



complexity simplified.

Quick Reference Card

Guava Nexin[®] Reagent 4500-0450 & 4500-0455

To determine the apoptotic percentages and concentrations of cellular samples

For Research Use Only. Not for use in diagnostic procedures.

Storage Conditions

Store the Guava Nexin[®] Reagent at 2 to 8°C, protected from light.

Kit Components

Guava Nexin[®] Reagent

- Catalog No. 4500-0450, 100 tests
- Catalog No. 4500-0455, 500 tests

Materials Recommended

- Guava[®] System with Guava Nexin[®] Software module
- Micropipettors
- Disposable micropipettor tips
- Vortex mixer
- Disposable gloves
- Centrifuge
- 37°C CO₂ incubator
- Deionized water
- Guava[®] Instrument Cleaning Fluid (ICF) [Cat. No. 4200-0140], optional
- 20% bleach solution

Assay Protocol

Culture cells, including for positive and negative controls, for appropriate time to induce apoptosis.



Remove Guava Nexin[®] Reagent from refrigerator and allow to warm to RT.



Prepare cell samples for incubation with Guava Nexin Reagent. Ensure prepared cell samples contain at least 1% BSA, 1% FBS, or 10% NHS.



Pipette 100 µL of each cell sample into appropriate tube/well. Cell concentration should be between 2×10^4 and 1×10^5 cells/sample, or 2×10^5 and 1×10^6 cells/mL.



Add 100 µL Guava Nexin Reagent to each tube/well.



Incubate for 20 minutes at RT in the dark.



Acquire samples on a Guava[®] System.

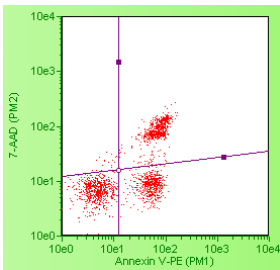
Expected Results

Figure 1 shows an example of results obtained using the Guava Nexin[®] Reagent to stain Jurkat cells treated with staurosporine to induce apoptosis.

Events in each of the four quadrants are as follows:

- Upper-left quadrant: mostly nuclear debris [Annexin V-PE (-) and 7-AAD (+)]
- Upper-right quadrant: cells in the late stages of apoptosis or dead cells (by necrotic or apoptotic mechanisms) [Annexin V-PE (+) and 7-AAD (+)]
- Lower-left quadrant: viable cells, not undergoing detectable apoptosis [Annexin V-PE (-) and 7-AAD (-)]
- Lower-right quadrant: cells in the early to mid stages of apoptosis [Annexin V-PE (+) and 7-AAD (-)]

Figure 1. Jurkat cells were induced to undergo apoptosis by incubation with 1 μ M staurosporine for 3 hours at 37°C. After incubation, cells were mixed with heat-killed Jurkats, stained with Guava Nexin Reagent, and acquired on a Guava[®] System. The results show 30% early apoptotic cells (lower right) and 35% late apoptotic/ dead cells (upper right) in this culture.



Apoptosis Quadrant Analysis Results

	Count	% Total	MFI-x	MFI-y
Upper left	54	2.9%	21	27
Upper right	467	25.2%	258	50
Lower left	914	49.4%	10	3
Lower right	417	22.5%	96	5
Annexin V+	884	47.7%	182	29
7-AAD+	521	28.1%	233	48

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