IncuCyte® Annexin V NIR Reagent for Apoptosis

Catalog number: 4768

Product Information

Presentation, storage and stability

IncuCyte® Annexin V NIR Reagent is supplied as a 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 NIR Reagent is a specially formulated highly-selective cyanine-based fluorescent dye ideally suited to a simple mix-and-read, real-time quantification of apoptosis. Addition of the IncuCyte Annexin V NIR Reagent 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 NIR Reagent, yielding a bright and photostable fluorescent signal. With the IncuCyte® integrated analysis software fluorescent objects can be quantified and background fluorescence minimized.

This pre-aliquoted reagent has been specially formulated and validated for use with the IncuCyte® S3 Live-Cell Analysis Systems for Neuroscience (Cat. No. 4763) configured with an Orange/NIR Optical Module. The IncuCyte Annexin NIR Reagent is **NOT** compatible with instruments configured with a Green/Red Optical Module (ie, Cat. No. 4647). This reagent enables 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 NIR Reagent can be combined with the IncuCyte® confluence metric for multiplexed measurements of apoptosis and cell proliferation in every assay well.

Recommended use

We recommend that the IncuCyte Annexin V NIR Reagent is solubilized by adding 100 µL of full media or PBS. The reagent 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 multiplexing with an orange fluorescent reagent, we recommend a spectral unmixing value of 2% NIR removed from the Orange channel. If bleed through into the Orange channel is still observed, an optimization assay should be conducted to determine the optimal Annexin NIR concentration to use. Please see full details on spectral unmixing and optimization on page 2.

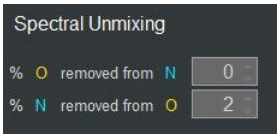
Multiplexing Optimization Protocol

Orange and NIR Multiplexing

When multiplexing orange and NIR fluorescent reagents, it is possible for the NIR fluorescence to bleed into the Orange channel. Spectral unmixing can be used within the software to remove this bleed through signal.

When setting up a multiplexed experiment for the first time, including wells containing only the NIR reagent is recommended. For the Annexin V NIR reagent, 2% NIR should be removed from Orange using the spectral unmixing tool as shown in Figure 1. There should be minimal orange fluorescence observed in the Annexin V NIR only control wells after spectral unmixing is applied.

Figure 1. 2% spectral unmixing of NIR removed from Orange



In some cases, the Annexin NIR signal may have a very high intensity and still be visible in the Orange channel, even after applying spectral unmixing. In this case, it may be necessary to dilute the reagent for use in a multiplex assay. The protocol below can be used to determine the optimal Annexin NIR reagent concentration.

Annexin NIR Concentration Optimization for Multiplex Assays

When optimizing Annexin NIR concentration for multiplexing, we recommend performing a serial dilution of the Annexin V NIR concentration independently in order to assess the effectiveness of spectral unmixing.

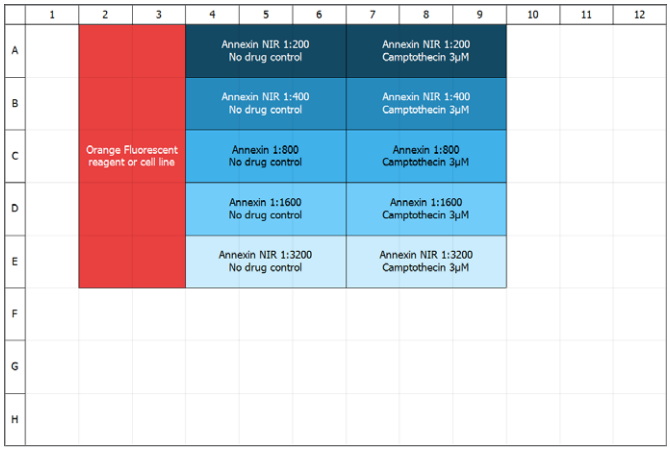
A high concentration of a positive control compound, such as Camptothecin, should be used to induce cell death and ensure that a robust Annexin V NIR signal is observed.

Wells containing only the orange reagent or cell line to be multiplexed should be included. This allows the use of vessel autoscaling to compare the intensity of Annexin V NIR bleed through to the desired orange signal.

Example experimental procedures:

- Begin by plating 100 µL of cell suspension into Columns 2-9 as shown in the plate map shown in Figure 2.
- Using a separate compound microplate, prepare reagent(s) and compound(s) to be added to cells. Add orange fluorescent reagent or cell lines of choice to Columns 2-3 only.
- Solubilize Annexin V NIR in 100 µL of media, then dilute 1:50 to a volume of 5 mL.
- Add 120 µL of Annexin V NIR solution into Wells A4 to A9 of the compound microplate.
- Add 60 µL of media into Rows B to E in Columns 4-9 and perform a 2-fold serial dilution by transferring 60 µL down the compound plate.
- Add 60 µL of 12 µM Camptothecin (or another positive control compound) into Columns 7-9. Add 60 µL of media to Columns 4-6 as a negative control.
- Transfer 100 µL from the compound plate to the cell plate.
- Add sufficient media to orange fluorescent wells to reach a total volume of 200 µL.

Figure 2. Plate map for optimization assay to determine optimal Annexin NIR concentration



Determining the Optimal Concentration

The optimal Annexin NIR concentration for multiplexing with an orange fluorescent reagent is a concentration where the fluorescent signal is bright enough to be masked in the NIR channel while any bleed through into the Orange channel is sufficiently removed by spectral unmixing.

To determine this concentration:

- Identify the time point of the maximal Annexin V NIR response.
- View this time point in the vessel view and set color autoscale to "vessel".
- Deselect the "Phase" and "NIR" channels to view the Orange channel only and set the spectral unmixing to remove 2% NIR from Orange.
- Select the concentration that shows minimal bleed through into the Orange channel after spectral unmixing is applied. Orange fluorescence should appear very dim or absent when scaled with the orange fluorophore wells on the plate.
- Finally, turn on the NIR channel and ensure that the intensity of the Annexin V NIR signal is bright enough to be identified and masked at the selected concentration.

In the example shown in Figure 3, HT-1080 cells were treated with Camptothecin and the suggested dilution range of Annexin NIR. The images were taken from the peak response at 24 hrs. At the 1:200 concentration, substantial bleed through into the orange channel remains after spectral unmixing. At the 1:1600 dilution, the bleed through signal remaining after spectral unmixing is negligible compared to the orange fluorophore control well on the right. The intensity of the NIR fluorescence at this concentration is still bright enough to be easily masked and analyzed. Using this, it is determined that the 1:1600 dilution is the optimal concentration for Annexin NIR assays with these HT-1080 cells.

Figure 3. Determination of optimal concentration of Annexin NIR from optimization assay images

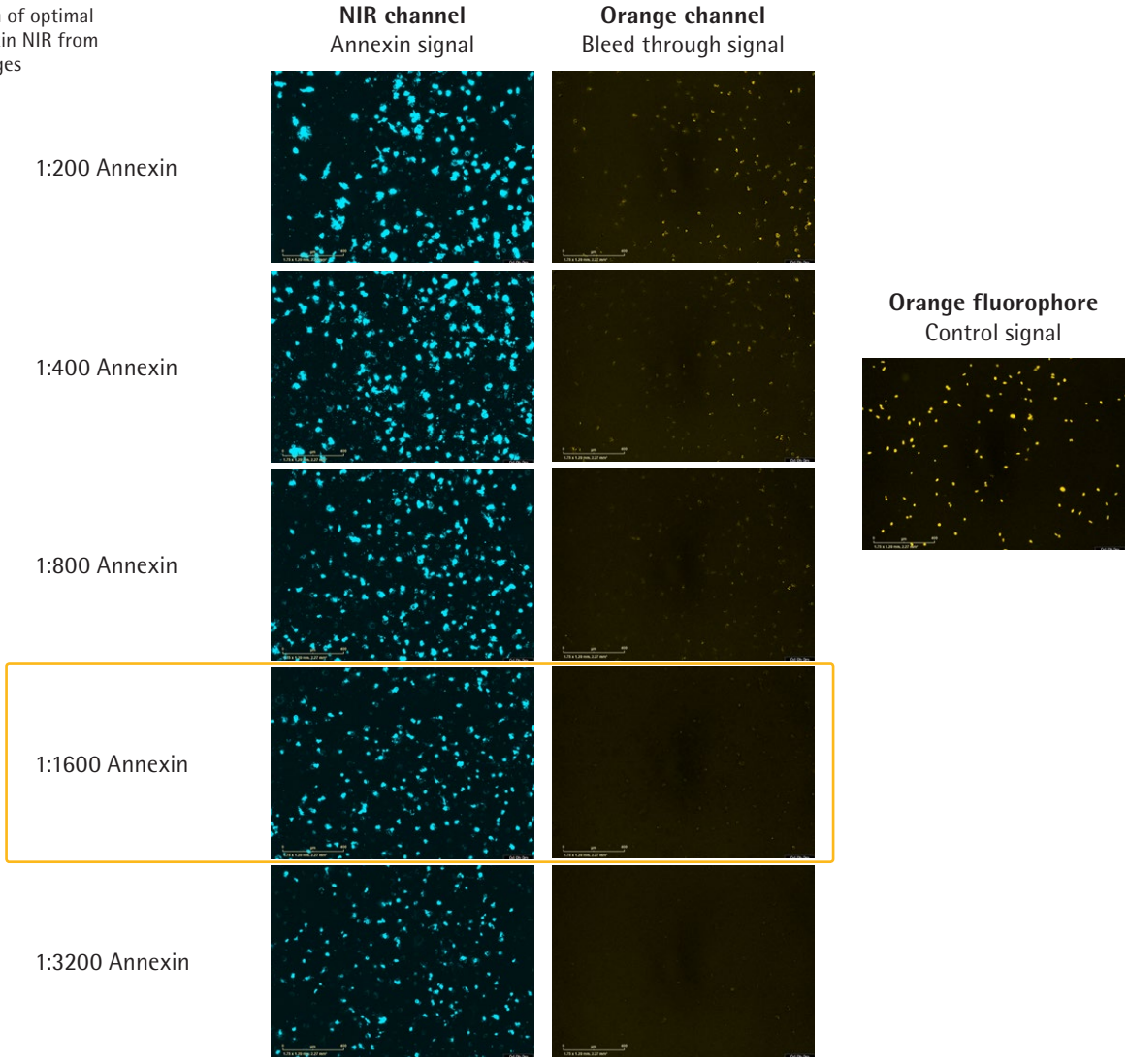


Figure 4. Excitation and emission spectra for Annexin V NIR Reagent

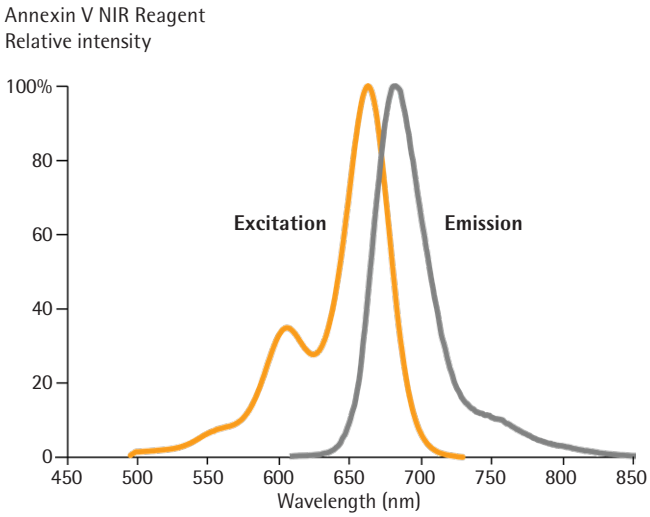
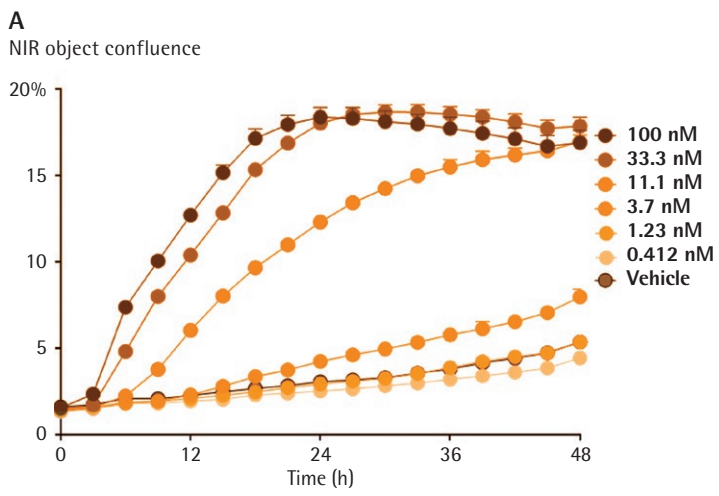
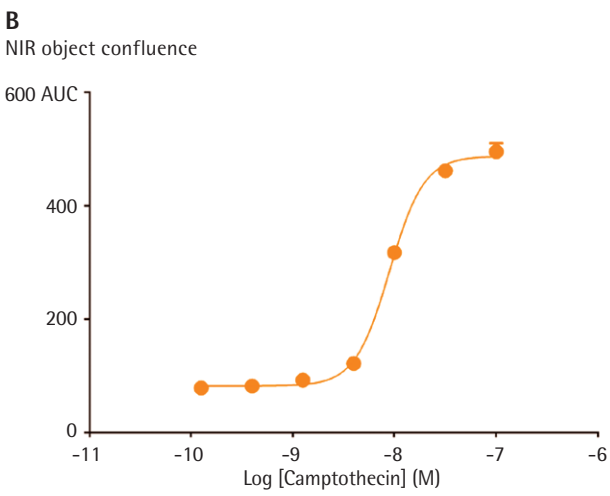


Figure 5. Concentration- and time-dependent increase of PS binding by IncuCyte Annexin V NIR following addition to Jurkat human T-cell leukemia cells treated with the topoisomerase inhibitor, camptothecin.



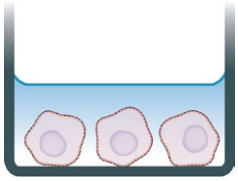
(A) Time-course for the effects of camptothecin on Jurkat cell death (NIR Object Confluence (%) presented as the mean ± SEM, n=3 wells).



(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).

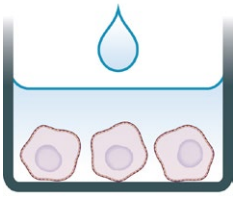
Quick Guide

1. Seed cells



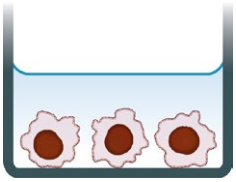
Seed cells (100 µL/well) into a 96-well plate.

2. Prepare apoptosis reagent and treat cells



Prepare the desired treatments at 1x in medium containing IncuCyte® Annexin V Reagent 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.

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

Product	Cat No.	Amount	Ex. maxima	Em. maxima
Annexin V NIR	4768	100 tests	663 nm	682 nm

Product label license

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