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Annexin V dead cells staining protocol

1. Prepare 1X Annexin V Binding Buffer by mixing 1 part of 10X binding buffer with 9 parts of distilled water.

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 and dead cells.

3. Harvest the cells after the apoptosis induction and wash cells twice by adding 2 ml of wash solution. Centrifuge at 300 xg 5 minutes and carefully aspirate the supernatant so as not to touch the cell pellet.

4. Resuspend cells in 1 X Annexin-binding buffer at a concentration 1 x 10^6 cells/ml.

5. Add the Annexin V reagent. Mix well and incubate cells for 15 minutes in the dark at room temperature (20-25°C) or for 30 minutes at 4°C.

6. Add the appropriate volume of viability dye for example Propidium Iodide or 7-Aminoactinomycin D.

7. Mix well and incubate cells for 5 minutes at room temperature (20-25°C) in the dark.

8. After incubation period, add 400 µl of 1X Annexin-binding buffer.

9. Analyze by flow cytometry within one hour.

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. BB10X-50ML
- Wash solution: 20 Mm NaH_2PO_4, 150 NaCl, pH 7.2 + 0,09% Sodium azide (NaN_3) + 0,5 % bovine serum albumin.
- 7-Aminoactinomycin D: ref. 7AAD-400T
- Propidium Iodide: ref. PI-400T