ACE Biolabs
Technical support: order@acebiolab.com
Phone: 886-3-2871151

Datasheet

Ver.1 Date : 20180207

Lymphocyte Separation Medium

Cat# CE1036 200 ml rxn

Storage at 4-25℃ for 2 year. Avoid light. **Do not freeze Lymphocyte Separation Medium**

INTRODUCTION

The ACE Biolabs Lymphocyte Separation Medium is designed for the separation of the portion of vital mononuclear cells from whole blood, buffy coats, bone marrow and several other starting materials, e.g. crude cell preparations, by means of low-density gradient centrifugation. Lymphocyte separation Medium has a density of 1.077 g/ml at 20°C. It is sterile, ready-to use and based on Ficoll 400 and sodium diatrizoate providing optimal physiological parameters and low cytotoxicity.

PHYSICAL PROPERTIES

Appearance: Clear solution
Density: 1.077±0.001g/mL
Osmotic pressure: 290±15mOsm

PROTOCOL

1. Restore Lymphocyte Separation Medium and all the buffer used for separation to room temperature.
2. Use a centrifuge tube to collect blood, and add the same amount of saline, Hanks solution or PBS without Calcium and Magnesium. (The blood can also be undiluted.)
3. Take a 15 mL centrifuge tube, add 3 mL of Lymphocyte Separation Medium, and slowly add diluted blood sample in step 2 to the upper layer of Lymphocyte Separation Medium
   Note: It is recommended to spread the blood sample at a moderate speed, especially at the beginning. Avoid damaging the interface between the Lymphocyte Separation Medium and the blood sample.
4. Centrifuge at 800 g for 20 min at room temperature
5. After centrifugation, the centrifuged solution is divided into 4 layers from top to bottom: plasma layer - **white membrane layer** - lymphocyte separation liquid layer-sedimentation of red blood cells and granulocytes. No need to discard the upper liquid, use a Pasteur tube to carefully draw the **white membrane layer** into another new centrifuge tube.
   Note: Due to the sample variation, some blood samples still have some red blood cells and granulocytes cannot be completely precipitated and remaining in the separation liquid layer after centrifugation. Therefore, when using the Pasteur tube to suck the cells of the white membrane layer, try to suck as little as possible into the separation liquid layer.
6. Resuspend **white membrane layer** containing peripheral blood mononuclear cell (PBMC) with

10 mL saline, Hanks solution or PBS without calcium and magnesium, then, centrifuge at 300 g for 10 min.

7. Repeat operation step "6" once, the precipitate is PBMC.

NOTICE

1. This product is sensitive to light. Continuous exposure of this product to light will separate the iodide ions in sodium diatrizoate and affect the separation effect. However, during daily use, this effect is negligible.
2. If the product is stored in the refrigerator, it needs to be restored to room temperature before use.
3. The freshness of the blood sample is closely related to the separation effect. In order to obtain the best separation effect and cell viability, it is best to perform the separation operation **within 2 hours after the blood is drawn**. The longer the storage time of the blood sample, the worse the separation effect.
4. Due to the sample variation, in order to obtain the best separation effect, it may be necessary to adjust the centrifugal force and centrifugation time, but usually the maximum centrifugal force does not exceed 1000 g.

PRODUCT USE LIMITATION

These products are intended for research use only.