Technical Brief

Catalogue No. MCH100108

Muse™ Caspase-3/7 Assay

A Mix-and-Read Assay for Apoptosis Detection

Assay Features

- Specific detection of caspase-3/7 activity in individual cells
- Quick determination of live, mid-apoptotic, late apoptotic cells and dead cells
- No-wash, mix-and-read format, rapid assay
- Simplified acquisition and analysis
- Minimal number of cells required
- Validated with both adherent and suspension cells
- Accurate and precise

Rapid, Sensitive Detection of Caspase-3/7 Activity

Caspases (cysteinyl-directed aspartate-specific proteases) are a family of enzymes that play a central role in the apoptotic process and result in the cleavage of protein substrates causing the disassembly of the cell. Caspase 3 and caspase 7 are "executioner caspases" that are activated downstream in the apoptosis cascade by a sequence of intrinsic or extrinsic signals. Once activated, these enzymes cause degradation of many key cellular proteins and influence chromatin condensation and DNA damage during apoptosis. Activation of caspase-3/7 is thus a hallmark and confirmation of the apoptotic process.

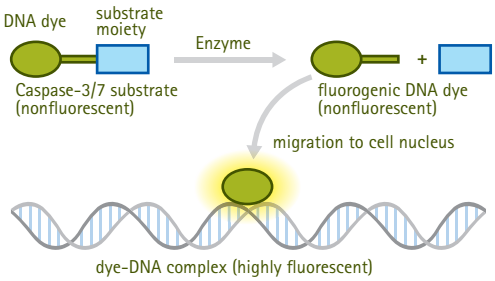

Figure 1.

Principle of the Muse™ Caspase-3/7 Assay.

Assay Principle

The Muse™ Caspase-3/7 Assay enables simultaneous measurement of caspase-3/7 activity and cell death in a single assay. The assay determines the count and percentage of cells in various stages of apoptosis based on caspase 3/7 activity in combination with a dead cell dye. Included in the kit are:

(1) The novel, fluorogenic Muse™ Caspase-3/7 reagent for detecting caspase-3/7 activity

(2) A cell death dye, 7-AAD, that provides information on membrane integrity.

The cell membrane-permeable, nontoxic Muse™ Caspase-3/7 reagent contains a DNA-binding dye that is linked to a DEVD peptide substrate. While still conjugated to DEVD, the dye is unable to bind DNA. Cleavage by active caspase-3/7 in the cell results in release of the dye, translocation to the nucleus, binding of the dye to DNA and high fluorescence. The dead cell marker, 7-AAD, is excluded from live (healthy) and early apoptotic cells, but enters membrane-compromised later stage apoptotic and dead cells. Late apoptotic and dead cells thus show increased fluorescence in the viability axis.

Four populations of cells can be distinguished in the assay

1. Live cells: caspase-3/7(-) and 7-AAD(-)
2. Mid-apoptotic cells exhibiting caspase-3/7 activity: caspase 3/7(+) and 7-AAD(-)
3. Late Apoptotic/Dead cells: caspase-3/7(+) and 7-AAD(+)
4. Dead cells: caspase-3/7(-) and 7-AAD(+)

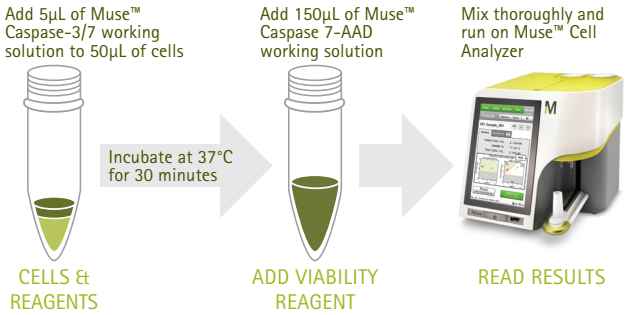

Figure 2.

The Muse™ Caspase-3/7 Assay uses a simple mix-and-read protocol, enabling easy determination of live, mid-apoptotic, late apoptotic/dead and dead cells.

Touchscreen Interface Greatly Simplifies Acquisition and Analysis of Apoptosis Data.

The Muse™ Caspase-3/7 software module guides you through setup, acquisition and analysis in a few simple steps.

- Intuitive touchscreen which guides users quickly to results.
- Results include count and percentage of populations automatically displayed after acquisition.
- Easy export of raw data to Excel® format enables archiving of results and additional analysis.

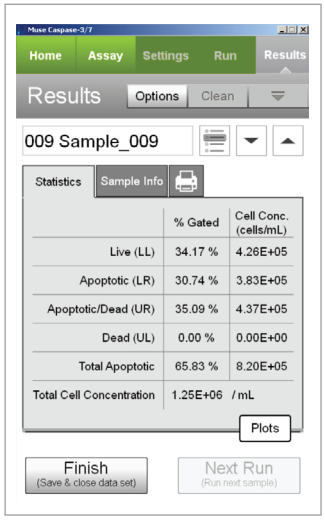

Figure 3. Results obtained using Jurkat cells induced to apoptosis with 1 µM staurosporine, stained with Muse™ Caspase-3/7 Assay and data acquired on the Muse™ Cell Analyzer.

Versatile and Accurate

The Muse™ Caspase-3/7 assay is versatile and works with both adherent and suspension cells and can be used with a variety of inducers to provide a measure of apoptotic populations. Figure 5 demonstrates that the Muse™ Caspase-3/7 Assay provides accurate percentages of caspase-active cell populations compared to data obtained using comparative platforms.

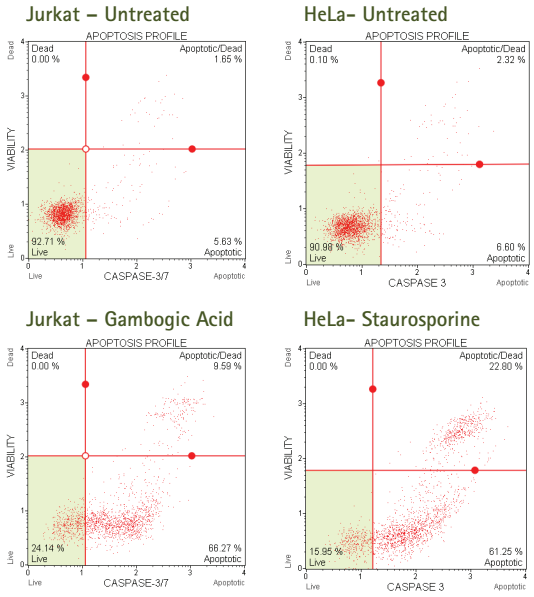

Figure 4. Impact of apoptosis-inducing compounds on HeLa cells and Jurkat cells analyzed using the Muse™ Caspase-3/7 Assay.

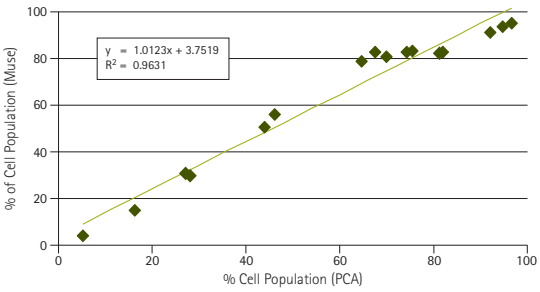

Figure 5. Correlation of caspase-active cell percentages determined using the Muse™ Cell Analyzer with percentages obtained from other flow cytometry platform (x-axis). Jurkat cells were treated with staurosporine and analyzed using the Muse™ Caspase-3/7 Assay

Ordering Information

Muse™ Caspase-3/7 Assay	MCH100108	Muse™ Cell Cycle Kit	MCH100106
Muse™ MultiCaspase Assay	MCH100109	Muse™ Count & Viability Kit	MCH100102
Muse™ MitoPotential Assay	MCH100110	Muse™ Annexin V & Dead Cell Kit	MCH100105
Muse™ System Check Kit	MCH100101		

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