solution per well to a 96-well plate.
2. Incubate the plate for 1 hour at ambient temperature.
3. Remove solution from wells and allow the plate to dry for 30–60 minutes prior to cell addition.

Prepare Cytotox Dye and Treatments

1. Prior to cell seeding, dilute Cytotox Dye to 2X final concentration in desired medium formulation.
   Note: All test agents will be diluted in this dye containing medium, so make up a volume that will accommodate all treatment conditions. The volumes and dilutions added to cells may be varied; however, a volume of 100 µL per well is generally sufficient for the duration of the assay.
2. Prepare cell treatments at 2X final assay concentration in enough dye-containing cell culture medium to achieve a volume of 100 µL per well.

Seed Cells and Add Prepared Treatments

1. Seed your choice of cells (100 µL per well) at an appropriate density into a 96-well plate in culture medium. The seeding density will need to be optimized for the cell line used; however, we have found that 5,000 to 25,000 cells per well (50,000–250,000 cells/mL seeding stock) are reasonable starting points.
2. Immediately add treatments and controls (100 µL per well) to appropriate wells of the 96-well plate containing cells. Triturate wells to appropriately mix the treatment to ensure cell exposure at 1X.

Live-Cell Imaging

Place the cell plate into the Incucyte® Live-Cell Analysis System and allow the plate to warm to 37° C for 30 minutes prior to scanning.

1. Objective: 10X or 20X
2. Channel selection: Phase Contrast and appropriate fluorescence channel
3. Scan type: Standard (2–4 images per well)
   Scan interval: Typically, every 2 hours, until your experiment is complete

Optional Cytotoxic Index

A cytotoxic index can be calculated on Incucyte® Live-Cell Analysis System using the Incucyte® Cell-by-Cell Analysis Software Module (Cat. No. 9600-0031). This enables individual cell identification and subsequent classification into subpopulations based on properties including fluorescence intensity. These subpopulations can then be expressed as a percentage of the total population to generate the cytotoxic index. To use this module the following settings should be used:

a. Scan type: Standard/Adherent or Non-Adherent Cell-by-Cell
b. Objective: 10X (for adherent cells) or 20X (for non-adherent cells)

For further details of this analysis module and its application see: **www.essenbioscience.com/cell-by-cell**

Multiplexing Optimization

When multiplexing with multiple fluorescent reagents, spectral unmixing may be required to account for signal that has been contributed from one of the given channels. Spectral unmixing values must be applied prior to running an analysis job.

- **Cytotox Green**: No spectral unmixing required
- **Cytotox Red**: 1–2% recommended to remove Red contributing to Green
- **Cytotox NIR**:
  - 7-10% recommended to remove NIR contributing to Orange;
  - 2% recommended to remove NIR contributing to Green

A complete suite of cell health applications is available to fit your experimental needs. Find more information at **www.sartorius.com/incucyte**

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