Technical Brief

Catalogue No. MCH100105

A mix-and-read assay for apoptosis detection: Muse™ Annexin V and Dead Cell Assay

Muse™ Annexin V and Dead Cell Assay Features

- Quick determination of live cells, early and late apoptotic cells, and dead cells
- No-wash, mix-and-read assay
- Highly simplified acquisition and analysis
- Minimal number of cells required
- Validated with both adherent and suspension cells
- Accurate and precise

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 from the inner to the outer leaflet of the plasma membrane and 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 and 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 and Dead Cell Assay is based on the detection of phosphatidylserine (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 Ca^{2+}-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 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 (–) and dead cell marker (–)
- Early apoptotic cells: Annexin V (+) and dead cell marker (–)
- Late apoptotic cells: Annexin V (+) and dead cell marker (+)
- Cells that have died through non-apoptotic pathway: Annexin V (–) and dead cell marker (+)

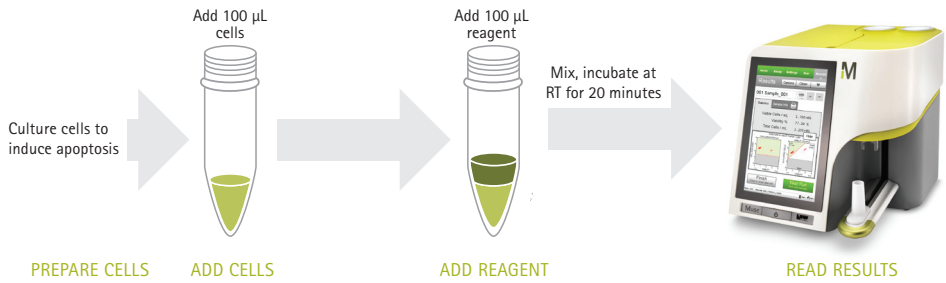

Figure 1.
The Muse™ Annexin V and Dead Cell protocol steps.

Touchscreen Interface Greatly Simplifies Apoptosis Data Acquisition and Analysis

The Muse™ Annexin V and Dead Cell software module guides you through setup, acquisition, and analysis in a few simple steps.

- Intuitive touchscreen guides users to the answers.
- Results include count and percentage of populations automatically displayed after acquisition. Results displayed with or without dot plots. (Figure 2)
- Easy raw data and Excel® export features enable archiving of results and additional analysis.

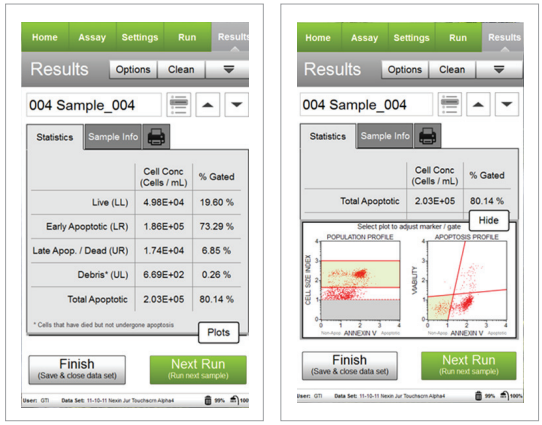

Figure 2. Results obtained for Jurkat cells induced to apoptosis with 1 µM staurosporine, stained with Muse™ Annexin V and Dead Cell Kit, data acquired on the Muse™ Cell Analyzer, and analyzed with the Muse™ Annexin V and Dead Cell software module.

Versatile and Accurate

The Muse™ Annexin V and Dead Cell Assay is versatile and works with both adherent and suspension cells and 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 provides comparable results for % of populations when compared to flow cytometric methods for apoptotic measurement (PAC).

Versatile: Applicable to Multiple Cell Types and Treatment Conditions

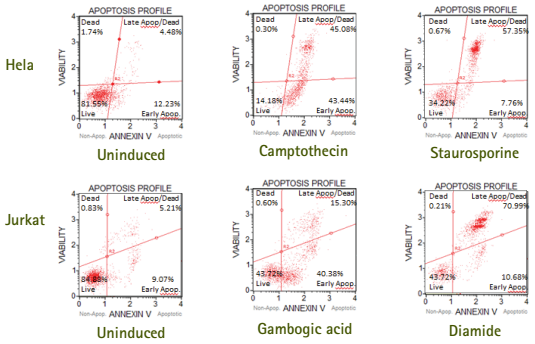

Figure 3. Impact of apoptosis-inducing compounds on HeLa cells (adherent lines) and Jurkat cells (suspension lines) analyzed using the Muse™ Annexin V and Dead Cell Assay.

Dose Response of Treatment

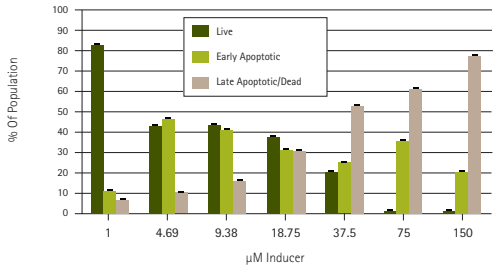

Figure 4. Response of Jurkat cells treated with gambogic acid and analyzed with the Muse™ Annexin V and Dead Cell Assay.

Results Comparable to Traditional Methods

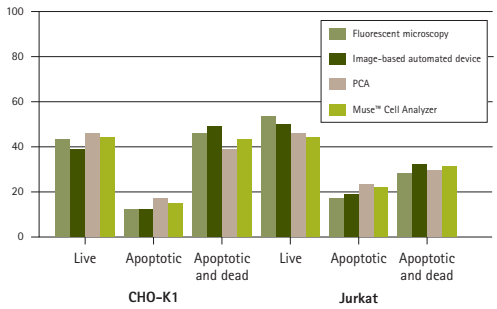

Figure 5. The Muse™ Cell Analyzer provides equivalent cell population measurement results, compared to results from other predicate analysis methods, for both adherent (CHO-K1) and suspension (Jurkat) cell lines. Cellular samples from the two cell lines were prepared in triplicates and analyzed by four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

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