Enzo Life Sciences

APOPTOSIS/NECROSIS DETECTION

GFP-Certified™ Apoptosis/Necrosis Detection Kit

ENZ-51002-25 — 25 Reactions
ENZ-51002-100 — 100 Reactions

HIGHLIGHT

- True multiplexing capabilities with GFP and other green fluorescent probes
- Readily distinguishes between healthy, early apoptotic, late apoptotic and necrotic cells
- Optimized for both fluorescence microscopy and flow cytometry applications
- Suitable for death pathway analysis and drug/toxin studies
- Stringently manufactured, to control and eliminate non-specific assay artifacts

Enzo Life Sciences' GFP-Certified™ Apoptosis/Necrosis Detection Kit is specifically designed to minimize fluorescence signal overlap with probes that emit in the green region of the spectrum, such as GFP and FITC. Conjugation of Annexin V with an enhanced Cyanine 3 fluorphore enables simple detection of apoptosis upon binding to phosphatidylserine on the extracellular surface of suitably induced cells. Similarly, the Necrosis Detection Reagent (Red) included in this kit detects loss of plasma membrane integrity by generating a far-red emission profile localized to the nucleus and distinct from both GFP and the Annexin V–Cyanine 3 conjugate. Staurosporine is included as a positive control for induction of apoptosis.

The GFP-Certified™ Apoptosis/Necrosis Detection kit readily distinguishes between early apoptosis, late apoptosis and necrosis, allowing detailed analysis of death pathways. This multiplexed system is useful for R&D applications ranging from target identification and validation to small molecule efficacy and toxicity, and enables both accurate and selective benchmarking of cell state.

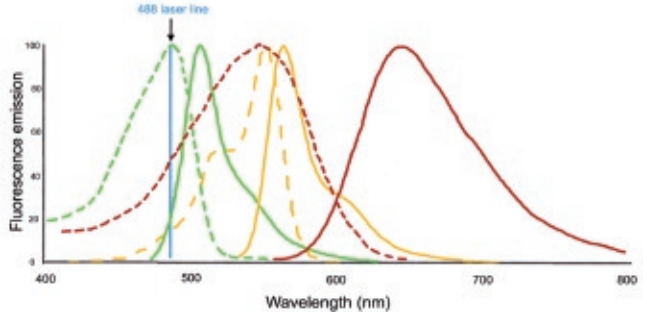

FIGURE 1: Excitation (hatched) and emission (solid) spectra for GFP, Annexin V-Cyanine 3 conjugate and Necrosis Detection Reagent (Red). All three dyes are readily excited with a 488 nm laser source. The emission maxima of all fluorophores are well separated from one another.

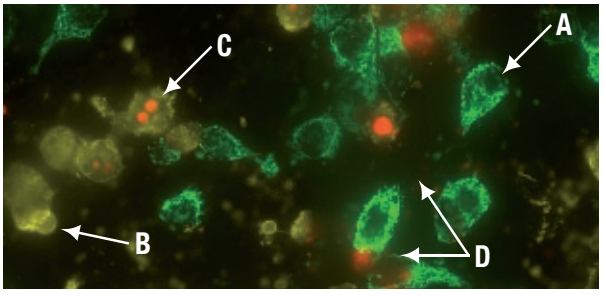

FIGURE 2: Apoptosis/necrosis induction in mitochondrial GFP-expressing HeLa cells. The Apoptosis Detection Reagent (Annexin V-Cyanine 3) and Necrosis Detection Reagent (Red) specifically detect cell state with clear spectral separation from mitochondria-associated GFP signal. Healthy cells (A), cells undergoing apoptosis (B), cells undergoing late-stage apoptosis (C), and necrotic cells (D). Induction with 2 μM Staurosporine was shown to be time-dependent, and yielded four distinct cell states after 4 hours.

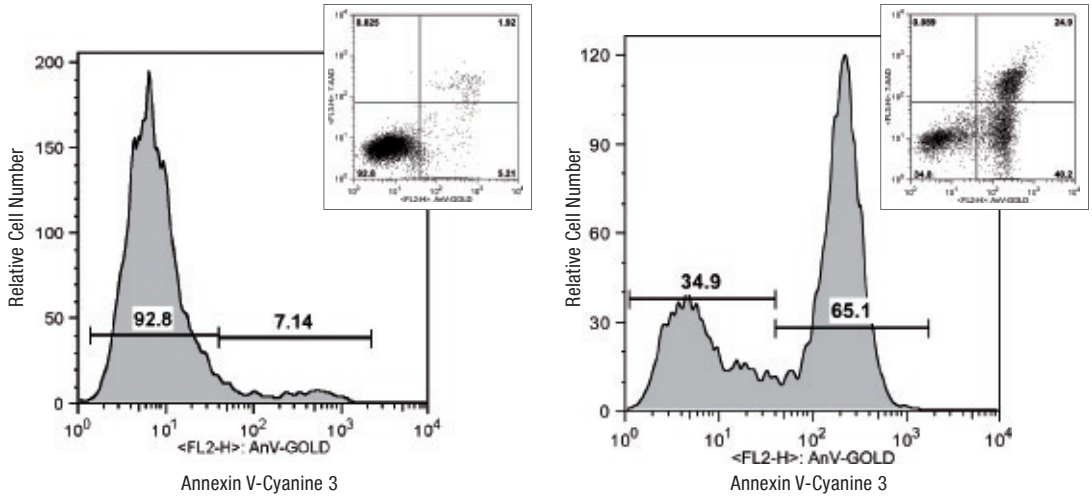

FIGURE 3: FLOW CYTOMETRY. Jurkat cells were mocked-induced with 0.2% DMSO (panel A) or induced with 2 μM Staurosporine (panel B) for 4 hrs. at 37°C. After treatment, cells were incubated with a buffer containing Annexin V-Cyanine 3 and the Necrosis Detection Reagent (Red), a far red emitting DNA-intercalating dye, then analyzed by flow cytometry using a 488 nm laser with fluorescence detection with FL2 (Apoptosis Detection Reagent) and FL3 (Necrosis Detection Reagent) channels. Mock-induced cells were primarily negative for apoptosis and necrosis. After a 4 hour treatment there were three populations of cells: (1) cells that were viable and not apoptotic or necrotic (Annexin V-Cyanine 3 and Necrosis Detection Reagent negative); (2) cells undergoing apoptosis (Annexin V-cyanine 3 positive and NDR negative); and (3) cells undergoing late-stage apoptosis and early necrosis (Annexin V-Cyanine 3 and Necrosis Detection Reagent positive).

Related Products

Product	Prod. No.	Size
5-Carboxyfluorescein (Ultra Pure)	ENZ-52051	100 mg
5-Carboxyfluorescein succinimidyl ester (Ultra Pure)	ENZ-52053	10 mg
Coelenterazine (Ultra Pure)	ENZ-52054	250 µg
JC-1 (Ultra Pure)	ENZ-52304	5 mg
JC-10 [Enhanced JC-1] (Ultra Pure)	ENZ-52305	5 mg
Nile Red (Ultra Pure)	ENZ-52551	25 mg

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