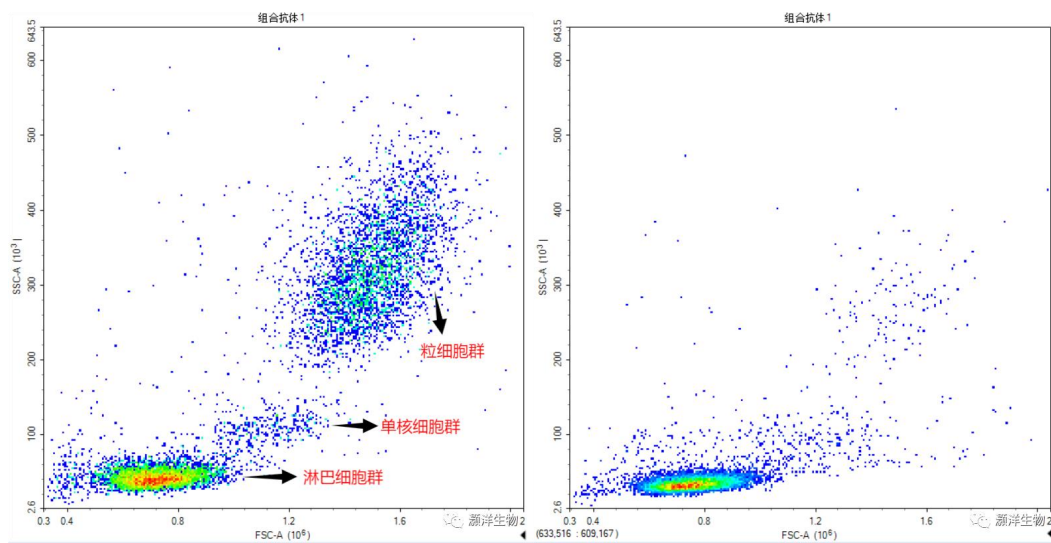


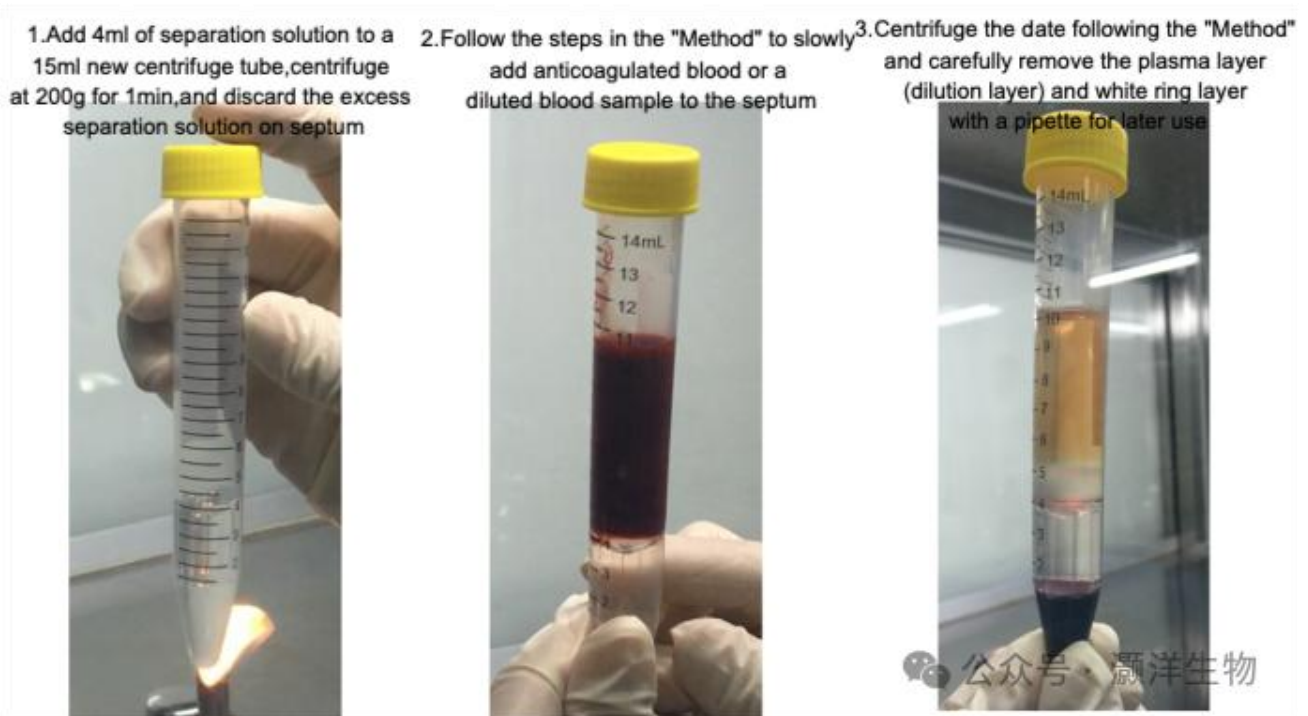
PBMC high-efficiency centrifuge tubes (15ml/50ml)

instructions for use

Product advantages: A septum is placed in the centrifuge tube to facilitate the extraction of PBMC and improve the extraction rate. Flow cytometry after blood splitting versus flow cytometry after separation by sample density separation:

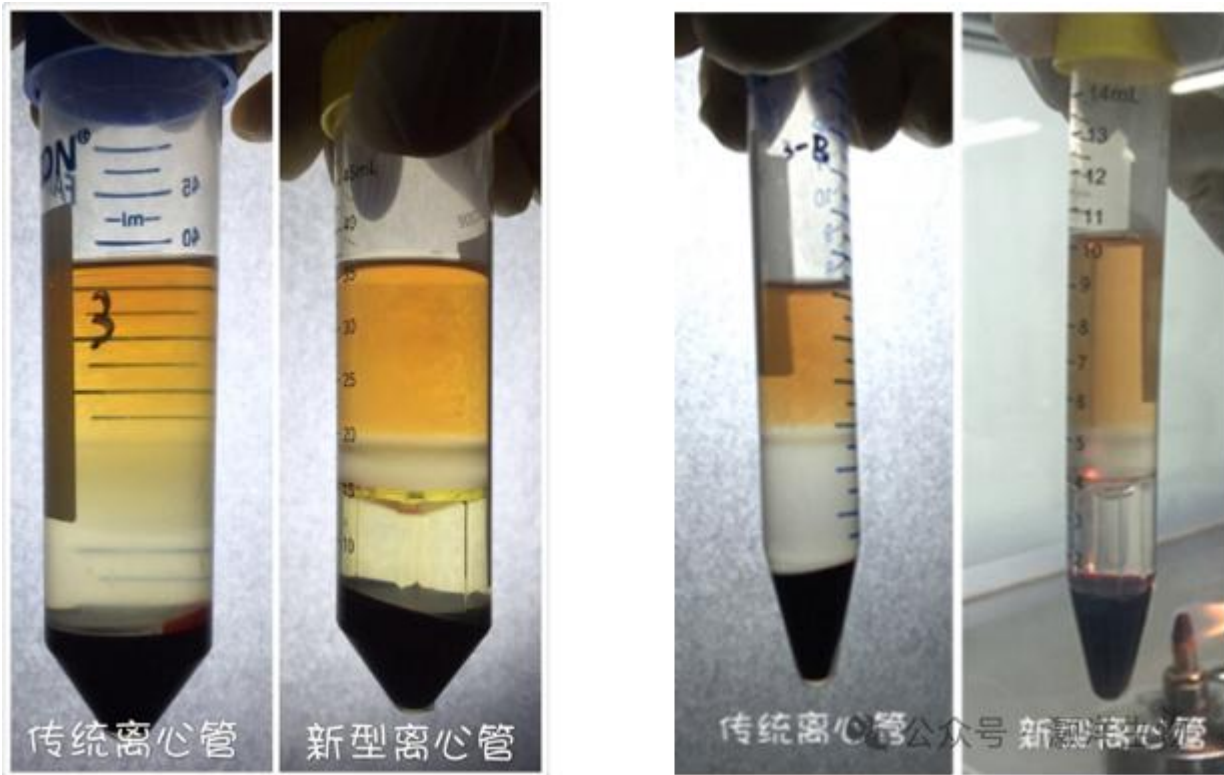


【Separation steps】



【Separation effect of PBMC high-efficiency centrifuge tube】

Under the same conditions, the comparison chart of the separation effect of the traditional centrifuge tube and the high-efficiency centrifuge tube shows that the separation effect of the high-efficiency centrifuge tube is better, and the white ring layer is clear.



Precautions for the use of various separators

一. Centrifuge selection

Swing-out rotor centrifuges must be used, and angular rotor centrifuges must not be used

二. The best sample donor for controlled experiments

1. Healthy adult male blood donors between the ages of 20-60; Avoid using female or umbilical cord blood samples.
2. Healthy women, 5% of women suffer from mild iron deficiency anemia (does not affect daily life), and their red blood cell indicators will affect the separation effect, which may lead to non-sedimentation or incomplete sedimentation of red blood cells. Because umbilical cord blood is mixed with maternal blood, if the mother has mild iron deficiency anemia during pregnancy or because the volume of red blood cells in umbilical cord blood is small, red blood cell residue and incomplete sedimentation will also occur. Therefore, the use of female or umbilical cord donor blood as normal controls is not recommended.

三. Choice of anticoagulant

1. The isolated PBMCs need to be cultured in the next step (such as culturing NK, CIK cells): heparin anticoagulant is selected. Due to the activation of fibrinogen in plasma into active fibrin, fibrin cross-links into fibrin blood clots, which is easy to form cell clumps in PBMCs, which is not conducive to cell activation. Heparin sodium anticoagulants can reduce the chance of these occurring.
2. The isolated PBMCs do not need to be cultured, but are used for other detection: sodium citrate anticoagulant is selected for the best separation effect.

四. Requirements for blood

The optimal time for PBMC isolation is within 2 hours after the blood is separated from the body. If the condition for blood separation is not reached within 2 hours, please be sure to perform the blood separation step within 4 hours, as it is difficult to perform the separation smoothly after 4

hours.

Blood out of vivo time	Separation effect
Within 2 hours	optimum
2-4 hours	acceptable
4-6 hours	Cell viability decreases and the isolation effect is poor
More than 6 hours	The isolation effect is extremely poor, until the cells cannot be isolated

五. The environment in which the separator will be used

1. The separation solution should be stored at room temperature (37 ° C-15 ° C) away from light, and refrigeration and freezing are strictly prohibited;
2. Strictly abide by the aseptic operation specifications when using, and operate at an ambient temperature of 18 ° C-22 ° C, and the separation effect is best under the condition of 20 ° C. Outside of this temperature range, the density of the separator may change, resulting in poor separation.

六. Blood sample dilution method

1. Dilution method: 1 part of PBS or normal saline plus 2 parts of blood for dilution (PBS: anticoagulant blood = 1:2);
2. Dilution requirements: Blood dilution with buffer or medium that does not contain calcium and magnesium ions.

七. How to use centrifuge tubes of various sizes

1. 15 ml centrifuge tubes (including high-efficiency centrifuge tubes):
 - 1) Optimal ratio: 5ml of separating solution + 4ml of diluted blood
 - 2) Optimal centrifugation conditions: 500g centrifugation for 25min at 20 ° C

3) When the centrifuge is used, it is adjusted to slow rise and fall (please consult the centrifuge manufacturer for specific parameters)

It is recommended that the time of speed increase (referring to the time of starting → reaching the set centrifugal force) and the time of speed reduction (referring to the completion of the set centrifugal time → complete stop of the machine) are controlled at about 3 minutes.

2. 50 ml centrifuge tubes (including high-efficiency centrifuge tubes):

1) Optimal ratio: 20ml of separating solution + 20ml of diluted blood

2) Optimal centrifugation conditions: 650g centrifugation for 30min at 20 ° C

3) When the centrifuge is used, it is adjusted to slow rise and fall (please consult the centrifuge manufacturer for specific parameters)

It is recommended that the time of speed increase (referring to the time of starting → reaching the set centrifugal force) and the time of speed reduction (referring to the completion of the set centrifugal time → complete stop of the machine) are controlled at about 3 minutes.

3. Undiluted anticoagulant apheresis method (**recommended for cell culture or plasma preservation**):

Use 50 ml centrifuge tubes (or 50 ml high-efficiency centrifuge tubes)

1) The optimal ratio of 50ml ordinary centrifuge tubes: 20ml of separation solution + 20ml of anticoagulant blood;

The optimal ratio of 50ml high-efficiency centrifuge tubes: 15ml of separating solution + 20ml of anticoagulant blood;

2) Optimal centrifugation conditions: 600g centrifugation for 30min at 20 ° C;

3) Obtain plasma with high purity: After centrifugation, aspirate the plasma layer from top to bottom 2/3 of the plasma, the lower 1/3 of the plasma may contain part of PBMC and a small amount

of fraction of liquid components.

Use a 15 ml centrifuge tube (or a 15 ml high-efficiency centrifuge tube).

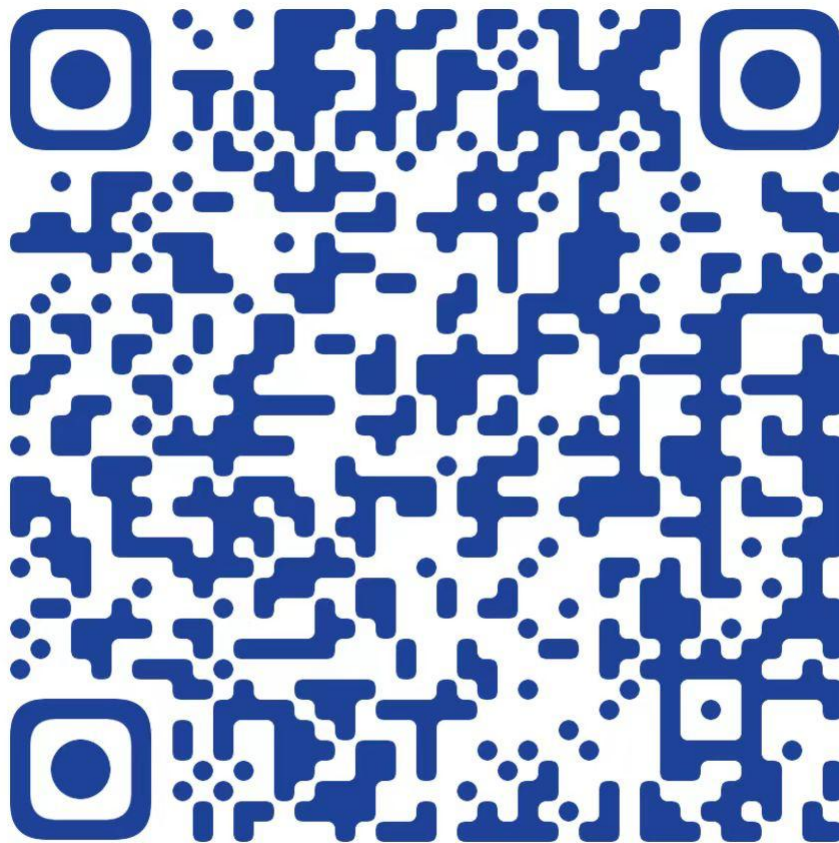
1) The optimal ratio of 15ml ordinary centrifuge tubes: 5ml of separating solution + 5ml of anticoagulant blood;

The optimal ratio of 15ml high-efficiency centrifuge tubes: 4ml of separating solution + 5ml of anticoagulant blood;

2) Optimal centrifugation conditions: 600g centrifugation for 25min at 20 ° C;

3) Obtain plasma with high purity: after centrifugation, aspirate the plasma layer from top to bottom 2/3 of the plasma, the lower 1/3 of the plasma may contain part of PBMC and a small amount of separating fluid components;

4) If the amount of blood collected is small, a small amount of separation is required, the amount of separation solution shall not be less than 3ml, and the amount of anticoagulant blood shall be between 0.2-3ml.



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