Human Endometrial Tissue Lymphocyte Separation Solution kit

V02

Cat: P5680
Size: 200mL/kit
Storage: Store at room temperature, valid for 2 years. This product is easy to be infected by bacteria, and should be operated under aseptic conditions. Operate under aseptic conditions, open Seal and store at room temperature. If stored at 4℃, the separation solution is easy to appear white crystals, affecting the separation effect.

Kit Compositions

Name	Specifications
Separating Solution 1	200mL
Separating solution 2	200mL
Sample diluent	200mL
Cleaning solution	200mL
The homogenate was flushed	200mL

Preparation before the experiment:

1、 Applicable instrument: Horizontal rotor centrifuge with maximum centrifugal force up to 1200g

Preparation of tissue samples *(only for reference)* :

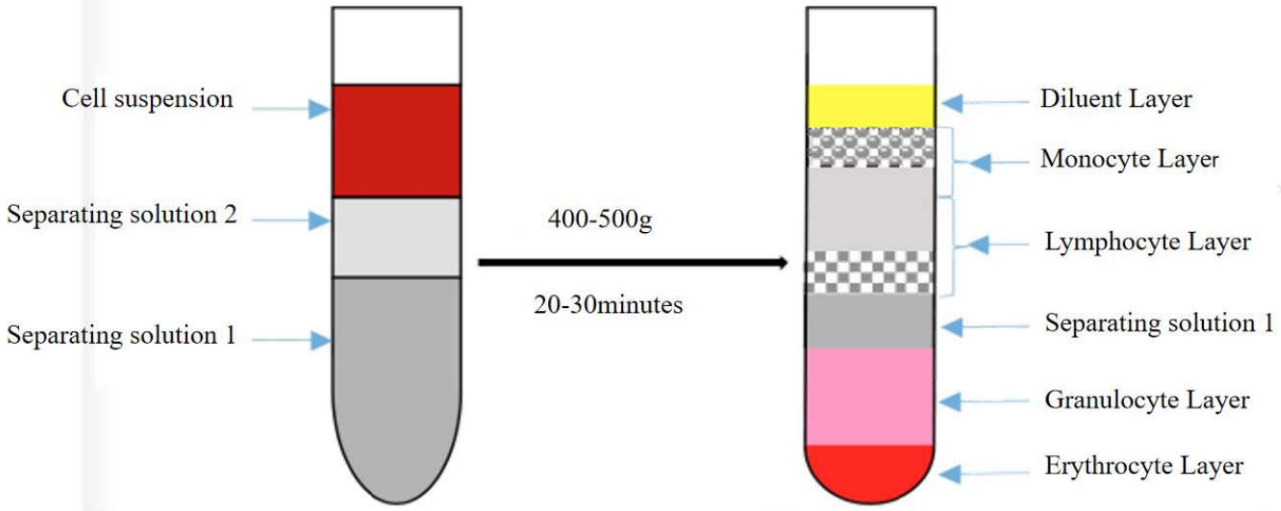
1、 Animals were sacrificed in an appropriate manner, the femur or tibia was stripped out, and both ends of the bone were cut with scissors to expose the internal cavity.
2、 Flush out the bone marrow in the inner cavity by sucking the appropriate amount (depending on the size of the animal) of homogenate irrigating solution with a syringe.
3、 The suspension was collected into a suitable centrifuge tube, blown repeatedly to form a single cell suspension, and filtered through a 70μm cell screen (self supplied).
4、 At 450g, the suspension was centrifuged for 10min and the supernatant was discarded.
5、 A single cell suspension with a cell concentration of 2×10^{8}-1×10^{9}/ml was resuspended with the sample dilution for later use (in the case of mice, 1mL of sample dilution was generally used to resuspend bone marrow cells).

Protocols *(only for reference)* :

The whole process of samples, reagents and experimental environment should be carried out at 20±2℃.
1、 Take a centrifuge tube and carefully add solution 1 and solution 2 in turn (the volume ratio is 3:1, and the volume ratio of the total reagent to the cell suspension is 2:1). If the cell suspension is 2ml, then add 1:3ml of separation solution and 2:1ml of separation solution successively. The total amount of reagent should be at least 4ml.) The gradient interface is made, and the layering of each liquid level must be clear.
2、 Carefully absorb the cell suspension with a straw and add it to the liquid surface of the separation liquid, the horizontal rotor is 400-500g, and the centrifugation time is 20-30minutes(Note: the centrifugation conditions are determined according to the sample volume, the more the blood sample volume, the greater the centrifugal force and the longer the centrifugation time, the specific centrifugation conditions need to be found by the customer to achieve the best separation effect).
3、 After centrifugation, the centrifuge tube is divided into six layers from top to bottom. The first layer is the dilution layer. The second layer is the annular milky white monocyte layer (the first white ring and the upper 50% separation solution 2). The third layer was the annular milky lymphocyte layer (lower 50% solution 2 and the second white ring). The fourth layer was clear separation solution 1 liquid layer. The fifth layer is the granulocyte layer. The sixth layer is the red blood cell layer.
4、 Use a straw to carefully draw the second layer into another 15ml centrifuge tube. Add 10ml of cleaning solution to the resulting centrifuge tube and mix the cells.

Tel: 400-968-6088 https://www.solarbio.com E-mail: sales-china@solarbio.com
Address: No. 85, Liandong U Valley, Middle Zone, 101102, Tongzhou Dist, Beijing, China

5、 The samples were centrifuged at 250g for 10minutes.
6、 Discard the supernatant.
7、 Use a straw to resuspend the resulting cells in 5-10ml of washing solution.
8、 At 250g, the cells were centrifuged for 10minutes.
9、 Repeat 7, 8, and 9, and discard the supernatant and resuspend the cells with 0.5ml of the corresponding liquid required for subsequent experiments.



Precautions:

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 whole process of samples, reagents and experimental environment should be carried out under the condition of 20±2℃. In order to obtain the best experimental results, it is best to carry out the experiment within 2 hours of sampling. The longer the sample is stored, the worse the cell separation effect is. When the samples were placed for more than 6 hours, the separation effect was worse or even could not achieve the purpose of separation.
C. In this experiment, it is better not to use plastic products with high polymerization materials (such as polystyrene). Instead, non-electrostatic, low-electrostatic centrifuge tubes and glass products without alkali treatment should be used, because electrostatic interaction will lead to cell adhesion, and the surface of alkali treated glass will become rough, which will affect the effect of cell separation.
D. When the amount of separation solution is greater than the sample volume of single cell suspension of organ tissue, the separation effect is better.

Possible problems and solutions:

1、 The possible problems and solutions due to differences in blood viscosity and cell density are shown in the following table:

Situations that arise	Reason for occurrence	Suggested solutions
After centrifugation, the target cells are present in the plasma or diluent layer	The speed is too small or the centrifugation time is too short	Increase or decrease the speed appropriately
After centrifugation, the target cells were present in the separation solution	Excessive rotating speed or centrifugation time	
Dispersion of the white ring layer after centrifugation	Excessive cell density	Adjust cell density
The white ring layer is too shallow or invisible after centrifugation	The cell density is too small	

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2、 The principle of this separation solution for cell separation is density gradient centrifugation, and its density is closely related to temperature and atmospheric pressure. Customers in different areas can adjust the centrifugation conditions according to local conditions. It is recommended to adjust the centrifugal conditions, constant centrifugal time, and adjust the centrifugal speed.

3、 This separation solution in accordance with the international standards, all use pharmaceutical grade raw materials, performance indicators and domestic similar products are slightly different, may appear incomplete erythrocyte sedimentation, can be appropriate to increase the centrifugal speed.

Note: When adjusting the centrifugal conditions, the centrifugal speed should be increased or decreased by 50-100g until the best separation effect is achieved. The minimum centrifugal force should not be less than 400g, and the maximum centrifugal force should not be greater than 1200g. The centrifugation time should be 20-30min.

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