SARTORIUS

Product Guide

Incucyte® Caspase-3/7 Dyes

For Detection of Apoptosis in Live Cells

Product Information

Presentation, Storage and Stability

The Incucyte® Caspase-3/7 Dyes are supplied as single vial solutions in dimethyl sulfoxide (DMSO), with each vial providing sufficient quantity for performing 100–200 tests (1 test = 1 well of a 96-well microtiter plate). Upon receipt, the solution should be stored at 4° C.

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® Caspase-3/7 Green Dye	4440	500 nm	530 nm	20 µL	5 mM	4° C	6 months
Compatible with Incucyte® Live-Cell Analysis Systems configured with a Green | Red optical module							
Incucyte® Caspase-3/7 Red Dye	4704	630 nm	650 nm	20 µL	0.5 mM	4° C	6 months
Compatible with Incucyte® SX5 Live-Cell Analysis Systems configured with the SX5 Metabolism Optical Module							
Incucyte® Caspase-3/7 for Metabolism Dye	4776	528 nm	563 nm	20 µL	1 mM	4° C	6 months

Background

The Incucyte® Caspase-3/7 Dyes for Apoptosis couple the activated caspase-3/7 recognition motif (DEVD) to a DNA intercalating dye and are ideally suited to the mix-and-read, real-time quantification of cells undergoing caspase-3/7 mediated apoptosis. Addition of the Incucyte® Caspase-3/7 Dyes to normal healthy cells is non-perturbing to cell growth and morphology. When added to tissue culture medium, the inert, non-fluorescent substrate crosses the cell membrane where it is cleaved by activated Caspase-3/7 resulting in the release of the DNA dye and fluorescent staining of the nuclear DNA. With the Incucyte® integrated analysis software, fluorescent objects can be quantified and background fluorescence minimized. These reagents have been validated for use with the Incucyte® Live-Cell Analysis System and enables the real-time evaluation of cell apoptosis induced by pharmacological agents and | or genetic and environmental factors.

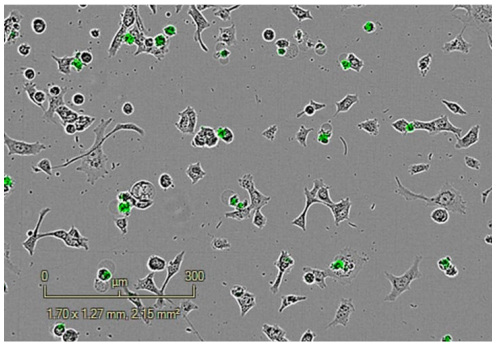
Recommended Use

We recommend diluting the Incucyte® Caspase-3/7 Dyes in complete culture media and adding directly to cells in culture, as per the guidelines below:

- Caspase-3/7 Green: 1:1000 in culture media, for a final assay concentration of 5 µM
- Caspase-3/7 Red: Optimize final assay concentration by diluting the reagent 1:200 in complete medium, then make a series of 2-fold dilutions for final assay concentrations of 2.5, 1.25 and 0.5 µM
- Caspase-3/7 for Metabolism: 1:1000 in culture media, for a final assay concentration of 1 µM

Example Data

A. HT-1080 Cells



B. Caspase-3/7

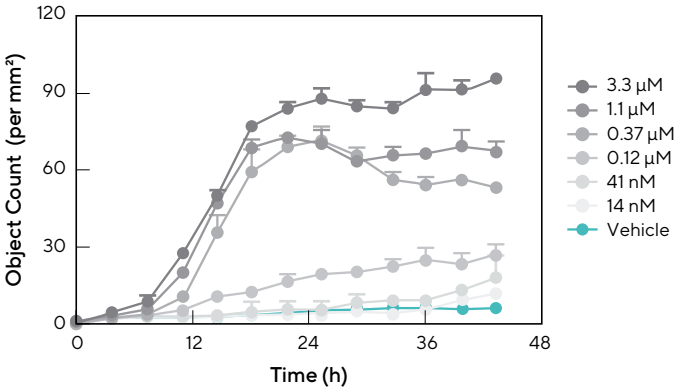
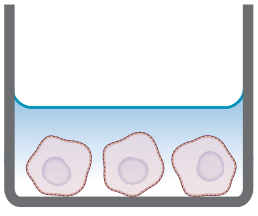


Figure 1. (A) Representative image of fibrosarcoma (HT-1080) cells treated with camptothecin in the presence of Caspase-3/7 Green Dye. (B) Time course of apoptosis of HT-1080 fibrosarcoma cells in the presence of 5 µM Caspase-3/7 Green Dye and increasing concentrations of camptothecin.

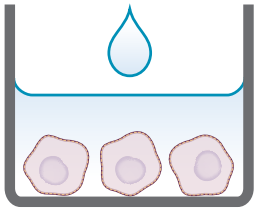
Quick Guide

1. Seed cells



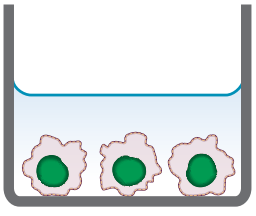
Seed cells (100 µL/well) into a 96-well plate and incubate overnight.

2. Prepare apoptosis reagent and treat cells



Prepare the desired treatments at 1X in medium containing Incucyte® Caspase 3/7 Dyes and add treatment.

3. Live-cell fluorescent imaging



Capture images every 2–3 hours (20X or 10X) in the Incucyte® Live-Cell Analysis System for 24–120 hours. Analyze using integrated software.

Protocols and Procedures

Materials

- Incucyte® Caspase-3/7 Dye
- Flat bottom tissue culture plate (e.g., Corning Cat. No. 3595)
- 0.01% Poly-L-ornithine solution (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, F-12K, and Eagles MEM have low riboflavin (<0.2 mg/L). DMEM and RPMI have high riboflavin (>0.2 mg/L).
- Following cell seeding, place plates at ambient temperature (15 minutes for adherent cell lines and 45 minutes for non-adherent cell lines) 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.

Adherent Cell Line Protocol

Seed Cells

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. 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: Monitor cell growth using the Incucyte® Live-Cell Analysis System to capture phase contrast images every 2 hours and analyze using the integrated confluence algorithm.

Prepare Apoptosis Reagent and Treat Cells

2. Dilute Caspase-3/7 Dye in full culture medium:
   a. Caspase-3/7 Green: dilute 1:1000 (final assay concentration is 5 µM)
   b. Caspase-3/7 Red: Optimize final assay concentration by diluting the reagent 1:200 in complete medium, then make a series of 2-fold dilutions for final assay concentrations of 2.5, 1.25 and 0.5 µM
   c. Caspase-3/7 for Metabolism: dilute 1:1000 (final assay concentration is 1 µM).

   Note: All treatments will be performed in this medium containing the Caspase-3/7 Dye, thus prepare a volume that will accommodate all treatment conditions. The volumes/dilutions added to cells may be varied. A volume of 100 µL per well is generally sufficient for the duration of the assay.

3. Remove the cell plate from the incubator and aspirate off culture medium.
4. Add treatments and controls to the appropriate wells of the 96-well plate.

Live-Cell Imaging

5. Place the cell plate into the Incucyte® Live-Cell Analysis System to monitor apoptosis using the appropriate fluorescent channel.
   a. Objective: 10x or 20x
   b. Channel selection: Phase Contrast and Fluorescence (depending on apoptosis reagent used)
   c. Scan type: Standard (2-4 images per well)
   d. Scan interval: Typically, every 2 hours, until your experiment is complete

Non-Adherent Cell Line Protocol

Coat Plate

1. Prepare recommended coating matrix of either 0.01% poly-L-ornithine solution (Sigma Cat No. P4957) or 5 µg/mL fibronectin (Sigma Cat No. A7906) diluted in 0.1% BSA.
2. Aliquot 50 µL of coating matrix per well and incubate plate for 1 hour at ambient temperature.
3. Remove solution from wells and allow plates to dry for 30-60 minutes prior to cell addition.

Prepare Apoptosis Reagent and Treat Cells

4. Dilute Caspase-3/7 Dye in full culture medium:
   a. Caspase-3/7 Green: dilute 1:1000 (final assay concentration is 5 µM)
   b. Caspase-3/7 Red: Optimize final assay concentration by diluting the reagent 1:200 in complete medium, then make a series of 2-fold dilutions for final assay concentrations of 2.5, 1.25 and 0.5 µM.
   c. Caspase-3/7 for Metabolism: dilute 1:1000 (final assay concentration is 1 µM)

   Note: All treatments and cell seeding stocks will be prepared in this medium containing the Caspase-3/7 Dye, thus prepare a volume that will accommodate all treatment conditions. The volumes/dilutions added to cells may be varied. A volume of 200 µL per well is generally sufficient for the duration of the assay.

5. Prepare cell treatments at 2x final assay concentration in enough cell culture medium containing the Caspase 3/7 Dye to achieve a volume of 100 µL per well.

Seed Cells and Add Treatments

6. Seed your choice of cells (100 µL per well) at an appropriate density into a 96-well plate in medium containing the Caspase-3/7 Dye. The seeding density will need to be optimized for the cell line used. We have found that 5,000 to 25,000 cells per well (50,000–250,000 cells/mL seeding stock) are reasonable starting points.
7. Immediately add treatments and controls to appropriate wells of the 96-well plate containing cells.
8. Triturate to mix the treatments with cells evenly.

Live-Cell Imaging

9. Place the cell plate into the Incucyte® Live-Cell Analysis System to monitor apoptosis using the appropriate fluorescent channel.
   e. Objective: 10x or 20x
   f. Channel selection: Phase Contrast and Fluorescence (depending on apoptosis reagent used)
   g. Scan type: Standard (2-4 images per well)
   h. Scan interval: Typically, every 2 hours, until your experiment is complete

Optional Apoptotic Analysis

An apoptotic 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. To use this module, the following settings should be used:

a. Scan type: Standard/Adherent Cell-by-Cell
b. Objective: 10x

For further details of this analysis module and its application see **www.sartorius.com/en/applications/life-science-research/cell-analysis/live-cell-assays/cell-movement-morphology/morphological-analysis/cell-by-cell-analysis.**

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