Apoptosis

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Abstract

Determination of apoptotic cell in lymphocyte popullation using flow-cytometry

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Protocol

Step 1.
Take 2 ml of peripheral blood with 300 µl of heparin at room temperature

Step 2.
Dilute 1 mL of Erythrocyte-Lysing Reagent (DAKO, Glostrup, Denmark) by 19 mL of deionized water

Step 3.
Add 2 mL of diluted Erythrocyte-Lysing Reagent to 100 µL of peripheral blood and mix immediately

Step 4.
Incubate it for 10 minutes at room temperature

Step 5.
Centrifuge the sample (300 g, 5 mins, 24oC), than remove the supernatant

Step 6.
Wash the sample twice in 5 mL of cold phosphate-buffered saline

Step 7.
Dilute 1mL of 10X concentrated Binding Buffer (BD Pharmingen™, USA) by 9 mL of deionized water

Step 8.
Resuspend cells of the sample in 1X Binding Buffer (BD Pharmingen™, USA) at a concentration of 5 x 10^6 cells/mL

Step 9.
Transfer 100 µL of the solution (5 x 10^5 cells) to a 5 mL culture tube

Step 10.
Add 5 µL of FITC Annexin V APC (BD Pharmingen™, USA) and gently vortex the cells

Step 11.
Incubate it for 10 min on ice in the dark

Step 12.
Wash the sample by 5 mL of cold 1X Binding buffer at 300g for 5 minutes at 5°C

Step 13.
Add 5 µL of Propidium Iodide (250 µg/ml) (BD Pharmingen™, USA)

Step 14.
Analyze by flow cytometr immediately.