Technical Brief

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A Mix-and-Read Assay for Apoptosis Detection: Cytek® Muse® Annexin V & Dead Cell Assay

Cytek® Muse® Annexin V & Dead Cell Assay features

- Quick detection of live cells, early and late apoptotic cells, and dead cells allows for the determination of major cell health pathways
- No-wash, mix-and-read assay format allows for rapid testing
- Simplified acquisition and analysis reduces time to results
- Minimal cell numbers required saves precious samples
- Validation using both adherent and suspension cells increases confidence in robust results
- Accurate and precise measurements ensure reliable results—the first time

Apoptosis and cell death: key parameters of cell health

Apoptosis—or programmed cell death—is an important regulator of cell growth and proliferation. Induction of apoptosis is characterized by a progressive series of cellular biochemical and morphological changes. One of the hallmarks of apoptosis is the translocation of phosphotidylserine (PS) from the inner to the outer leaflet of the plasma membrane, and its exposure to the outer surface of the cell. This universal phenomenon is independent of species, cell type, and induction system, and occurs early in the apoptotic process.

The Muse® Annexin V & Dead Cell Assay is a simple, sensitive, and easy-to-perform test for the quantitative detection of apoptosis in cellular samples (**Figure 1**).

Principle of the assay

The Muse Annexin V & Dead Cell Assay is based on the detection of PS on the surface of apoptotic cells, using fluorescently labeled annexin V in combination with the dead cell marker, 7-AAD. Annexin V is a Ca2+-dependent phospholipid binding protein that has a high affinity for 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 can readily bind to them. Late-stage apoptotic cells show a loss of membrane integrity. The membrane-impermeant dye 7-AAD is used to distinguish dead cells from early apoptotic cells. The assay can thus distinguish four populations:

- Viable cells, not undergoing detectable apoptosis:
  Annexin V (–), 7-AAD (–)
- Early apoptotic cells:
  Annexin V (+), 7-AAD (–)
- Late apoptotic cells:
  Annexin V (+), 7-AAD (+)
- Cells that have died through a non-apoptotic pathway:
  Annexin V (–), 7-AAD (+)

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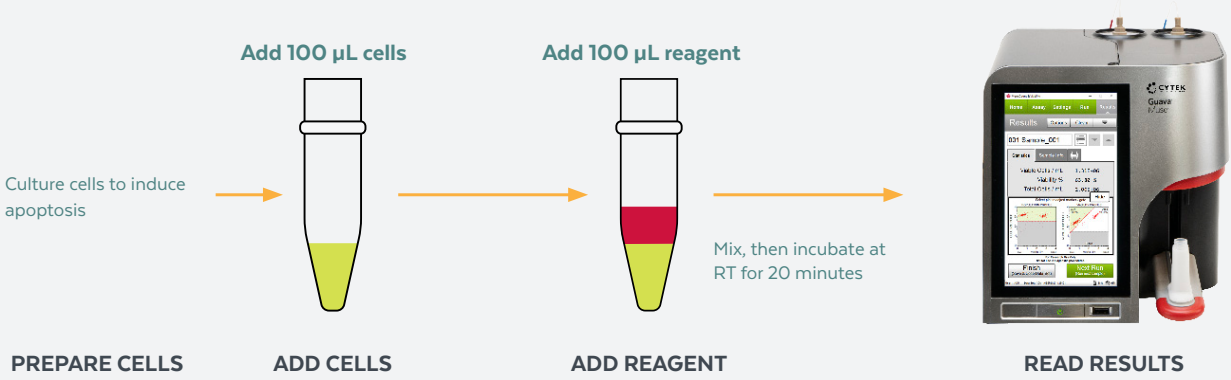
Figure 1. The Muse® Annexin V & Dead Cell protocol steps



An intuitive touchscreen interface greatly simplifies apoptosis data acquisition and analysis

The Cytek® Muse Annexin V & Dead Cell Software Module guides you through setup, acquisition, and analysis in a few simple steps:

- Intuitive touchscreen guides users through acquisition

Results include the count and percentage of populations, which is automatically displayed after acquisition and can be viewed with or without dot plots (**Figure 2**)

- Easy raw data and Excel export features enable result archiving and additional analysis

Figure 2. Example results

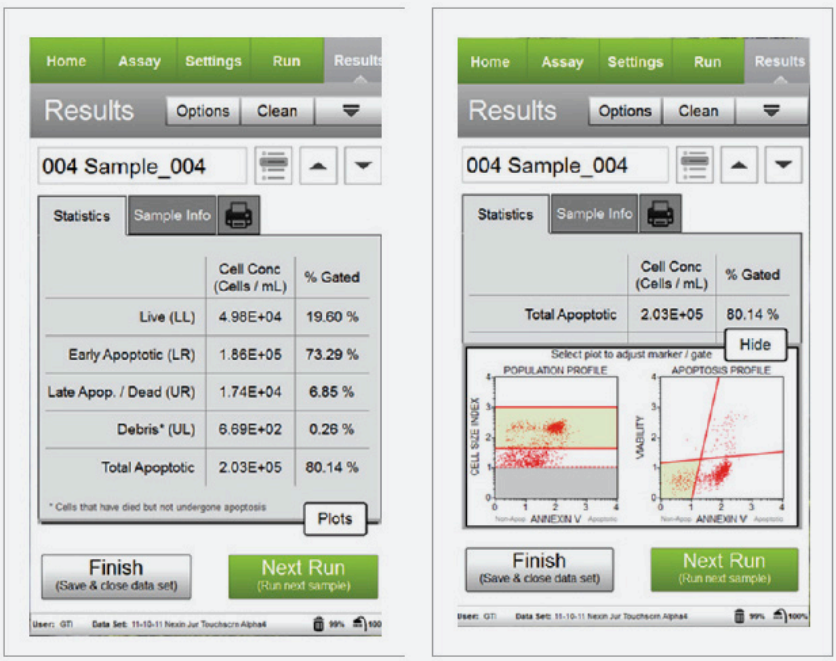


Figure 2. Results were obtained from Jurkat cells treated with 1 µM staurosporine, which were then stained using Muse® Annexin V & Dead Cell Kit, acquired on the Cytek® Guava® Muse® cell analyzer, and analyzed with the Cytek® Muse Annexin V & Dead Cell Software Module.

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Versatile and accurate

The Muse Annexin V & Dead Cell Assay is versatile and works with both adherent and suspension cells for multiple treatment conditions (**Figure 3**). The assay is useful for generating dose-response data on cells treated with apoptosis inducers (**Figure 4**). **Figure 5** shows the assay can provide comparable results for a percent of populations when compared to flow cytometric methods for apoptotic measurement.

Figure 3. Versatile workflows: applicable to multiple cell types and treatment conditions

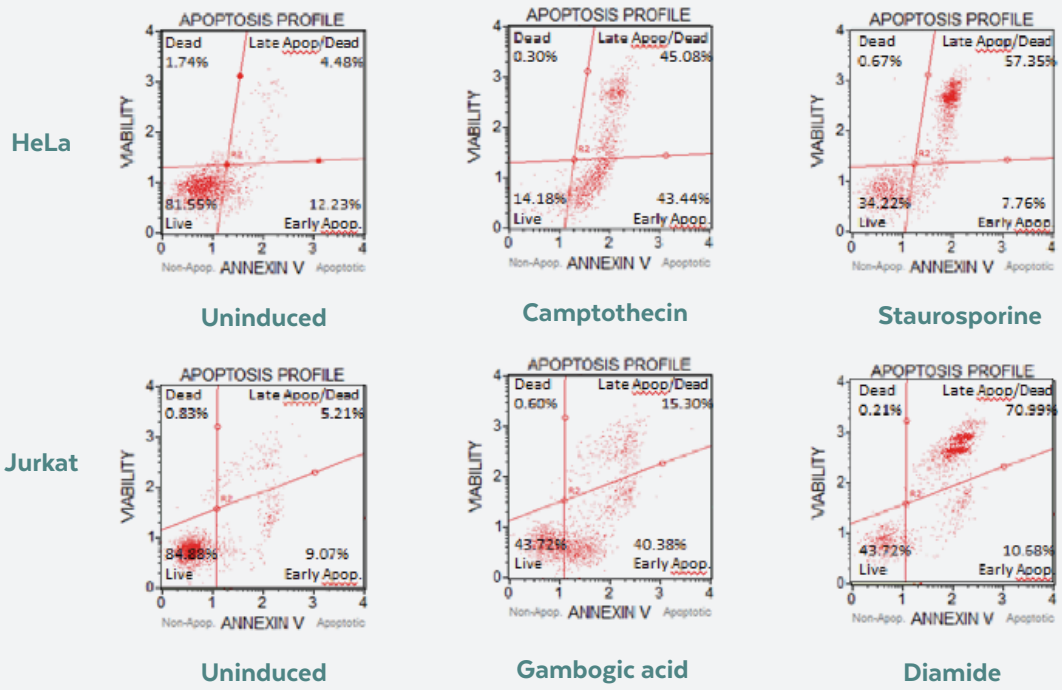

Figure 3. The impact of apoptosis-inducing compounds on HeLa cells (adherent lines) and Jurkat cells (suspension lines) was analyzed using the Muse® Annexin V & Dead Cell Assay.

Figure 4. Dose response of treatment

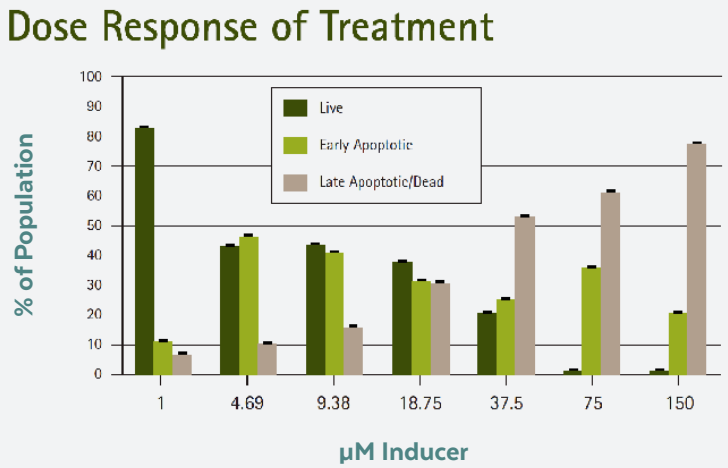

Figure 4. The response of Jurkat cells treated with gambogic acid was analyzed with the Muse® Annexin V & Dead Cell Assay.

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Figure 5. Results comparable to traditional methods

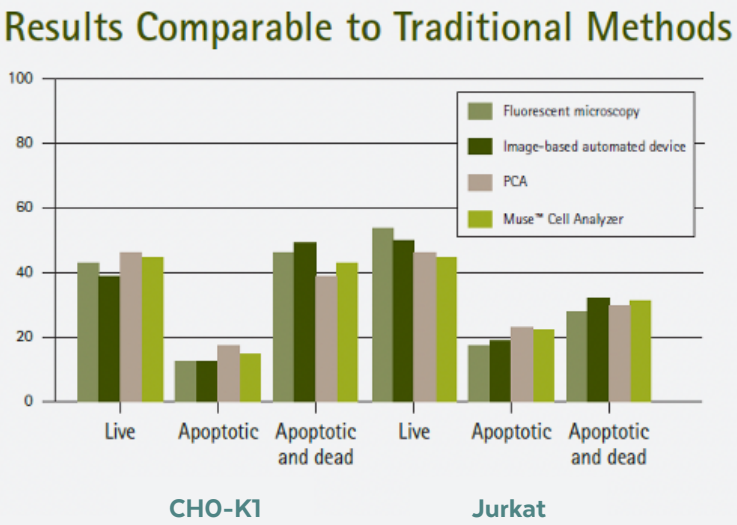

Figure 5. The Cytek® Guava® Muse® cell analyzer provides equivalent cell population measurement results compared to results from traditional analysis methods in both adherent (CHO-K1) and suspension (Jurkat) cell lines. Cellular samples from the two cell lines were prepared in triplicate and analyzed by four methods: fluorescent microscopy, image-based fluorescent analysis, the Cytek® Guava® PCA, and the Cytek® Muse® cell analyzer. The results indicate that the Muse Annexin V & Dead Cell Assay on the Cytek® Muse cell analyzer provided equivalent results to traditional methods for obtaining cell population measurements for both cell lines.

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