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Isolation of chicken splenocytes

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Diffusion : FrAgEncode project		

Key-words : *splenocytes*

CONTENT

1. Object
Isolation of splenocytes from birds: protocol used on chickens for Fr-AgEncode

2. Domain
Cell biology

3. Principle
Splenocytes are separated from the whole spleen by density gradient using Histopaque 1.077 in order to remove nucleated erythrocytes

4. Reagents

-Histopaque 1.077 (SIGMA ALDRICH-10771)
-RPMI 1640 (FISHER 11554516)
-Fetal Calf Serum (FISHER 10309433)
-DPBS Ca- Mg- (FISHER 11590476)

5. Material and equipments
- metallic circular grids
- Cell Strainer 100µm for 50 mL tubes
- Scalpels, pliers, scissors
- 15mL & 50mL Falcon tubes
- petri dishes
- Centrifuge (for 15mL& 50mL tubes)
- Syringe 10mL
- a microscope, a Malassez or Thoma cell counter

6. Operating mode

NB histopaque 1.077 must be kept at room temperature at least 2h before use

I/ Tissue sampling

The spleen of a chicken is sampled after euthanasia (electronarcosis + bleeding, without anesthetic injection). The spleen is gently removed with a plier with smooth end, scissors and a scalpel, without touching other organs at proximity (liver, gall blader…).
Spleen is kept in sterile PBS in a 50 mL tube placed in crushed ice. It may be kept as such during 6 hours.

II/ Isolation of splenocytes from the spleen.

1- Spleen is placed in a sterile petri dish : the conjunctive capsula is removed with scissors and pliers, and the spleen is cut into small pieces with scissors
2- Add 10mL of RPMI in the dish.
3- Within the petri dish, crush the spleen on the metallic grid with the piston of a 10mL syringe then filter the crushed tissues on a 100 µm Cell Strainer on top of 50 mL Falcon tube. Do not hesitate to add RPMI1640 medium to dilute and rince the crushed tissue. Change the Cell Strainer if it gets clogged. Adjust the final volume so that it is a multiple of 6.

4- In 15mL tubes, gently distribute 6mL of the material obtained in 3 on a 6mL pad of Histopaque 1.077.
5- Centrifuge 30 min at 700g at room temperature, (acceleration 1, deceleration 1).
6- Collect the white cells at the interface in a 50mLtube and add DPBS for washing.
7- Centrifuge 10 min at 450-500g at room temperature (acceleration 9, deceleration 9)
8- Repeat 6 + 7 once.
9- After the second wash, collect the cell pellet in 1mL DPBS.
10- After counting, calculate the number of ml you need to sample according to the number of cells you wish to store per aliquot.

III/ Freezing the cells

Prepare the buffer = decomplemented FCS (56°c 30 minutes in a water bath) + 5% DMSO
Add 5 x 10^6 to 1 x 10^7 cells per ml of SVF-DMSO
Immediately store at -20°C ; keep for 24h at -20°C, then store in liquid nitrogen.

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