

Staining cells protocol with DiIC1(5), Annexin V and Non-Viable cells solutions (PI and 7-AAD).

1. Prepare Annexin V Binding Buffer: 10 mM Hepes/NaOH (pH 7,4) 140 mM NaCl, 2,5 mM CaCl₂.
2. Induce apoptosis in cells using the desired method. A negative control should be prepared by untreated cells, that is used to define the basal level of apoptotic and necrotic or dead cells.
3. Harvest the cells after the apoptosis induction.
4. Wash cells twice with temperate PBS and resuspend cells in temperate phosphate-buffered saline (PBS) at a concentration 1 x 10⁶ cells/ml.
5. Add 5 µl of 10µM DiIC1(5).
6. Incubate the cells at 37 °C, 5% CO₂, for 15 minutes
7. Wash cells twice with temperate PBS and resuspend cells in 1 X Annexin-binding buffer at a concentration 1 x 10⁶ cells/ml.
8. Add 5 µl of the Annexin V-FITC and 5 µl of PI, to each 100µl of cell suspension.
9. Incubate the cells at room temperature for 15 minutes at room temperature (25°C) in the dark.
10. After incubation period, add 400 µl of 1X Annexin-binding buffer. Analyze by flow cytometry within one hour.

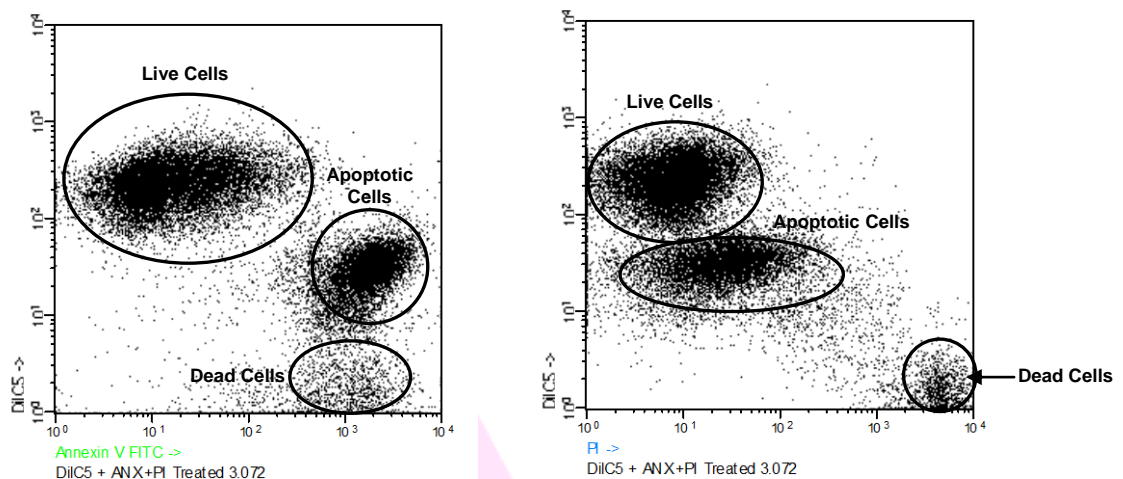


Figure 1. Jurkat cells (T-cell leukemia, human) treated with 6 µM camptothecin for four hours (both panel).

Possible combinations:

PRODUCT	LASER EXCITATION WAVELENGTH (NM)	EMIT (NM)
DiICl(5)	633	658
Annexin V- FITC	488	520
PI	488 & 595	617

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DiICl(5)	633	658
Annexin V- PE	488	575
7-AAD	488	645

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. (tech@immunostep.com)

Reagent list:

- Annexin V Binding Buffer: Ref. BBIOX-50ML
- Wash solution: 20 Mm NaH₂PO₄, 150 NaCl, pH 7.2 + 0,09% Sodium azide (NaN₃) + 0,5 % bovine serum albumin.
- 7-Aminoactinomycin D: ref. 7AAD-400T
- Propidium Iodide: ref. PI-400T
- Annexin V FITC: Ref. ANXVF-200T
- Annexin V PE: Ref. ANXVPE-200T
- 10µM DiICl(5): Ref. MITO-100T