DETECTION OF DNA FRAGMENTATION BY TUNEL TECHNICAL

Reagents and materials

- 96-well plates with conical bottoms.
- Cytometer acquisition tubes.
- phosphate buffered saline (PBS) 1X.
- Distilled water.
- Trypsin 1X.
- Paraformaldehyde at 4%.
- Chilled 70% (v / v) ethanol.
- Deoxynucleotidyl transferase (TdT) terminal at 25 U/µl.
- dUTP-FITC (concentration varies according to supplier).
- Cobalt chloride (CoC12) at 25 mM.
- Glycerol.
- Reaction buffer containing 1 M potassium cacodylate, 125 mN trisHCl (pH = 6.6), and 1.25 mg/ml BSA.
- Rinse buffer containing 0.1% Triton X-100 (v/v), BSA at 4 mg/ml.
- Tris-glycerol buffer (30 mg Tris / HCl and 5 ml glycerol in 10 ml distilled water);
- DNase-I at 1 µg/ml in Tris-glycerol buffer.

Marking of cells in suspension

This test is carried out on 500,000 to 106 cells per sample.

- Centrifuge the cells at 300 G for 5 min. Remove the supernatant.
- Resuspend the cell pellets in 200 µl of PBS 1X and distribute in a 96-well plate.
- Centrifuge at 300 G for 5 min. Remove the supernatant.
- Fix the samples in 200 µl of PBS containing 1% PF A, for 15 min at 4°C.
- Centrifuge at 300 G for 5 min. Remove the supernatant.
- Wash the wells with 200 µl of PBS.
- Centrifuge at 300 G for 5 min. Remove the supernatant.
- Permeabilize the samples in 200 µl of ice-cold 70% ethanol for 1 min at 4°C, and centrifuge immediately for 5 min at 300 G at 4°C. Remove the supernatant.
- Wash the wells with 200 µl of PBS.
- Centrifuge at 300 G for 5 min. Remove the supernatant.
- Resuspend each pellet in 50 µl of labeling solution containing :

- 10 µl of reaction buffer;
- 5 µl of cobalt chloride at 25 mM;
- 0.5 µl of dUTP-FITC diluted to the appropriate concentration (conditions of the supplier);
- 0.5 µl of TdT at 25 U/µl;
- 34 µl of distilled water.

- Incubate the cells for 60 min at 37°C.
- Add 150 µl of rinsing buffer and centrifuge at 300 G for 5 min.
- Resuspend and fix each pellet in 200 µl of PBS containing 1% PFA for 15 min at room temperature and in the dark.
- Analyze the samples with a cytometer.

Different witnesses are to be realized :

- the positive control, which consists of a sample which will have been treated before fixing with 20 µl of the DNase-1 solution at 1 µg/ml for 1 hour;
- the negative control, which consists of a sample which will have been treated before fixing with 20 µl of the DNase-1 solution at 1 µg/ml for 1 hour, but for which the TdT has been omitted.

Note: permeabilization with ethanol drastically changes the appearance of cells. It is therefore

normal for these to appear in the form of translucent caps, or even hardly visible to the eye after centrifugation in a conical bottom.

Labeling of adherent cells

To determine the percentage of apoptosis in this type of culture, it is necessary to analyze the adherent cells and the cells of the supernatant.

- Collect the supernatant from each culture and place it in a specifically annotated cytometer acquisition tube.
- Wash the wells with 200 µl of PBS without calcium or magnesium.
- Trypsinize the adherent cells with 1X trypsin. Stop the action of trypsin.
- Transfer the adherent cells into the tube containing the cells of the corresponding supernatant.
- Centrifuge at 300 G for 5 min. Remove the supernatant.
- Carry out the TUNEL marking as described for the cells in suspension.
- Acquire with the cytometer.