IncuCyte™ Annexin V Reagents for Apoptosis

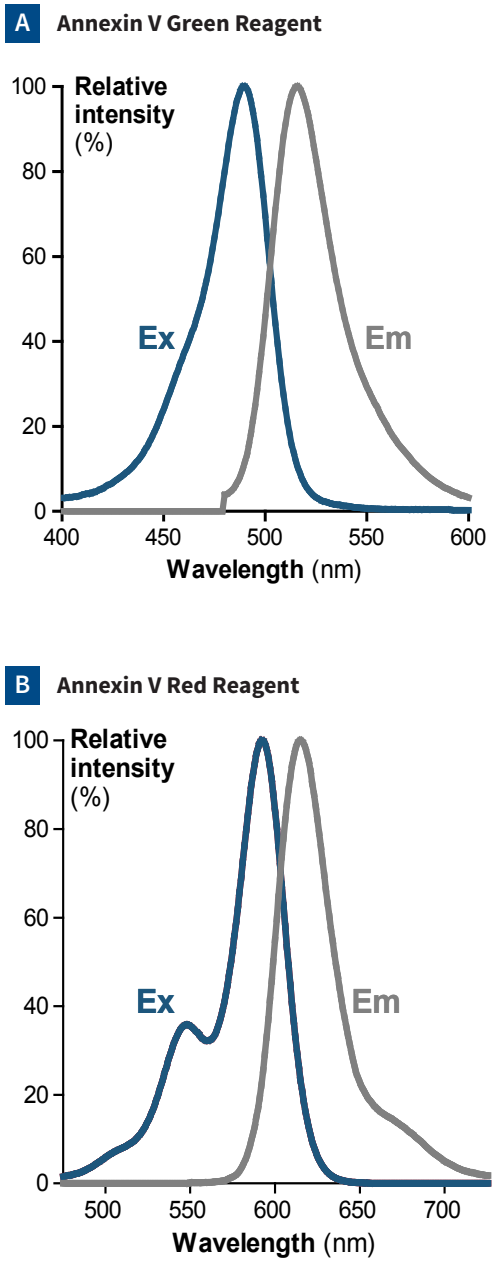


Figure 1. Excitation and emission spectra for the **(A)** Annexin V Green and **(B)** Annexin V Red fluorophores conjugated to goat anti-mouse IgG in PBS (pH 7.4).

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 ZOOM® 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® ZOOM live cell imaging 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 ZOOM® 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 $CaCl_2$ for direct addition to cells seeded in a 96-well plate to yield a final dilution of 1:200. When used in an IncuCyte ZOOM® live cell imaging system we recommend data collection every 2-3 hours.

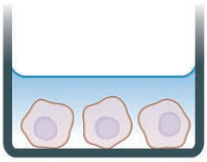
Please see the relevant protocol published on our website:
essenbioscience.com/apoptosis

Safety data sheet (SDS) information

The SDS can be found on our website:
essenbioscience.com/apoptosis

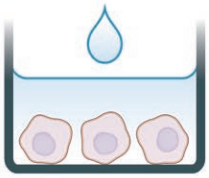
Quick guide

1 SEED TARGET CELLS



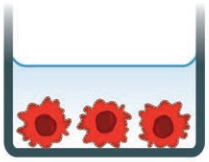
Cell Seeding
Seed adherent or suspension cells (100 µL/well, 1 x10^3 to 1 x10^4 cells/well) into a 96-well plate and leave to adhere or settle overnight.

2 TREAT CELLS AND ADD INCUCYTE™ ANNEXIN V REAGENT



Sample Treatment and IncuCyte™ Annexin V Reagent Addition
Prepare the desired treatments at 3x final assay concentration in diluted IncuCyte™ Annexin V Reagents for Apoptosis. Add to cells (50 µL/well).

3 LIVE CELL FLUORESCENT IMAGING



Automated Imaging and Quantitative Analysis
Capture images every 2-3 hours (20x or 10x) in IncuCyte® ZOOM for 2 to > 120 hours. Analyze using integrated software.

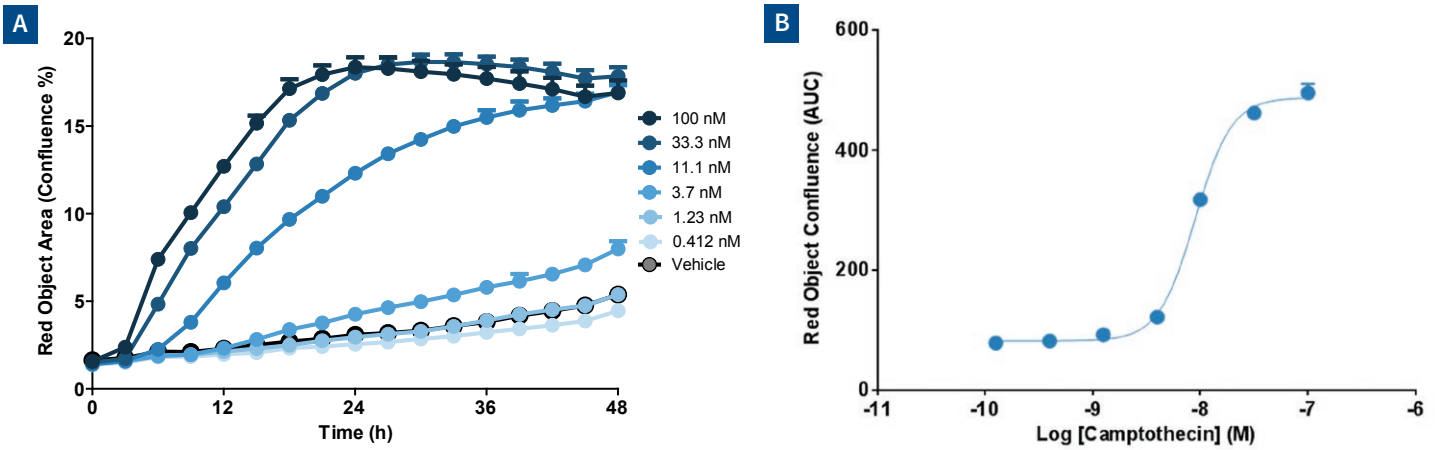


Figure 2. Concentration- and time-dependent increase of PS binding by IncuCyte™ Annexin V Red Reagent following addition to Jurkat human T-cell leukemia cells treated with the topoisomerase inhibitor, camptothecin. (A) Time-course for the effects of camptothecin on Jurkat cell death (Red Object Confluence (%) 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 pIC_{50} values (camptothecin pIC_{50} = 8.01).

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

Product	Cat No.	Amount	Ex. maxima	Em. maxima
IncuCyte™ Annexin V Red Reagent	4641	100 tests	593 nm	614 nm
IncuCyte™ Annexin V Green Reagent	4642	100 tests	490 nm	515 nm

Product label licence

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