T-Cell *Xtend*®

Oxford Immunotec

For use in the preparation and isolation of purified lymphocytes directly from whole blood

PACKAGE INSERT

For *In Vitro* Diagnostic Use

This Package Insert covers use of:

T-Cell *Xtend* (Catalogue number: TTK.610)

Intended Use

The T-Cell *Xtend* reagent is intended for use in the preparation of T cells purified from whole blood. The reagent will enable laboratories to isolate lymphocytes from whole blood for up to 32 hours following venepuncture for assay by a commercial ELISPOT procedure.

Introduction

In general, ELISPOT techniques have been validated for the processing of blood samples within 8 hours of venepuncture. This timescale for blood handling may impact on laboratory staff and procedures by restricting the work flow for conducting the assay. The incorporation of the T-Cell *Xtend* reagent into the ELISPOT process leads to increased flexibility for the laboratory. Blood samples may be shipped and/or stored overnight with no impact on T cell function or performance.

Principle of Method

The use of the T-Cell *Xtend* reagent, as an aid in the separation of lymphocytes from whole blood, improves the logistics of processing ELISPOT assays with stored samples. T cells isolated from whole blood stored overnight appear to show reduced responses to stimulation with antigens in ELISPOT assays, but this is primarily due to contaminating cell populations in the Peripheral Blood Mononuclear Cell (PBMC) layer. The T-Cell *Xtend* reagent contains bispecific monoclonal antibodies which are directed against cell surface markers on selected inhibitory white blood cells and red blood cells. The T-Cell *Xtend* reagent cross-links the selected white blood cells with the red blood cells, which increases the density of the selected cells. When a density gradient is applied during FICOLL extraction, the selected white blood cells remain separated in the red blood cell layer away from the PBMC layer. Non-selected cells, including T cells and antigen presenting cells, are contained in the PBMC layer. Studies have shown that the functionality of T cells, prepared using the T-Cell *Xtend* reagent after overnight storage of blood, is comparable to that obtained from fresh blood.

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 Cell Preparation Tubes (CPT™, Becton Dickinson) or EDTA blood collection tubes, as they are incompatible with the T-Cell *Xtend* reagent.
7. Do not refrigerate or freeze whole blood samples. Store and transport blood samples to the laboratory between 10-25 ˚C.
8. Add T-Cell *Xtend* reagent to the whole blood prior to sample processing.
9. Do not dilute or add other components directly to the T-Cell *Xtend* reagent.
10. Only use single-use containers for venous blood specimen collection.
11. Do not mix different lots in a single patient sample.
12. Do not use beyond expiration date.
13. Do not use with a whole blood sample that has been stored for more than 32 hours.
14. Use aseptic techniques when using this product.
15. Do not use if the vials appear to be damaged or already open as first usage.
16. Do not use if the fluid within vials appears discoloured or has precipitate.
17. T-Cell *Xtend* contains 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:

Three (3) 2 mL vials of T-Cell *Xtend* monoclonal antibodies (TT.610).

Storage and Stability

Store unopened vials of the T-Cell *Xtend* reagent at 2-8 ˚C, until the expiration date shown on the box. Store opened and resealed vials at 2-8 ˚C and use within 12 weeks of opening, unless this period exceeds the expiration date on the box.

Equipment and Materials Required but not Provided

1. Heparinised blood collection tubes.
2. FICOLL or alternative PBMC separation materials, i.e. Accuspin™ and Leucosep tubes.
3. A centrifuge for the isolation of PBMCs capable of at least 1800 RCF (g) and able to maintain the samples at ambient room temperature (18-25 ˚C), if using density centrifugation methods to separate the PBMCs.
4. Biosafety Level 2 (BL 2) cabinet (recommended).
5. Pipettes and sterile pipette tips.
6. ELISPOT kit.

Procedure

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

1. Collect whole blood in lithium heparin blood collection tubes and store for up to 32 hours at 10-25 ˚C.
2. Immediately prior to use in a commercial ELISPOT assay, add 25 µL of the T-Cell *Xtend* reagent per mL of whole blood by removing the collection tube cap and pipetting in the recommended volume.
3. Replace the cap and gently invert the blood collection tube 8 to 10 times.
4. Incubate the whole blood with the T-Cell *Xtend* reagent for 20 ± 5 minutes at ambient temperature (18-25 ˚C).
5. Isolate the PBMC fraction using FICOLL density gradient centrifugation or an alternative PBMC isolation method.
6. Prepare PBMCs for the ELISPOT assay following the ELISPOT kit manufacturer's instructions for use.

Note: Individual laboratories should validate their procedures for collection and separation of PBMCs to obtain sufficient numbers. It is recommended that:

- Blood samples are collected into lithium heparin blood collection tubes with PBMCs being subsequently separated using standard separation techniques, such as a FICOLL density gradient. Alternative methods to purify the PBMC fraction may be employed if desired, e.g., Accuspin or Leucosep tubes pre-filled with FICOLL.
- A patient's cells can be pooled, if necessary, to obtain sufficient cells from multiple tubes of blood which have been collected and processed concurrently.

Typically, for an immunocompetent patient, sufficient PBMCs to run the assay can be obtained from venous blood samples according to the following guidelines:

- Adults and children over 2 years old: one lithium heparin 6mL tube
- Children up to 2 years old: one 2mL pediatric tube

Reagent Preparation

The T-Cell *Xtend* reagent is supplied ready to use. No reagent preparation is required.

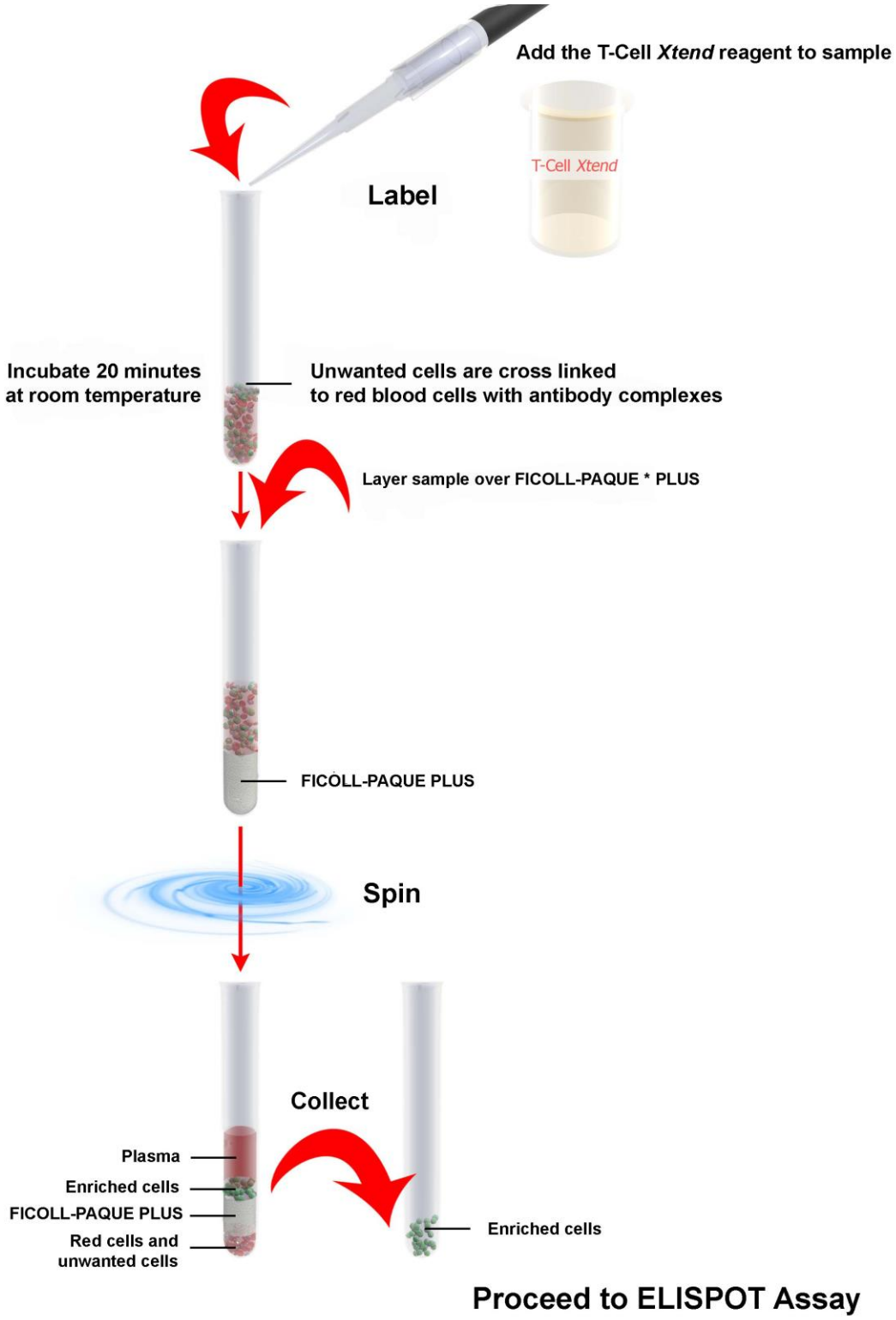

Figure 1: *Diagram showing how the T-Cell Xtend reagent should be incorporated into an ELISPOT protocol for use with stored/shipped whole blood up to 32 hours post-venepuncture.*

Limitations

1. The T-Cell *Xtend* reagent is a diagnostic aid. Test results should be interpreted in conjunction with the results of the diagnostic test being utilized.

Quality Control

In-house testing of the T-Cell *Xtend* reagent has shown no significant decrease in PBMC yields or T cell populations, when comparing whole blood samples stored for less than 8 hours post-venepuncture with whole blood samples stored for up to 32 hours treated with the T-Cell *Xtend* reagent. 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

Clinical studies were conducted with and without the T-Cell *Xtend* reagent added prior to cell separation for the processing of whole blood samples with an ELISPOT assay (T-SPOT®.*TB* test) stored for up to 32 hours post-venepuncture.

Overall agreement for the clinical study data (3 sites) between the T-SPOT.*TB* test with and without the T-Cell *Xtend* reagent was 96.6 % (340/352) [95 % CI 94.1-98.2 %].

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.

Customer Service Contact Information

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www.oxfordimmunotec.com

Troubleshooting Guidance in the Preparation of PBMCs for ELISPOT

Problem	Potential Cause	Possible Solution
Low cell yield	Leucopenia	Add an additional blood collection tube
	Incorrect blood collection	Do not use Cell Preparation Tubes (CPT, Becton Dickinson) or blood collection tubes containing the anticoagulant EDTA
	Blood collection tube is not at ambient room temperature (18-25 ˚C)	Ensure blood collection tube has equilibrated to room temperature prior to sample collection
	Blood storage is not at 10-25 ˚C	Make sure blood shipment is at 10-25 ˚C
	Blood storage is over the recommended time	Collect another blood sample and repeat test
Red blood cell contamination	Blood collection tube is not at ambient room temperature (18-25 ˚C)	Ensure blood collection tube has equilibrated to room temperature prior to sample collection
	Incorrect centrifugation	Increase centrifugation time to 30 minutes
		Check the centrifuge is refrigerated
		Check the centrifuge has a working brake and ensure that these steps are carried out in accordance with the manufacturer's instructions for FICOLL separation
No defined or distinct mononuclear layer	Centrifuge is not correctly calibrated	Have centrifuge calibrated
	Centrifuge speed too low	Increase centrifuge speed to produce 1500-1800 RCF
	Centrifuge time too short	Increase time of centrifugation to 30 minutes
	Hyperlipemic sample	Collect fasting blood sample
Invalid results	Invalid results can be caused by a number of incorrect sample handling issues	Refer to the sections above

Literature References

1. NCCLs procedure H3 – A5, Procedures for the collection of diagnostic blood specimens by venepuncture

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
EC REP	EU Authorised Representative

Manufacturer:
Oxford Immunotec Ltd
143 Park Drive East, Milton Park, Abingdon
Oxfordshire, OX14 4SE, UK
www.oxfordimmunotec.com

EC REP European Union Authorised Representative:
Wallac Oy
Mustionkatu 6,
FI-20750 Turku,
Finland

Revision Number	Date of Issue	Modifications
1 - 6	Details available upon request from Oxford Immunotec.	
7	June 2022	Change of manufacturer address. Addition of revision history. Addition of instructions to report serious incidents, EC REP and EU Importer details.
8	October 2022	Remove EU Importer detail
9	November 2023	Removal of 'a PerkinElmer company' from logo
10	August 2024	Change EU Authorised Representative

PI-TT.610-UK (DOC-717) Ver. 2

Approved By:

(CO-591) Update only to swap out incorrect document attached to record for PI-TT.610-UK

Description

Update only to swap out incorrect document attached to record for PI-TT.610-UK, which was incorrectly published with PI-TS-IVD-UK attached instead of PI-TT.610-UK. Correct document had been reviewed and approved outside of GG.

Justification

It was noted by a member of the QC team that the incorrect document was attached to the GG record for PI-TT.610-UK v1, and as the GG versioning is not in use for Pack Inserts, it is possible to update the record and swap the document for the correct one without full approvals, which were given under CO-451. IFU numbering continued from EtQ due to version traceability for registration purposes as although the change is specific to EU territories, some of the translated and UK IFUs are submitted at registration in ROW territories. This document will remain as it was in the update under CO-451, which is v10.

Assigned To:	Initiated By:	Priority:	Impact:
Sophie Francis	Sophie Francis	Low	Minor

Version History:

Author	Effective Date	CO#	Ver.	Status
Sophie Francis	October 24, 2024 11:30 AM GMT	CO-591	2	Published
Rhea Antony	October 17, 2024 9:42 AM GMT	CO-451	1	Superseded
Jacob Caudle	February 15, 2024 12:01 PM GMT	CO-15	0	Superseded