Various Animal Tumor Infiltrating Tissue Monocyte Isolation solution Kits

V02

Size:200mL/kit

Storage: This product is sensitive to light, should avoid light storage at room temperature, shelf life of 2 years. After sterile opening, save at room temperature.

Kit compositions

Kit components	Specifications	Storage conditions
Reagent A	200mL	Hide from light at room temperature
Reagent D	200mL	Hide from light at room temperature
Cell washing solution	200mL	Room temperature
Whole blood and tissue dilution solution	200mL	Room temperature

Protocols(*only for reference*)

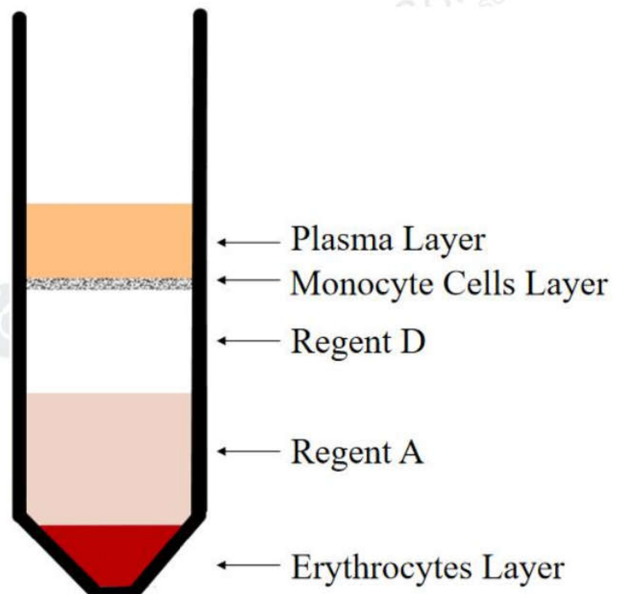
1、 A single cell suspension of the tumor infiltrating tissue was prepared.

2、 Take a sterile centrifuge tube and add reagent A first, followed by reagent D, to form a gradient interface (the volume ratio of reagent A:reagent D is 3:2, if the volume of cell suspension is less than 5ml, add 3ml of reagent A and 2ml of reagent D; if the diluted blood sample is greater than 5ml, the total volume of the reagents should be equal to the volume of the diluted blood sample). The layering of the two reagents must be clear.

3、 The cell suspension is tiled to the separation liquid above the liquid level, pay attention to keep the interface of the two liquid levels clear. (You can use a Pasteurized straw to draw the blood, and then carefully spread the blood on the separation solution, because the density difference between the two will form an obvious layered interface. If more samples are added for a long time, it is normal for red blood cells to agglomerate and sink before centrifugation.)

4、 Centrifuge for 20-30minutes at room temperature with horizontal rotor at 500-800g. (The larger the amount of single cell suspension, the larger the centrifugal force and the longer the centrifugation time, the specific centrifugation conditions can be explored by yourself, and the maximum centrifugal speed does not exceed 1200g).

5、 After centrifugation, there will be a clear layering: the first layer is the plasma layer; the second layer is the layer of white mononuclear cells; the third layer is the layer of transparent reagent D; the fourth layer is the semi-transparent reagent A layer; and the fifth layer is the red blood cell layer (as shown in the figure).



6、 Carefully transfer the second layer of white mononuclear cells and regent D to another sterile 15ml centrifuge tube, add 10mL of cell washing solution or PBS, shake well, and centrifuge at 250g for 10 minutes.

7、 The supernatant was discarded, and the cells were resuspended by adding 10mL of cell wash solution, 250g, and centrifuged for 10minutes.

8、 The supernatant was discarded and the cells were resuspended for later use.

9、 Purify cells by differential adherence: suspend the cells in mononuclear cell culture medium at a density of 10^6cells/ml and lay them on a cell plate or cell bottle, and incubate them in

a cell culture incubator for adherence.

1) Cells will adhere to the surface within 2-4hours, and these are mononuclear cells.

2) Cells will adhere to the surface within 10-24hours, and these are endothelial cells, endothelial progenitor cells, and stem cells.

3) Cells that do not adhere to the surface are lymphocytes.

Due to the differences in adherence time of different cells, this method can achieve a simple purification of mononuclear cells. This method is cost-effective and easy to operate. If you need to obtain high-purity target cells, you can use immunomagnetic bead sorting or apply flow cytometry to select cells.

Preparation of Tumor Infiltrating Tissue Cell Suspension(*only for reference*)

Methods for tumor infiltrating tissue grinding:

1、 The tissue is extracted under sterile conditions, the peritoneum is removed, and the tissue is cut into small pieces with an ophthalmic scissors..

2、 A nylon tumor infiltrating tissue or cell sieve was placed on a plate, and a small amount of whole blood and tissue diluant was added (to ensure that the tumor infiltrating tissue and the cells obtained were in liquid condition).

3、 Place the tumor infiltrating tissue on the screen and grind the organ tissue using a syringe piston or sterile tweezers (try to control the grinding force, keep the screen suspended, and avoid grinding directly on the bottom of the dish and causing a large number of cell deaths)

4、 After complete grinding, rinse the screen with whole blood and tissue dilution, collect the cell suspension, and then filter it through the filter.

Notes:

A. A single cell suspension can be obtained by enzymatic digestion using collagenase to digest the tumor infiltrating tissue.

B. If the resulting cells need to be cultured, the reagents and equipment required for the whole process must be sterile.

C. The concentration of single cell suspension was controlled at 10^8-10^9cells/mL according to the volume of tumor infiltrating tissue.

Note

A. Mix it upside down before opening. This separation solution is a sterile product. In order to prolong the storage time of the separation solution, please unseal it under sterile conditions to avoid microbial contamination.

B. The separation solution should always be kept at room temperature (18℃~25℃) when used. If the indoor temperature is low, the separation solution can be preheated. Centrifugation at 4℃ or lower temperature may cause the white film layer to be unclear.

C. Blood samples should preferably be fresh anticoagulated (within 2h of blood collection). In order to maintain the activity of neutrophils, freezing and cold storage should be avoided.

D. Dilute blood or wash cells, do not use buffer and culture medium containing Ca, Mg ions, its formation will lead to blood cell agglutination, greatly reduce the cell yield and purity.

E. Due to the electrostatic interaction of some plastic products (such as polystyrene), it may cause the cell to hang on the wall, affecting the separation effect.

F. The viscosity or temperature difference of blood samples may affect the separation effect, so the number of centrifugation and centrifugation time can be adjusted to find the best separation condition.

G. If the separated cells are to be further cultured, pay attention to maintain aseptic operation throughout the process to avoid microbial contamination.

H.Excessive suction of cell layers and separation fluid layers can result in the removal of granulocytes at the interface between the separation fluid and the suction cup, thereby increasing the number of contaminated granulocytes. Excessive suction of plasma layers may result in contamination of granulocytes with plasma proteins and platelets.

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