

PRODUCT DATA SHEET



ENZ-51041

Cyto-ID[®] Autophagy/ apoptosis/ necrosis detection kit for flow cytometry

A three-parameter cell-based assay to detect autophagy, early stage apoptosis, and late-stage apoptosis/necrosis.

Product Number/Sizes

ENZ-51041-K100

1 Kit

- Flow cytometry application allows an easy quantitation of autophagy, apoptosis and necrosis
- Simple assay does not require non-physiological protein mutations or genetically engineered cell line
- Identify and confirm compounds involved in cell death pathways Suitable for screening compounds that are of potential therapeutic value

Cyto-ID[™] Autophagy/Apoptosis/Necrosis Detection Kit is a three-parameter assay utilizing an argon laser excitable, green-emitting fluorophore to highlight the various vacuolar components of the autophagy pathway, a violet laser-excitable, blue fluorophore conjugate of Annexin V to measure phosphatidyl serine exposure in early stage apoptosis, and an argon laser excitable, cell-impermeable red fluorescent DNA intercalation dye to measure membrane disintegration in late-stage apoptosis/necrosis. The assay has been validated using a range of conditions known to modulate autophagy, apoptosis and necrosis pathways. The assay provides a rapid, information-rich read-out of the three principal PCD pathways by flow cytometry and should facilitate better drug activity profiling and clearer kinetic analysis of these fundamental processes in living cells.

Product Specifications

QUANTITY:

100 assays

QUALITY CONTROL:

A sample from each lot of Cyto-ID[™] Autophagy/ Apoptosis/ Necrosis Detection Kit is used to stain Jurkat cells and analyzed by flow cytometry, using the procedures described in the user manual.

- Percentage of Apoptotic cells after treatment with 1 μ M staurosporine for 4 hours is greater than 40%.
- Mean fluorescence of apoptotic cells/mean fluorescence of viable cells is greater than 30-fold using the Staurosporine treatment described in the user manual.
- The autophagy activity factor (AAF) values of Autophagy for the samples is greater than 30 using using the 1-hour starvation treatment described in the user manual.

KIT/SET CONTAINS:

Apoptosis Detection Reagent (Annexin V-Atlantic Blue), 500 μ L
Cyto-ID[®] Green Autophagy Detection Reagent, 25 μ L
Necrosis Detection Reagent (Red), 600 μ L
Apoptosis Inducer (Staurosporine), 50 nmol
10X Binding Buffer, 6 mL
Earle's Balanced Salt Solution (starvation media), 25 mL

APPLICATION:

Cyto-ID[™] Autophagy/ Apoptosis/ Necrosis Detection Kit provides a rapid, information-rich read-out of the three principal PCD pathways by flow cytometry and should facilitate better drug activity profiling and clearer kinetic analysis of these fundamental processes in living cells.

SHIPPING:

SHIPPED ON BLUE ICE

LONG TERM STORAGE:

-20°C

USE/STABILITY:

Upon receipt, store the Cyto-ID[™] Green Autophagy Detection Reagent and Apoptosis Inducer (Staurosporine) at -20°C. Store all other reagents are 4°C, protected from light.

HAZARD:

HARMFUL.

HANDLING:

Avoid freeze/thaw cycles.
Protect from light.

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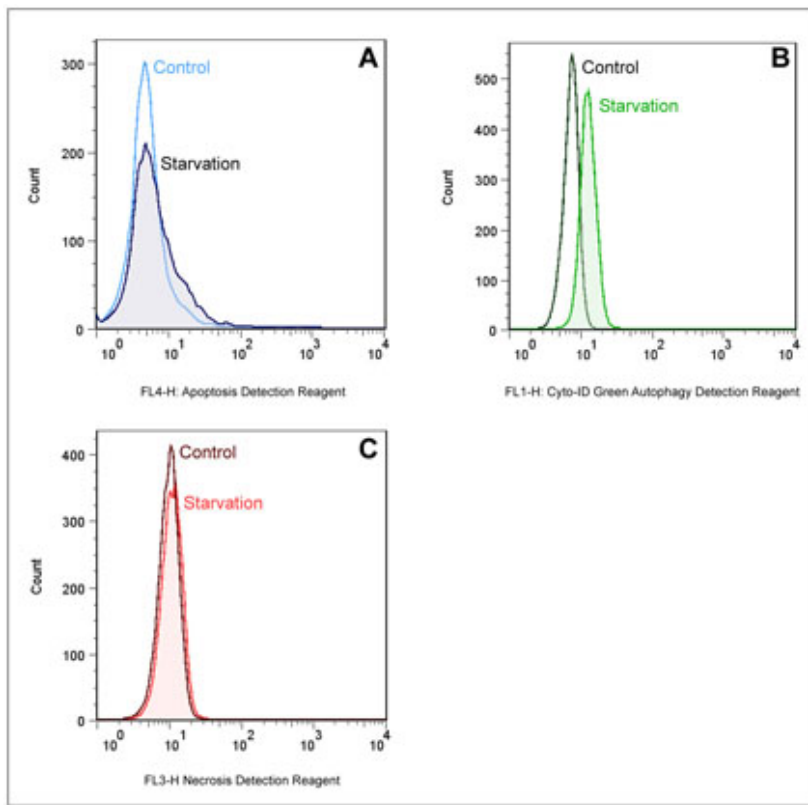


Figure 1. Jurkat cells (T-cell leukemia, human) treated with starvation for 1 hour or untreated control. Cells were treated with the reagents in the kit and analyzed by flow cytometry. Note that the Cyto-ID™ green autophagy dye signal in starvation treated cells increases about 2-fold (panel B). The starvation treated cell has similar percentage of apoptotic cells (panel A) and necrosis (panel C) to the basal level seen in the control cells.

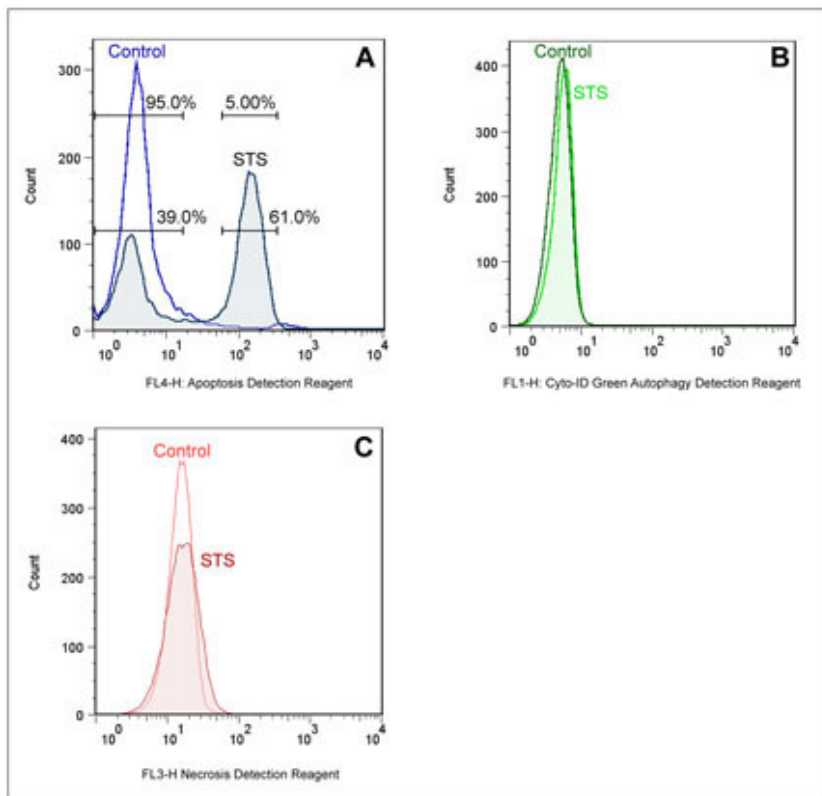


Figure 2. Jurkat cells (T-cell leukemia, human) treated with 1 μ M Staurosporine for 4 hours or left untreated. Cells were treated with the reagents in the kit and analyzed by flow cytometry. Note that the Staurosporine treated cells have a higher percentage of apoptotic cells than the basal level of apoptosis seen in the control cells (panel A). The Cyto-ID™ green autophagy dye signal remained at control levels after Staurosporine treatment (panel B). The Staurosporine treated sample has no significant increases in percentage of necrosis cells compare to basal level in the control sample (panel C).

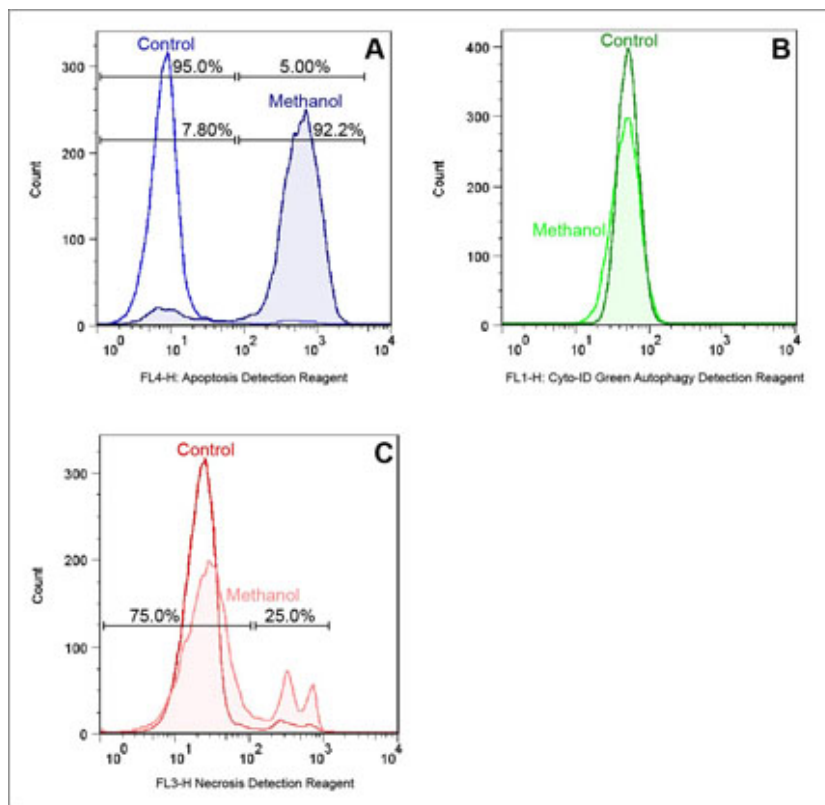


Figure 3. Jurkat cells (T-cell leukemia, human) treated with 100 mM methanol for overnight or left untreated. Cells were treated with the reagents in the kit and analyzed by flow cytometry. Necrosis involves compromise of the plasma membrane's integrity, leading to Atlantic Blue-Annexin V binding to phosphatidyl serine exposed to the cytoplasmic side of the plasma membrane during necrosis. (panel A). It is readily apparent that the plasma membrane is compromised, since the red necrotic stain can bind to the nuclear DNA within the cells (panel C). Necrosis is distinguishable from apoptosis based upon analysis of both the blue and red emission channels.

Background/Technical Information

The Cyto-ID™ Autophagy/ Apoptosis/ Necrosis Detection Kit is a member of the CELLestial® product line, reagents and assay kits comprising fluorescent molecular probes that have been extensively benchmarked for live cell analysis applications. CELLestial® reagents and kits are optimal for use in demanding cell analysis applications involving confocal microscopy, flow cytometry, microplate readers and HCS/HTS, where consistency and reproducibility are required.

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WARNING: THIS PRODUCT IS NOT INTENDED OR APPROVED FOR HUMAN, DIAGNOSTICS OR VETERINARY USE. USE OF THIS PRODUCT FOR HUMAN OR ANIMAL TESTING IS EXTREMELY HAZARDOUS AND MAY RESULT IN DISEASE, SEVERE INJURY, OR DEATH.

MATERIAL SAFETY DATA: This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. This information contains some, but not all, of the information required for the safe and proper use of this material. Before use, the user must review the complete Material Safety Data Sheet.

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