Various Animal Bone Marrow Lymphocyte Isolation Solution Kits

V02

Size: 3X200mL/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

Various Animal Bone Marrow Lymphocyte Isolation solution Kits	200mL
Whole Blood and Tissue Thinning Fluid	200mL
Cell Washing solution	200mL

Lymphocyte Isolation Protocols(*only for reference*)

1、 A single cell suspension of the bone marrow was prepared.

2、 Take an appropriate centrifuge tube and add the same amount of separation solution as the single cell suspension of the bone marrow (the minimum amount of separation solution should not be less than 3mL, and the total volume should not exceed two-thirds of the centrifuge tube, otherwise the separation effect will be affected).

3、 Carefully absorb the single-cell suspension and add it to the liquid surface of the separation liquid, paying attention to keeping the interface of the two liquid surfaces clear. (The single-cell suspension can be drawn using a Pasteur pipette and carefully laid over the separation solution, which will form a distinct layered interface due to the density difference between the two.)

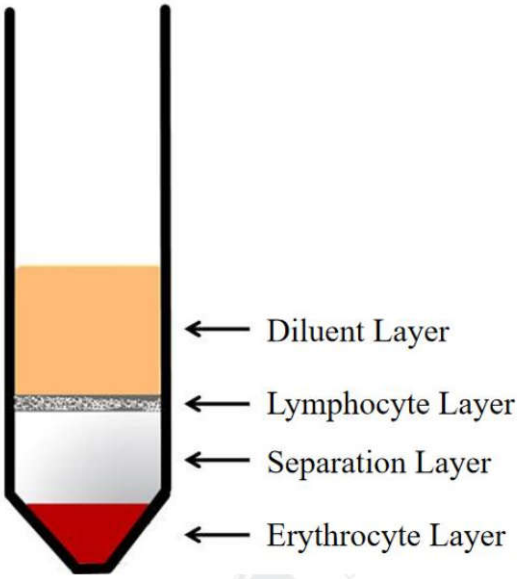
4、 At room temperature, horizontal rotor 500~1000g, centrifugation for 20~30minutes (The larger the single-cell suspension volume, the greater the centrifugal force, and the longer the centrifugation time. You can experiment with the specific centrifugation conditions to achieve the best separation results.).

5、 After centrifugation, there will be obvious stratification: the top layer is the diluted plasma layer, the middle is the clear separation liquid layer, the white membrane layer between the plasma and the separation liquid is the lymphocyte layer, and the bottom of the centrifuge tube is the red blood cells and granulocytes.

6、 Carefully suck the tunica albuginea cells into a 15mL clean centrifuge tube, and wash the tunica albuginea cells with 10mL PBS or cell wash solution. Centrifuge at 250g for 10minutes.

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

8、 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 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 (2×10^8-1×10^9/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,

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the suction was stopped. The cell concentration was adjusted to 2×10^8-1×10^9/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℃~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
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.

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