

Leucosep™ Tubes



For use in the collection and separation of peripheral mononuclear cells from whole blood

PACKAGE INSERT

For *In Vitro* Diagnostic Use

This Package Insert covers use of:

Leucosep™ Tubes (Catalogue number: LTK.615)

Intended Use

Leucosep tubes are intended for use in the collection and separation of peripheral blood mononuclear cells (PBMCs) from whole blood. The tubes facilitate the separation of PBMCs from whole blood using a polysucrose-based gradient separation solution, prior to the use of these cells in ELISPOT assays.

Introduction

ELISPOT techniques utilise T cells in PBMCs that have been separated from whole blood. One method for separating these cells requires the use of a polysucrose-based gradient separation solution. This procedure involves a number of steps which should be carried out with care to ensure optimal results. The use of Leucosep tubes reduces the number of steps, makes the procedure more robust and reduces the amount of time required to perform the separation procedure.

Principle of Method

Leucosep tubes have been developed to achieve optimal separation of PBMCs from human whole blood by means of density gradient centrifugation. Leucosep tubes incorporate a porous barrier in a transparent polypropylene tube. This biologically inert barrier consists of high grade polyethylene. The Leucosep tube eliminates the time-consuming and laborious layering of the sample material over a polysucrose-based gradient separation solution. Anticoagulated whole blood is mixed with culture media and is then poured into the Leucosep tube. The porous barrier prevents mixing of the blood with the separation medium. During centrifugation, PBMCs are separated from unwanted erythrocytes due to their differing densities and collected in the interface above the separation medium. When separation is complete, the barrier prevents recontamination of the PBMC layer during its removal.

Warnings and Precautions

1. For *In vitro* diagnostic use only.
2. For professional use only; operators must be trained in this procedure.
3. Blood samples should be considered as potentially hazardous. Care should be taken when handling material of human origin.
4. Handling of whole blood samples and assay components, during use, storage and disposal should be in accordance with procedures defined in appropriate national biohazard safety guidelines or regulations.
5. Any deviation from recommended procedures for pipetting, washing techniques, centrifugation times and/or temperatures may alter the purity of PBMC isolations and influence subsequent test results.
6. Do not collect blood in Cell Preparation Tubes (CPT™, Becton Dickinson) or EDTA blood collection tubes, as they are incompatible with Leucosep tubes.
7. Do not refrigerate or freeze whole blood samples. Store and transport blood samples to the laboratory at temperatures between 18-25 °C or 10-25 °C if samples are to be treated with the T-Cell *Xtend*® reagent.
8. Blood should not be stored for longer than 8 hours, or 32 hours if later treated with the T-Cell *Xtend* reagent.
9. Only use single-use containers for venous blood specimen collection.
10. Do not use beyond expiration date.
11. Use aseptic techniques when using this product.
12. Do not store Leucosep tubes in direct light.
13. Do not use if the Leucosep tubes are damaged or if the polysucrose-based gradient separation solution has deteriorated, as indicated by the appearance of a distinct yellow colour or particulate matter in the clear solution.
14. Do not reuse Leucosep tubes. Each tube is designed for single use.
15. Follow blood specimen collection tube instructions for whole blood collection.

Materials Provided

Each box contains:

50 Leucosep tubes pre-filled with a polysucrose-based gradient separation solution.

CD containing the Package Insert and MSDS.

Storage and Stability

Store at 4-30 °C unopened until the expiration date shown on the box. Store the opened product at room temperature (18-25 °C) and use within 12 weeks of opening the outer bag, or before the expiration date on the box, whichever is earlier.

Equipment and Materials Required but not Provided

1. Heparinised blood collection tubes.
2. AIM-V® and RPMI cell culture media.
3. 15 mL centrifuge tubes.
4. A centrifuge for the fractionation of PBMCs capable of at least 1000 RCF (g) and able to maintain the samples at ambient room temperature (18-25 °C).
5. Biosafety Level 2 (BL 2) cabinet (recommended).
6. Pipettes and sterile pipette tips.
7. ELISPOT Kit.
8. T-Cell *Xtend* reagent may be required if samples older than 8 hours are used.

Procedure

Note: The following steps should be performed using the principles of Good Laboratory Practice:

1. Ensure that the required number of Leucosep tubes are at room temperature (18-25 °C) prior to use.
2. If the polysucrose-based gradient separation solution has collected above the porous barrier (frit), centrifuge the Leucosep tubes at 350 x g for one minute to move the polysucrose-based gradient separation solution below the frit.
3. Invert the whole blood sample then dilute at a ratio of 5 parts blood to 3 parts AIM-V or RPMI cell culture medium in a 15 mL centrifuge tube. Invert the centrifuge tube several times to mix.
4. Pour the diluted blood directly into the Leucosep tube and centrifuge at 1000 x g for 10 minutes at 18-25 °C with no brake.
5. Aspirate the cloudy PBMC layer and make up to ~10 mL with AIM-V or RPMI in a 15 mL centrifuge tube. Invert the centrifuge tube to mix.
6. Centrifuge at 600 x g for 7 minutes. Discard the supernatant.
7. Resuspend the cell pellet with AIM-V or RPMI, make up to ~10 mL with AIM-V or RPMI and centrifuge at 350 x g for a further 7 minutes.
8. Discard the supernatant and resuspend the cell pellet in the volume of AIM-V (**not** RPMI) required by the ELISPOT assay.
9. If cells are required for antigen stimulation in an ELISPOT assay, dilute cells accordingly with AIM-V (**not** RPMI).

Limitations

1. As with other separation methods, Leucosep tubes may alter the proportion of some PBMC subsets (e.g. T and B cells) from those in non-separated whole blood. This alteration is believed to be relatively insignificant in normal cases.

Quality Control

As part of an individual laboratory's quality control activity, cell counting methods should be designed and validated to ensure that sufficient PBMCs have been obtained for the relevant test system. In addition, quality control activities should employ the use of positive and negative controls developed to ensure the expected performance of the T cells within the relevant test system.

Performance Characteristics

Fresh Blood

6 mL of blood was obtained from 15 donors. Samples from each donor were processed within 8 hours of venepuncture. Samples were processed using Leucosep tubes filled with polysucrose-based separation media. Isolated cells were viable and functional in the ELISPOT assay.

Stored blood using T-Cell *Xtend* Reagent

Blood samples from 205 donors were obtained and used for assaying cell recoveries using Leucosep tubes filled with polysucrose-based separation media. 6 mL of blood was collected per donor and blood was processed between 29-32 hours post venepuncture using T-Cell *Xtend* reagent.

Isolated cells were viable and functional in the ELISPOT assay.

Note: it is recommended that 6ml of blood is used per donor. A decrease in the blood volume may impact cell recoveries.

Reporting of Serious Incidents

If a serious incident has occurred in relation to this device, it should be reported to Customer Service. In European Union Member States, serious incidents should also be reported to the competent authority (the government department responsible for in vitro diagnostic medical devices) in your country. Please refer to your government website for details of how to contact your competent authority. A 'serious incident' means any incident that directly or indirectly led, might have led or might lead to:

- the death of a patient, user or other person;
- the temporary or permanent serious deterioration of a patient's, user's or other person's state of health;
- a serious public health threat.

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Troubleshooting Guidance in the Collection and Separation of PBMCs for ELISPOT

Problem	Potential Cause	Possible Solution
Low cell yield	<p>Leucopenia</p> <p>Incorrect blood collection</p> <p>Blood collection tube is not at ambient room temperature (18-25 °C)</p> <p>Blood storage is not at the required temperature</p> <p>Use of volumes or dilutions other than those recommended</p>	<p>Add an additional blood collection tube</p> <p>CPT and blood collection tubes containing EDTA are not compatible with Leucosep tubes</p> <p>Allow blood collection tube to equilibrate to room temperature</p> <p>Make sure blood shipment is at 18-25 °C or 10-25 °C if T-Cell <i>Xtend</i> reagent is used</p> <p>Ensure correct instructions are followed</p>
Red blood cell contamination	<p>Blood storage is not at the required temperature</p> <p>Blood collection tube is not at ambient room temperature (18-25 °C)</p> <p>Blood is not diluted</p> <p>Inversion of blood sample within Leucosep tube</p> <p>Incorrect centrifugation</p> <p>Sample separation incomplete</p>	<p>Ensure blood is transported at 18-25 °C or 10-25 °C if T-Cell <i>Xtend</i> reagent is used</p> <p>Allow blood collection tube to equilibrate to room temperature</p> <p>Ensure blood is diluted according to the procedure</p> <p>Ensure that Leucosep tube remains upright prior to centrifugation</p> <p>Ensure centrifugation instructions are followed</p> <p>Check the centrifuge is capable of refrigeration at 18-25 °C</p> <p>Check the centrifuge brake is switched off</p> <p>Increase centrifugation step by 10 min.</p>
No defined or distinct mononuclear layer	<p>Sample separation incomplete</p> <p>Centrifuge is not correctly calibrated</p> <p>Incorrect centrifugation</p> <p>Hyperlipemic sample</p>	<p>Increase centrifugation step by 10 min.</p> <p>Have centrifuge calibrated</p> <p>Ensure centrifugation instructions are followed</p> <p>Collect fasting blood sample</p>
Invalid results	Invalid results can be caused by a number of incorrect sample handling issues	Refer to the sections above

Glossary of symbols

	Use by/Expiration date (Year-Month-Day)
	Lot number
	Catalogue number
	Attention, see instructions for use
	Manufacturer
	Sufficient for “n” tests
	<i>In vitro</i> diagnostic device
	Temperature limitation/Store between
	Consult instructions for use
	EU Authorised Representative

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Leucosep is a trademark of Greiner Bio-One
AIM-V is a trademark of the Life Technologies Corporation.
CPT is a trademark of Becton, Dickinson and Company.

The use of the T-Cell *Xtend* reagent is protected by the following patents:
EP2084508, US9090871, CN101529221, AU2007-303994, JP5992393, IN289117, CA2665205

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Revision Number	Date of Issue	Modifications
1 - 4	Details available upon request from Oxford Immunotec.	
5	June 2022	Change of manufacturer address. Addition of revision history. Addition of instructions to report serious incidents and details for EC REP and EU Importer.
6	October 2022	Remove EU Importer.
7	November 2023	Removal of ‘a PerkinElmer company’ from logo



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