

## Various Animal Bone Marrow Isolation solution Kits

**Size:**200mL/kit

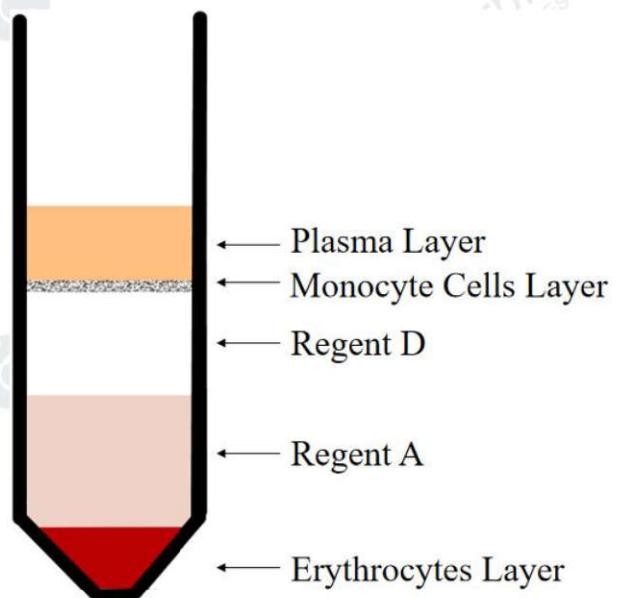
**Storage:** This product is sensitive to light, should avoid light storage at room temperature, shelf life of 2 years. After sterile opening, save at room temperature.

### 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 bone marrow 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 reagent 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  $10^6$ cells/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 Bone Marrow Single Cell Suspension (*only for reference*)

#### Collection of small animal bone marrow:

- 1、 The animals were sacrificed, the femurs and tibs 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 1ml 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°C~25°C) when used. If the indoor temperature is low, the separation solution can be preheated. Centrifugation at 4°C 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.

#### Related products

- YA0902 Disposable Pasteurized Straw
- S9020 Superior Fetal Bovine Serum
- 31800 RPMI Medium 1640
- A Variety of Other Animal and Other Cell Separations and Kits

