Various Animal Tumor Infiltrating Tissue Neutrophil 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.

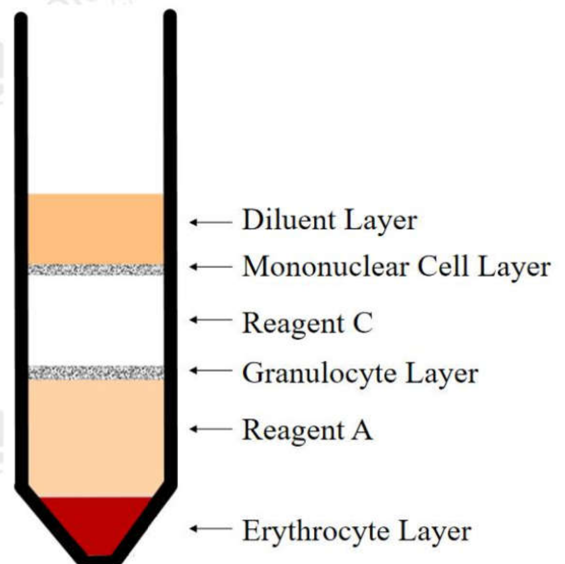
Kit compositions

Kit components	Specifications	Storage conditions
Reagent A	200mL	Hide from light at room temperature
Reagent C	100mL	Hide from light at room temperature
Cell washing solution	200mL	Room temperature
Red blood cell lysate	100mL	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、 When the volume of cell suspension is less than 5mL, add 4mL of reagent A to the centrifuge tube first, and then 2mL of reagent C is carefully superimposed on reagent A to form A gradient interface (cell suspension volume is greater than or equal to 5mL, the ratio of reagent A to reagent C is 2:1, and the total amount of reagent is equal to the diluted sample volume. However, the total volume of the two should not exceed two-thirds of the centrifuge tube, otherwise the separation effect will be affected), the cell suspension was tiled to the top of the separation liquid surface, and the interface between the two liquid surfaces was kept clear. (You can use a Pasteurized pipette to draw the cell suspension, and then carefully lay the cell suspension on the separation solution, because the density difference between the two will form an obvious layered interface. If there are many samples and the sample is added for a long time, it is normal for red blood cells to agglomerate and sink before centrifugation.)



3、 Centrifuge for 20-30minutes at room temperature with horizontal rotor at 500-1000g. (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).

4、 After centrifugation, there will be two layers of circular milky white cell layer in the centrifuge tube, the upper cell layer is the mononuclear cell layer, and the lower cell layer is the granulocyte layer, as shown in the figure (individual differences or different separation conditions, the separation of the granulocyte layer is not obvious).

5、 The neutrophils between reagent C and reagent A and some of the neutrophils in reagent A were carefully sucked into a 15mL clean centrifuge tube with a pipette, and the cells were washed with 10mL PBS or cell washing solution. 250g, centrifuge for 10minutes (add appropriate amount of red blood cell lysate if there are red blood cells mixed).

6、 The supernatant was discarded, and the cells were resuspended by adding 5mL of cell wash solution, 250g, and centrifuged for 10minutes.

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

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. The tissue to be separated should be fresh and avoid freezing and refrigeration.

D. Some plastic products (such as polystyrene) may cause cells to hang on the wall due to their electrostatic interaction, affecting the separation effect.

E. If the isolated cells are to be further cultured, aseptic operation should be paid attention to during the preparation of single cell suspension and separation to avoid microbial contamination.

Related products

YA0902 Disposable Pasteurized Straw
R1018 Cell Wash Solution
R1017 Whole Blood and Tissue Diluent
S9020 Superior Fetal Bovine Serum
31800 RPMI Medium 1640
A Variety of Other Animal and Other Cell Separations and Kits

Reference

[1] Boyum A. Separation of leucocytes from blood and bone marrow. Scand J Clin Lab Invest Suppl. 1968; 97: 7.

[2] Ting A, Morris PJ. A technique for lymphocyte preparation from stored heparinized blood. Vox Sang. 1971 Jun; 20(6): 561-3.

[3] Boyum A. Separation of Blood Leucocytes, Granulocytes and Lymphocytes Tissue Antigens. 1974; 4(4): 269-74.

[4] Weisbart RH, Webb WF, Bluestone R, Goldberg LS. A simplified method for lymphocyte separation. Vox Sang. 1972; 23(5): 478-80.

[5] Recalde HR. A simple method of obtaining monocytes in suspension. J Immunol Methods. 1984 Apr 13;69(1):71-7.

[6] BøyumA,LøvhaugD,TreslandL.Separation of leucocytes: improved cell purity by fine adjustments of gradient medium density and osmolality. Scand J Immunol. 1991 Dec; 34(6):697-712.

[7] Harris R, Ukaejiofo EO. Tissue typing using a routine one-step lymphocyte separation procedure. Br J Haematol. 1970 Feb; 18(2):229-35.

Note: For more literature on the use of this product, please refer to Solarbio's official website.

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