IncuCyte® Annexin V Reagents for Apoptosis
Catalog numbers: 4641 and 4642

Presentation, storage and stability
IncuCyte® Annexin V Reagents are supplied as lyophilized solid in sufficient quantity capable of performing 100-200 tests (1 test = 1 well of 96-well microtiter plate). The lyophilized solid should be stored at -20°C and once solubilized, the solution should be stored at +4°C and protected from light. When stored as described the lyophilized solid will be stable for at least 2 years and the solution for at least 1 week.

Background and intended use
The IncuCyte Annexin V Reagents are specially formulated highly-selective cyanine-based fluorescent dyes ideally suited to a simple mix-and-read, real-time quantification of apoptosis. Addition of the IncuCyte Annexin V Reagents to normal healthy cells is non-perturbing to cell growth or morphology and yields little or no intrinsic fluorescent signal. Once cells become apoptotic, plasma membrane phosphatidylserine (PS) asymmetry is lost leading to exposure of PS to the extracellular surface and binding of the IncuCyte Annexin V Reagent, yielding a bright and photostable fluorescent signal. With the IncuCyte® integrated analysis software fluorescent objects can be quantified and background fluorescence minimized.

These pre-aliquoted reagents have been specially formulated and validated for use with the IncuCyte® live-cell analysis system and enable real-time evaluation of cell membrane integrity and apoptosis in response to pharmacological or biological agents and/or genetic and environmental factors. Furthermore, the IncuCyte® Annexin V Reagents can be combined with the IncuCyte® confluence metric, IncuCyte® Caspase-3/7 Reagent, IncuCyte® NucLight™ nuclear labeling reagents or IncuCyte® Cytotox Reagents for multiplexed measurements of apoptosis, cell proliferation or cytotoxicity in every assay well.

Recommended use
We recommend that the IncuCyte Annexin V Reagents are solubilized by adding 100 µL of full media or PBS. The reagents may then be diluted in full media containing at least 1 mM CaCl₂ for direct addition to cells seeded in a 96-well plate to yield a final dilution of 1:200. 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
The SDS can be found on our website:
essenbioscience.com/apoptosis

Figure 1. Excitation and emission spectra for the (A) Annexin V Green and (B) Annexin V Red fluorophores conjugated to goat anti-mouse IgG in PBS (pH 7.4).
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.

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**Figure 2.** Concentration- and time-dependent increase of PS binding by IncuCyte Annexin V Red Reagent following addition to Jurkat human T-cell leukemia cells treated with the topoisomerase inhibitor, camptothecin.

(A) Time-course for the effects of camptothecin on Jurkat cell death (Red Object Confluence %) presented as the mean ± SEM, n=3 wells.

(B) Concentration response curve to camptothecin. Area under the curve (AUC) values have been determined from the time-course shown in panel A (0-36 hours) and are presented as the mean ± SEM, n=3 wells. Average AUC values were used to calculate pIC50 values (camptothecin pIC50 = 8.01).

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<table>
<thead>
<tr>
<th>Product</th>
<th>Cat No.</th>
<th>Amount</th>
<th>Ex. maxima</th>
<th>Em. maxima</th>
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<td>IncuCyte® Annexin V Red Reagent</td>
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<td>100 tests</td>
<td>593 nm</td>
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