

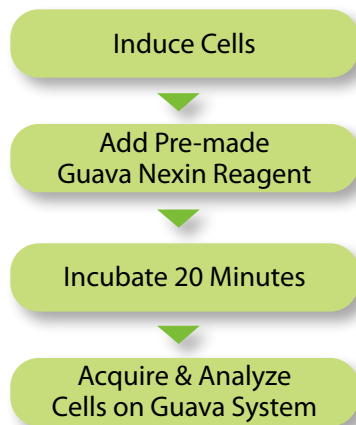
THE POWER OF

A No-Wash, Mix-and-Read Assay for Early and Late Apoptosis Detection: Monitor induction of apoptosis using annexin V binding and dead cells using 7-AAD

Assay Features & Benefits

- **Easy preparation:** pre-made reagent cocktail requires no reagent preparation
- **Mix-and-read protocol:** no wash steps
- **Minimizes assay development time:** reagent optimized for 96-well microplates or tubes
- **Robust and reproducible:** Z' factor >0.8
- **Sensitive:** discriminates live, early apoptotic, and late apoptotic/dead cells
- **Easy sample acquisition and data analysis:** dedicated software module
- **Flexible:** works with adherent and suspension cells

Easy Mix-And-Read Assay Format



Automated single-cell analysis is the technique of choice for monitoring apoptosis via Annexin V binding, due to its sensitivity and reproducibility. Guava Technologies, with its patented microcapillary flow cytometers*, along with the new Guava Nexin Reagent, has streamlined the overall approach so that these analyses can be performed routinely in your laboratory. The Guava Nexin Assay involves a simple, single-step reaction using a pre-mixed reagent cocktail that minimizes both sample manipulation and time. The end result is an assay that is easy, fast, highly sensitive, and reproducible.

Easily Distinguish the Important Populations of Interest

Apoptosis, or programmed cell death, is an important and active regulatory pathway of cell growth and proliferation. Cells respond to specific induction signals by initiating intracellular processes that result in characteristic physiological changes. Annexin V is a calcium-dependent phospholipid binding protein with high affinity for phosphatidyl serine (PS), a membrane component normally localized to the internal face of the cell membrane. Early in the apoptotic pathway, molecules of PS are translocated to the outer surface of the cell membrane where Annexin V conjugated to phycoerythrin (PE) can readily bind them (Fig 1). Late stage apoptotic cells show loss of membrane integrity. The membrane impermeant dye 7-AAD is used to distinguish these late stage apoptotic as well as dead cells from early apoptotic cells. With these two dyes, the Guava Nexin Assay is able to easily distinguish four populations:

- **Non-apoptotic (viable) cells:** Annexin V (-) and 7-AAD (-)
- **Early apoptotic cells:** Annexin V (+) and 7-AAD (-)
- **Late stage apoptotic and dead cells:** Annexin V (+) and 7-AAD (+)
- **Mostly nuclear debris:** Annexin V (-) and 7-AAD (+)

Fig. 1: Annexin V-PE binds to the PS molecules that are translocated to the outer surface of the cell membrane. 7-AAD is used to distinguish late apoptotic/dead cells from early apoptotic cells.

Principle of the Guava Nexin Assay



*The Guava Nexin Reagent is optimized for all Guava Instruments: Guava PCA and EasyCyte™ Mini Systems (single tube formats); Guava PCA-96 and EasyCyte Plus Systems (96-well microplate/10 tube format)

Dedicated Software Module Facilitates Ease of Use

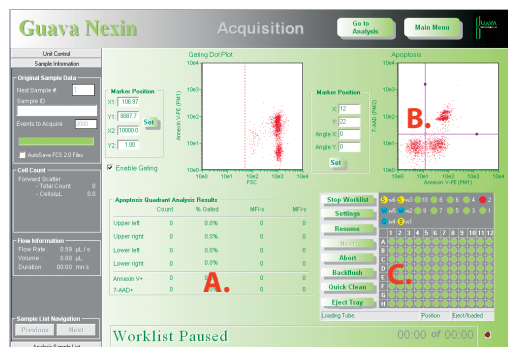


Fig. 2a: Guava Nexin Acquisition screen shot: A) Assay results automatically calculated and displayed with relevant statistics; B) Simple 4 quadrant dot plot analysis: discriminates up to 4 populations; C) System operation consistent across assay modules.

All necessary functions to run the assay are contained within one screen. Eliminates need to configure software for that assay.

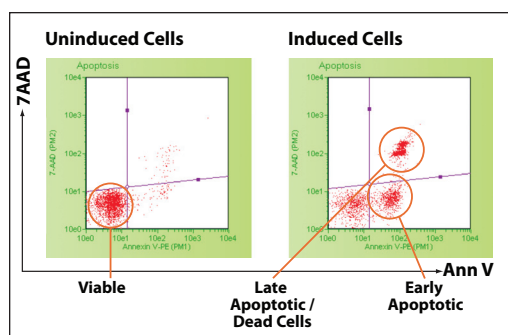


Fig. 2b: Guava Nexin Assay dot plots: Examples of dot plots from uninduced and induced cells.

Ordering information

Reagents

Guava Nexin Reagent (100 Tests)	4500-0450
Guava Nexin Reagent (500 Tests)	4500-0455

Guava Nexin Software Modules for:

Guava PCA System	0500-0250
Guava PCA-96 System	0500-0600
Guava EasyCyte Mini System	0500-1490
Guava EasyCyte System	0500-0990
Guava EasyCyte Plus System	0500-2030

For pricing, please contact Guava Technologies, Inc.

Guava Nexin Assay Is Robust and Ideal for Secondary Screening

The Z' factor is often used to assess the robustness and reliability of a cell-based assay. The Z' factor is a statistical measure that takes into account the variability of the positive and negative control results plus the dynamic range of the assay. For cell-based assays, a Z' value greater than 0.7 is typically considered acceptable for screening.¹ As depicted in the table below, the Guava Nexin Assay produced Z' values >0.8 for both adherent (HeLa) and suspended (Jurkat) cells.

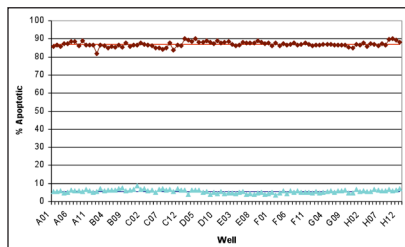


Fig. 3: Full plates of uninduced and staurosporine-induced Jurkat cells were stained with the Guava Nexin Reagent and assayed on two different days to determine percentage of cells that were Annexin V positive. The Z' factor was determined from results obtained for induced and uninduced samples on each of 3 replicate plates on multiple days. The Z' factors were 0.92 and 0.81 on days 1 and 2, respectively.

The robust day-to-day Z' factors and EC₅₀ values (data not shown), together with the simple assay preparation and easy-to-use software module, make the Guava Nexin Assay ideal for identifying apoptotic potential through chemical compound screens. Further, the pre-made reagent cocktail is uniquely optimized for microplates, minimizing assay development, and saving time and valuable resources.

Works with Many Different Cell Types

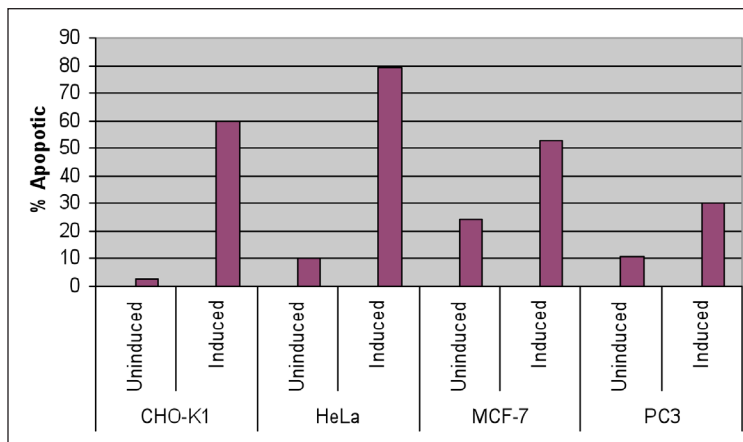


Fig. 4: Adherent cell lines were induced for varying times with optimal concentrations of camptothecin, then trypsinized and stained with Guava Nexin Reagent. The Guava Nexin Reagent could readily detect very low levels of apoptotic cells present in uninduced cultures as well as high levels of apoptotic cells in induced cultures.

¹ Zhang, J. et al. A Simple Statistical Parameter for use in Evaluation and Validation of High Throughput Screening Assays. *J Biomol Screening* 1999; 4:67-73.



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