

## Various Animal Bone Marrow Neutrophil Isolation solution Kits

V02

**Size:**200mL/kit

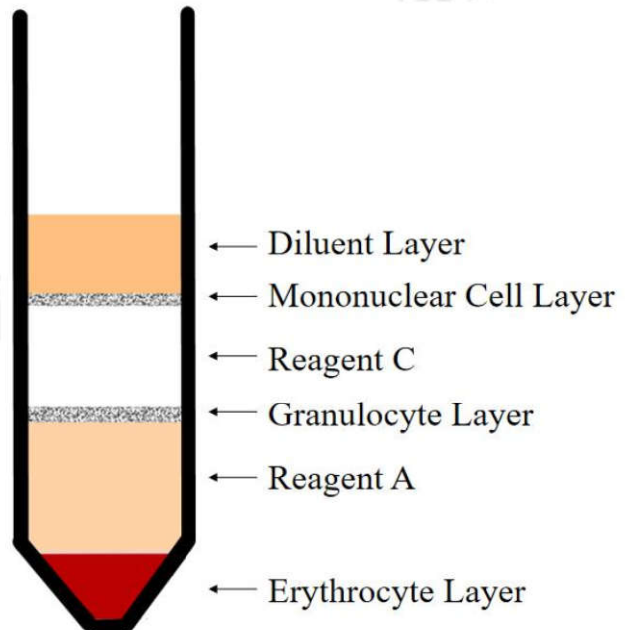
**Storage:** This product is sensitive to light, should avoid light storage at room temperature, shelf life of 2 years. After sterile opening, save at room temperature.

### 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 bone marrow 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 10 minutes.

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

### Preparation of Bone Marrow Single Cell Suspension (only for reference)

#### Collection of small animal bone marrow:

1. The animals were sacrificed, the femurs and tibiae were aseptically extracted, and the cartilage was cut off at both ends to expose the red bone marrow cavity (take care to remove as little bone marrow cavity as possible).
2. In a sterile 1 ml syringe, a small amount of diluent containing 10% standard fetal bovine serum or serum-containing medium was aspirated, and the marrow cavity was flushed to obtain bone marrow.
3. Single cell suspension ( $2 \times 10^8$ - $1 \times 10^9$ /ml) was prepared for later use.

#### Collection of bone marrow from large animals:

Large animal bone marrow can be collected by in vivo puncture method: first, the animal is anesthetized, fixed, debrided locally, and the skin is sterilized. Then the distance from the skin to the bone marrow is estimated, and the length of the bone marrow puncture needle is fixed. The operator tensed the skin around the puncture point with his left hand, and penetrated the needle vertically at the puncture point with his right hand. When the needle entered the bone marrow cavity, there was often a feeling of emptiness. The needle was connected to the syringe and the bone marrow tissue was aspirated slowly. When a little bone marrow was drawn into the syringe, the suction was stopped. The cell concentration was adjusted to  $2 \times 10^8$ - $1 \times 10^9$ /ml of single cell suspension with 10% standard fetal bovine serum.

#### Common bone marrow puncture sites:

Femur: the puncture site is in the inner side of the femur, on the concave surface of the lower end;

Sternum: the puncture site is the connection between the body of the sternum and the manubrium sternum;

Rib: the puncture site is the midpoint of the points of the fifth to seventh ribs;

Tibia: the puncture site is the concave surface of the medial and lower end of the femur. If the rib is used, tape should be used to seal the puncture hole after the puncture to prevent pneumothorax.

#### 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 ( $18^\circ\text{C}$ - $25^\circ\text{C}$ ) when used. If the indoor temperature is low, the separation solution can be preheated. Centrifugation at  $4^\circ\text{C}$  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



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V02

[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.

