IncuCyte® Caspase-3/7 Reagent for Apoptosis
Catalog number: 4440

Presentation, storage and stability
The IncuCyte® Caspase-3/7 Reagent is supplied as one vial (20 µl) of 5 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 4°C. When stored as described the stock solutions will be stable for at least 6 months.

Background and intended use
The IncuCyte Caspase-3/7 Reagent for Apoptosis couples the activated caspase-3/7 recognition motif (DEVD) to a DNA intercalating dye and is 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 Reagent 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 green fluorescent staining of the nuclear DNA. With the IncuCyte® integrated analysis software, fluorescent objects can be quantified and background fluorescence minimized.

This reagent has 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. Furthermore, our IncuCyte Caspase-3/7 Reagent for Apoptosis can be combined with the IncuCyte® system confluence metrics (phase), our range of IncuCyte® NucLight nuclear labeling reagents, or the IncuCyte® Cytotox Red Reagent to quantify cell proliferation or cytotoxicity alongside apoptosis in a single well.

Recommended use
We recommend that IncuCyte Caspase-3/7 Reagent be used at a final concentration of 5 µM (1:1000) in growth media and added directly to cells in culture. When used in an IncuCyte® live-cell analysis system, we recommend data collection every 2-3 hours.

Please see the relevant protocol published on our website: essenbioscience.com/apoptosis

Safety data sheet (SDS) information
Download the SDS from our website: essenbioscience.com/apoptosis

Figure 1. IncuCyte Caspase-3/7 Reagent

Excitation and emission spectra of enzymatically-cleaved IncuCyte Caspase 3/7 reagent in the presence of dsDNA. Absorption/emission maxima of IncuCyte Caspase 3/7 reagent bound to DNA: 500/530 nm.
Quick guide

1. SEED CELLS
   - Seed cells (100 µL/well) into a 96-well plate.

2. PREPARE APOPTOSIS REAGENT AND TREAT CELLS
   - Prepare the desired treatments at 1x in medium containing IncuCyte Caspase-3/7 or Annexin V Reagents and add treatment.

3. LIVE CELL FLUORESCENT ANALYSIS
   - Capture images every 2-3 hours (20x or 10x) in the IncuCyte® System. Analyze using integrated software.

**Figure 2.** Concentration dependent activation of the IncuCyte Caspase-3/7 Reagent by the DNA topoisomerase I inhibitor camptothecin in HT-1080 fibrosarcoma cells.

(A) Time course of apoptosis in the presence of increasing concentrations of camptothecin. Apoptosis has been quantified as the number of green fluorescent caspase-3/7 active objects for each time point.

(B) Concentration response curve to camptothecin. Area under the curve (AUC) values have been determined for the time course shown in panel A.

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<table>
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<tr>
<th>Product</th>
<th>Cat No.</th>
<th>Amount</th>
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<th>Em. maxima</th>
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<td>20 µl</td>
<td>500 nm</td>
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