

## Various Animal Spleen Mononuclear Cell Isolation solution Kits V02

**Size:** 3X200mL/kit

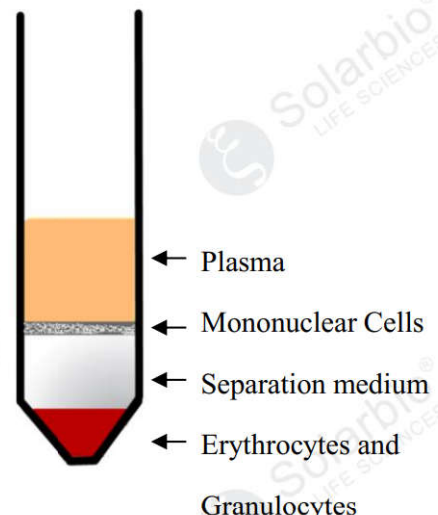
**Storage:** This product is sensitive to light, should avoid light storage at room temperature, shelf life of 2 years. After sterile opening, save at room temperature.

### Kit compositions

Various Animal Spleen Mononuclear Cell Isolation	200mL
Whole blood and tissue dilution solution	200mL
Cell washing solution	200mL

### Mononuclear Cell Isolation Protocols(*only for reference*)

- 1、 A single cell suspension of the spleen was prepared.
- 2、 Take an appropriate centrifuge tube and add the same amount of separation solution as the single cell suspension of the spleen (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 mononuclear cell 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 Spleen Single Cell Suspension(*only for reference*)

Methods for spleen grinding:

- 1、 The spleen was removed under sterile conditions, the spleen capsule was removed, and the spleen was cut into small pieces with ophthalmic scissors.
- 2、 A nylon screen or cell sieve was placed on a plate, and a small amount of whole blood and tissue diluant was added (to ensure that the spleen and the cells obtained were in liquid condition).
- 3、 Place the spleen on the screen and grind the spleen 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 spleen 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  $10^8$ - $10^9$  cells/mL according to the volume of spleen.

### 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 13;69(1):71-7.
- [6] Bøyum A, Løvhaug D, Tresland L. 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.

