

NC-3000[™] Caspase 3/7, 8 & 9 Assay

- For easy, fast and objective measurement of apoptosis

Caspases are the main executors of the apoptotic process, as caspases upon activation, mediate apoptosis by proteolysis of specific substrates. In this assay caspase activity is measured using a caspase specific inhibitor sequence linked to a fluorescent probe. This assay is known as Fluorochrome-Labelled Inhibitor of Caspases Assay (FLICA).

The non-cytotoxic caspase specific inhibitor is cell permeant and passes through the intact plasma membrane and covalently binds to the reactive cysteine residue on the large subunit of the active caspase heterodimer. Unbound caspase inhibitor diffuses out of the cell and is washed away, thus there is no interference from pro-caspases or inactive forms of the enzyme. The fluorescence measured thus gives a direct measure of the amount of active caspase in the whole living cell. Non-viable cells are identified using propidium iodide.



Key Benefits

of the NC-3000[™] Caspase 3/7, 8 & 9 Assay

Analysis time less than one minute!

- Measure caspase 3/7, caspase 8 or caspase 9
- ✓ User friendly protocol with predefined settings
- ✓ Obtain single caspase information on the cellular level
- ✓ Information about both early apoptotic and late apoptotic/necrotic cells provided
- ✓ Fast automated single cell analysis
- PlotManager for superior data presentation
- ✓ Automated PDF reports
- Export of data in FCS/ACS formats



















CLOGGING

Principle: NC-3000[™] Caspase 3/7, 8 & 9 Assay

Using fluorescence microscopy and image analysis, the NucleoCounter[®] NC-3000[™] system automates detection of apoptotic cells based on caspase activity. Cells are stained with Hoechst 33342, PI and carboxyfluorescein labeled FLICA reagent.

The total cell population is stained with Hoechst 33342, while early apoptotic and late apoptotic/necrotic cells are stained with carboxyfluorescein labeled FLICA reagent and PI, respectively.



Image acquired with the NucleoCounter® NC-3000™ for the Caspase 3/7, 8 & 9 Assay



Automated PDF reports



The NucleoCounter[®] NC-3000[™] - Next generation cell analysis

Results: Presented in PlotManager



Jurkat cells were grown in the absence (upper row) or in the presence (lower row) of camptothecin (CPT). Cells were stained with Hoechst-33342, FLICA reagent (FAM) and Propidium Iodide (PI) and analysed using the Caspase Assay and a NucleoCounter[®] NC-3000^M. Scatter plots and histograms were obtained from the NucleoView^M NC-3000^M software. Polygons and markers in the displayed plots were used to demarcate the various cell populations. In this example camptothecin causes a dramatic increase of early apoptoctic cells (Caspase FAM positive and PI negative cells).



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For more information, please visit www.chemometec.com/NC-3000

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