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Annexin V expression in apoptotic peripheral blood lymphocytes protocol

1. Separate mononuclear PMBC using a density gradient centrifugation protocol.

2. Induce apoptosis in leukocytes incubating 6 hours with an apoptosis inducting agent (e.g H_2O_2 200µM). A negative control should be prepared by untreated cells, that is used to define the basal level of apoptosis and necrosis cell death.

3. Harvest cells after apoptosis induction and wash them 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 100 µl of Wash solution at a concentration 1 x 10^6 cells/ml.

5. Add lymphocytes specific conjugated monoclonal antibody and incubate for 15 minutes in the dark at room temperature (20-25°C) or for 30 minutes at 4°C.

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

7. Wash lymphocytes once with temperate wash solution and resuspend cells in 500 µl of 1 X Annexin-binding buffer.

8. 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.

9. Add the appropriate volume of viability dye (e.g Propidium Iodide or 7-Aminoactinomycin D).

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

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

12. Analyze by flow cytometry immediately.

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
- Conjugates CD19 monoclonal antibody: Ref. 19A1-100T
- Wash solution: 20 Mm NaH_2PO_4, 150 NaCl, pH 7.2 + 0,09% Sodium azide (NaN_3) + 0,5 % bovine serum albumin.
- H_2O_2 200 µM
- 7-Aminoactinomycin D: ref. 7AAD-400T
- Propidium Iodide: ref. PI-400T