

# IncuCyte<sup>®</sup> Cytotox Reagents for Counting Dead Cells

Catalog numbers: 4632 and 4633

## Presentation, storage and stability

IncuCyte<sup>®</sup> Cytotox Reagents are supplied as five vials (5  $\mu$ L per vial) of 1 mM solution in dimethylsulfoxide (DMSO), with each vial providing sufficient quantity for performing 100-200 tests (1 test = 1 well of 96-well microtiter plate). Upon receipt, the solution should be stored at -20°C in a desiccator. When stored as described the stock solutions will be stable for 6 - 12 months.

## Background and intended use

IncuCyte Cytotox Reagents are highly sensitive cyanine nucleic acid dyes ideally suited to a simple mix-and-read, real-time quantification of cell death. Addition of the IncuCyte Cytotox Reagents to normal healthy cells is non-perturbing to cell growth or morphology and yields little or no intrinsic fluorescent signal. Once cells become unhealthy plasma membrane integrity diminishes, allowing entry of the IncuCyte Cytotox Reagent and yielding a 100-1000-fold increase in fluorescence upon binding to deoxyribonucleic acid (DNA). With the IncuCyte<sup>®</sup> integrated analysis software fluorescent objects can be quantified and background fluorescence minimized.

These pre-aliquoted reagents have been validated for use with the IncuCyte<sup>®</sup> 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 Reagents can be combined with the IncuCyte<sup>®</sup> confluence metric, our IncuCyte<sup>®</sup> Annexin V reagents, our range of IncuCyte<sup>®</sup> NucLight nuclear labeling reagents, or the IncuCyte<sup>®</sup> Caspase 3/7 Reagent for multiplexed measurements of proliferation and apoptosis alongside cytotoxicity in a single well.

## Recommended use

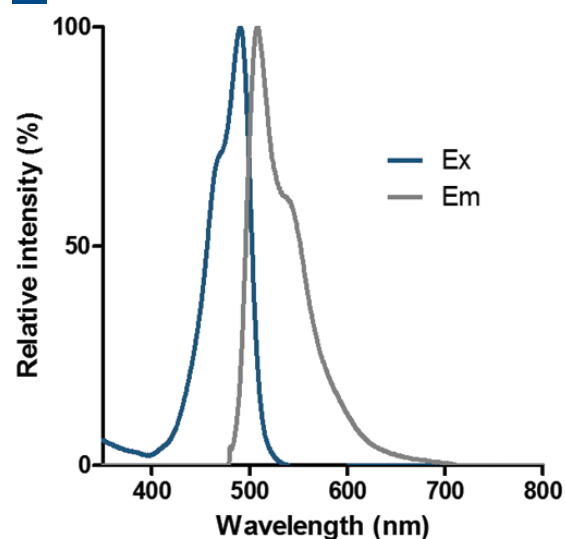
We recommend that each vial of IncuCyte<sup>®</sup> Cytotox Reagent is diluted to a stock concentration of 100  $\mu$ M in full media or PBS. This may then be diluted further in full media for direct addition to cells seeded in a 96-well plate to yield a final concentration of 250 nM. When used in an IncuCyte<sup>®</sup> live-cell analysis system, we recommend data collection every 2-3 hours.

Please see the relevant protocol published on our website:  
[essenbioscience.com/cytotox](http://essenbioscience.com/cytotox)

## Safety data sheet (SDS) information

The SDS can be found on our website:  
[essenbioscience.com/cytotox](http://essenbioscience.com/cytotox)

**A** Cytotox Green Reagent



**B** Cytotox Red Reagent

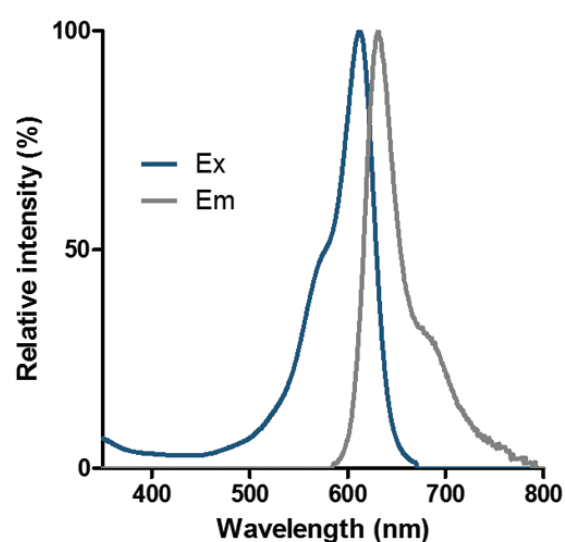
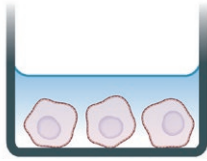


Figure 1. Excitation and emission spectra for the (A) Green and (B) Red fluorophores bound to double-stranded calf thymus DNA in aqueous solution.

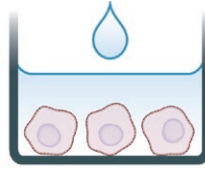
Quick guide

**1** SEED TARGET CELLS



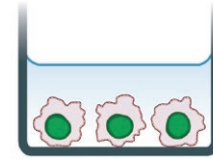
Seed adherent or non-adherent cells (100 µL/well) into a 96-well plate.

**2** PREPARE CYTOTOXICITY REAGENT AND TREAT CELLS



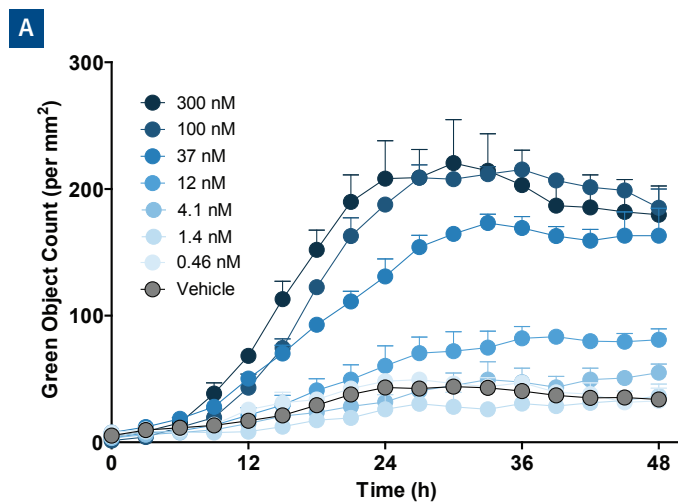
Prepare the desired treatments at 1x in medium containing IncuCyte<sup>®</sup> Cytotoxicity Reagent and add treatment.

**3** LIVE CELL FLUORESCENT IMAGING

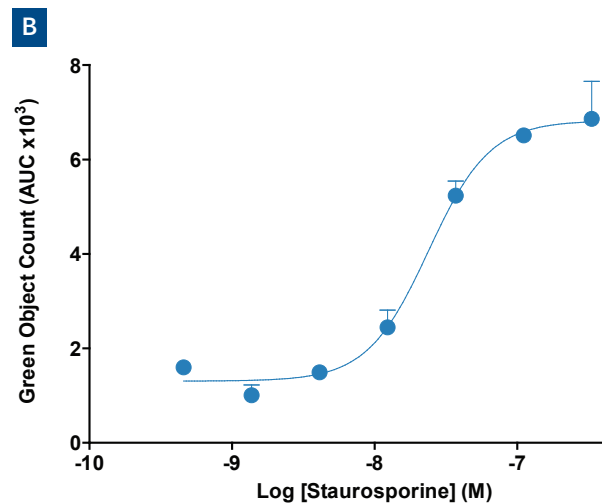


Capture images every 2-3 hours (20x or 10x) in IncuCyte<sup>®</sup> for 24 to 120 hours. Analyze using integrated software.

**Figure 2.** Concentration- and time-dependent increase of nucleic acid binding by IncuCyte<sup>®</sup> Cytotox Green Reagent following addition to HT-1080 cells treated with the protein kinase inhibitor, Staurosporine.



(A) Time-course for the effects of Staurosporine on HT-1080 cell death.



(B) Area under curve (AUC) analysis of green fluorescent time-course data used to generate concentration response curve for the effect of Staurosporine on HT-1080 cell death (Green object Count per mm<sup>2</sup>). Average AUC values were used to calculate EC<sub>50</sub> values.

**FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE.**

Product	Cat No.	Amount	Ex. maxima	Em. maxima
IncuCyte™ Cytotox Red Reagent	4632	5 µL x 5	612 nm	631 nm
IncuCyte™ Cytotox Green Reagent	4633	5 µL x 5	491 nm	509 nm