T-Cell *Select*™

Oxford Immunotec

For use in the preparation of purified mononuclear immune cells directly from whole blood

PACKAGE INSERT

For *In Vitro* Diagnostic Use

This Package Insert covers use of:

T-Cell *Select* (Catalogue number: TSK.910)

Intended Use

The T-Cell *Select*™ reagent kit is intended for the isolation of mononuclear immune cells from whole blood, using positive selection via a magnetic bead-based cell separation system, for use in cell-mediated immune assays.

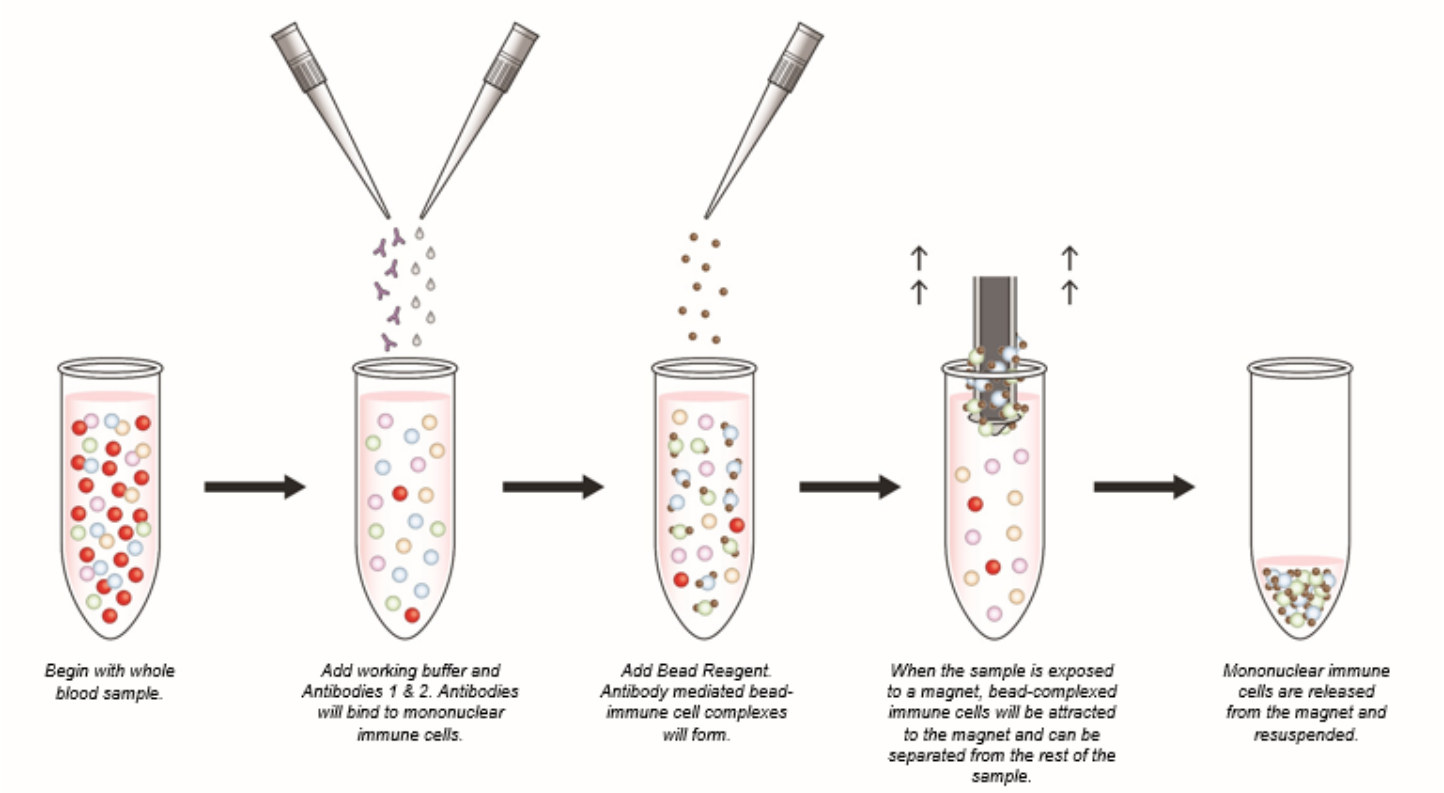
Introduction

For cell-mediated immune assays, such as enzyme-linked immunospot (ELISPOT) assays, peripheral blood mononuclear cells (PBMCs) are typically isolated using density gradient centrifugation methods. The T-Cell *Select* reagent kit allows PBMCs to be isolated using positive selection of the mononuclear immune cells with a magnetic bead-based cell separation system. The use of magnetic separation systems enables automation of the cell isolation process, which significantly reduces the hands on time required for sample preparation.

Cells isolated from blood stored for up to 54 hours using T-Cell *Select* reagent kit produce equivalent results to those isolated using density gradient centrifugation and T-Cell *Xtend*® reagent as assessed in the T-SPOT®.*TB* test.

Principle of Method

The use of the T-Cell *Select* reagent kit improves the logistics and workflow of preparing PBMCs for use in ELISPOT assays. The kit contains a proprietary set of reagents consisting of buffer concentrate, antibodies, and superparamagnetic beads. Diluted T-Cell *Select* buffer is added to the whole blood sample to facilitate cell purification and reduce red blood cell contamination, and then antibodies are added which bind to the requisite immune cells in the sample. Addition of superparamagnetic beads results in formation of complexes with the antibodies attached to the immune cells. The magnetic properties of the beads are utilized, with the aid of a suitable, validated magnetic separation system, to isolate the PBMCs from the sample for subsequent use in the ELISPOT assay. Laboratories should validate the positive selection method on their own specific equipment, including appropriate blood volumes and number of cycles that samples are exposed to the magnet.



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 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, incubation times and/or temperatures may influence test results.
6. Do not collect blood in EDTA blood collection tubes.
7. Do not refrigerate or freeze whole blood samples. Store and transport blood samples to the laboratory between 18 and 25 °C.
8. Do not dilute or add other components directly to the T-Cell *Select* reagents beyond the instructions in this package insert.
9. Validate any laboratory equipment via the laboratory's protocol before using the T-Cell *Select* reagent kit.
10. Only use single-use containers for venous blood specimen collection.
11. Do not mix different reagents from different lots in a single patient sample.
12. Do not use beyond the expiration date.
13. Do not use with a whole blood sample that has been stored for more than 54 hours.
14. Use aseptic techniques when using this product.
15. Do not use if the vials appears to be damaged or already opened.
16. Do not use if fluid within vials appears discoloured or has precipitate.
17. Antibody 1 and Antibody 2 contain substances of animal origin which are potentially infectious. Under normal conditions of use, these substances do not come into contact with the user.

Materials Provided

Each box contains:

144 test kit*

Reagent	Quantity
Buffer Concentrate (BU.910)	1 x 50 mL
Antibody 1 (AC.910)	3 x 2 mL
Antibody 2 (AH.910)	3 x 2 mL
Bead Reagent (BR.910)	1 x 10 mL

**please see Limitations section for an important note on test quantity per kit*

Storage and Stability

Store unopened kits of T-Cell *Select* at 2 to 8 ˚C until the expiration date shown on the box. Store opened and resealed vials of the T-Cell *Select* reagents at 2 to 8 ˚C, and use within 4 weeks of opening unless this period exceeds the expiration date on the box. Do not mix components between different kit lots.

Equipment and Materials Required but not Provided

1. Blood collection tubes; use of lithium or sodium heparin tubes or sodium citrate tubes is recommended.
2. Distilled or deionized water.
3. Sterile serum free cell culture medium, such as Gibco® AIM-V® Medium.
4. RPMI 1640 Medium.
5. Suitable magnetic particle purification equipment should be validated by the laboratory for use, such as:
   - A submerged magnetic rod head instrument, compatible for use with a deep-well microplate
   - A block magnet with test tube cavity
   - A plate magnet base, compatible for use with a deep-well microplate
6. Tubes or containers compatible with the laboratory's magnetic separation system.
7. Pipettes and sterile pipette tips.
8. Timer.
9. Biosafety Level 2 (BL 2) cabinet (recommended).

Procedure

Individual laboratories should validate their procedures for collection and separation of mononuclear immune cells to obtain sufficient numbers. This procedure demonstrates the T-Cell *Select* isolation method for use with laboratory-validated equipment. The blood volume per sample should be validated with the equipment used. Steps 11, 12 and 16 (and any additional cycles of magnetic exposure) can be optimized for the magnetic system used within the timings provided. Validation should ensure that appropriate quantities of PBMCs are isolated for use in the ELISPOT assay required.

Note: The following steps should be performed using the principles of Good Laboratory Practice. Ensure all reagents are at room temperature prior to use.

1. Thoroughly mix blood sample.
2. Aliquot blood into a container compatible with your laboratory's magnetic separation system. Typically, 3.5-5 mL of blood per sample is used in order to obtain sufficient numbers of cells for use in ELISPOT procedures.
3. Dilute T-Cell *Select* Buffer Concentrate in distilled or deionized water in the ratio 2:3, buffer concentrate: distilled or deionized water.
4. Add diluted T-Cell *Select* Buffer to whole blood in the ratio 1:7, buffer: blood.
5. Add 10 µL of Antibody 1 per mL of the combined volume of whole blood and buffer.
6. Add 10 µL of Antibody 2 per mL of the combined volume of whole blood and buffer.
7. Mix sample thoroughly.
8. Incubate sample for 15 minutes at room temperature.
9. Thoroughly mix the Bead Reagent immediately before use. Add 15 µL of Bead Reagent per mL of the combined volume of whole blood and buffer.
10. Mix sample gently.
11. Incubate sample for 15 minutes at room temperature mixing at least every 5 minutes.
12. Expose sample to a suitable magnet for a minimum of 10 minutes (Note: the incubation time needs to be validated in conjunction with the magnet system used). Antibody-labelled cells will migrate towards the magnet.
13. Discard supernatant according to your laboratory procedure while taking care to retain the beads with attached cells that have migrated to the magnet.
14. Remove sample from magnetic field.
15. Add a volume of RPMI equal to the original volume of whole blood and diluted T Cell *Select* buffer to the remaining cells. Mix to resuspend the sample.
16. Expose sample to a suitable magnet for a minimum of 10 minutes (Note: the incubation time needs to be validated in conjunction with the magnet system used). Antibody-labelled cells will migrate towards the magnet.
17. Discard supernatant according to your laboratory procedure while taking care to retain the beads with attached cells that have migrated to the magnet.
18. Remove sample from magnetic field.
19. Thoroughly resuspend the sample in AIM-V medium to achieve a final cell concentration of at least 2.5 x 10^6/mL. Sample is ready for cell counting and use in ELISPOT procedures (Note: sample may be exposed to magnet for additional cycles before addition of AIM-V medium if more cycles validated with magnet system used).

Note: Individual laboratories should validate their procedures for collection and separation of mononuclear immune cells to obtain sufficient cell numbers. Laboratories must validate their magnetic separation systems and any other equipment used in the cell isolation procedure. It is recommended that:

- A patient's cells can be pooled, if necessary, to obtain sufficient cells from multiple tubes of blood which have been collected and processed within 54 hours.
- Typically, for immunocompetent adults and children over 2 years old, sufficient cells to run cell-mediated immunoassay procedures can be obtained from 3.5 mL whole blood samples.

For children up to 2 years old, one 2 mL paediatric tube should be used.

Limitations

1. Although the T-Cell *Select* reagent kits are indicated for 144 tests, the number of tests each laboratory is able to perform with each kit will differ. Multiple variables affect the amount of reagents that will be needed and thus the number of tests supported by each kit. Such variables may include laboratory equipment used, starting volume of blood, and number of cycles samples are exposed to the magnet.
2. The T-Cell *Select* reagent kit is intended for use in the isolation of mononuclear cells from whole blood for use in cell-mediated immunoassay procedures. It is not a diagnostic test in itself. Test results should be interpreted in conjunction with the results of the diagnostic assay being utilized.

Quality Control

- In-house testing has shown no significant difference in mononuclear immune cell populations obtained from whole blood with use of the T-Cell *Select* reagent kit compared to density gradient separation methods.
- As part of an individual laboratory's quality control activity, magnetic separation and cell counting methods should be designed and validated to ensure that sufficient mononuclear immune cells can be obtained for the relevant diagnostic assay.
- Relevant positive and negative controls should be included to ensure that the mononuclear cells prepared by magnetic separation deliver the expected performance in the diagnostic assay.

Performance Characteristics

Clinical studies were conducted to demonstrate T-SPOT.*TB* test performance using cells isolated with the T-Cell *Select* reagent kit from blood stored for up to 54 hours post venepuncture in both a high and a low endemic setting. Split samples were processed using the T-Cell *Select* reagent kit (0-54 hours storage time) and density gradient centrifugation (0-32 hours storage time).

Overall agreement for the clinical study data between the T-SPOT.*TB* test between the T-Cell *Select* reagent kit and density gradient separation method was 97 % (644/664) [95 % CI 95.4-98.2 %].

Amongst the small number of discordant results between the methods, it was observed that a number of negative results from samples with cells isolated by the standard density centrifugation method which were positive when using the T-Cell *Select* reagent kits, were subsequently determined to be microbiologically confirmed TB positives (n=6). This indicates that the positive selection cell isolation method may confer improved sensitivity on the T-SPOT.*TB* test.

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.

Contact Information

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For product support downloads and further technical information, please visit our website:
www.oxfordimmunotec.com

Glossary of Symbols

	Use by/Expiration date (Year-Month-Day)
LOT	Lot number
REF	Catalogue number
	Attention, see instructions for use
	Manufacturer
	Sufficient for “n” tests
IVD	*In vitro* diagnostic device
	Temperature limitation/Store between
	Consult instructions for use
	Keep away from sunlight
EC REP	EU Authorised Representative

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