



T-Cell Xtend[®]



PACKAGE INSERT

For *In Vitro* Diagnostic Use

For use with the T-SPOT[®].TB test only.

Instructional Information

INTENDED USE

The T-Cell *Xtend*[®] reagent is intended for use with the T-SPOT[®].*TB* test for the pre-treatment of whole blood prior to lymphocyte separation. The reagent aids in the removal of selected white blood cells from whole blood stored at room temperature (18-25°C).

SUMMARY & EXPLANATION

The T-SPOT.*TB* test has been evaluated for the processing of blood samples within 8 hours of venipuncture. 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 process leads to increased flexibility for the laboratory. Blood samples may be shipped and/or stored overnight, at room temperature (18-25°C) and processed with the T-Cell *Xtend* reagent from 0 – 32 hours post-venipuncture.

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 of the T-SPOT.*TB* test with stored samples. T cells separated from whole blood stored overnight appear to show reduced responses to stimulation with antigens in the T-SPOT.*TB* test, but this is primarily due to contaminating cell populations in the Peripheral Blood Mononuclear Cell (PBMC) layer. T-Cell *Xtend* contains bispecific monoclonal antibodies which are directed against cell surface markers on selected white blood cells and glycophorin A on the 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 into the red blood cell layer away from the PBMC layer and 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 & PRECAUTIONS

1. T-Cell *Xtend* reagent has not been evaluated for uses other than with the T-SPOT.*TB* test.
2. For *In vitro* diagnostic use only.
3. For professional use only.
4. Do not use reagent beyond the expiration date.
5. Blood samples should be considered potentially hazardous. Care should be taken when handling material of human origin.
6. 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.
7. Observe aseptic technique when using this product to avoid contamination of the reagent.
8. 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.
9. Add T-Cell *Xtend* reagent to the whole blood prior to sample processing.
10. Do not dilute or add other components directly to the T-Cell *Xtend* reagent.
11. Only use single-use containers for venous blood specimen collection.
12. Do not mix different reagent lots.

MATERIALS PROVIDED

Each box contains:

Three (3) 2mL vials of the T-Cell *Xtend* monoclonal antibodies

STORAGE & STABILITY

Store unopened T-Cell *Xtend* reagent at 2-8°C until the expiration date shown on the box. Store the opened and resealed product 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. Lithium heparin blood collection tubes.
2. Ficoll or alternative PBMC separation materials.
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. T-SPOT.*TB* test kit.

PROCEDURE

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

1. Collect whole blood into a lithium heparin blood collection tubes and store between 0 and 32 hours post venipuncture at 18-25°C. Follow the guidelines in the T-SPOT.*TB* test package insert, Section 5, Specimen Collection and Handling Section.
 - a) A patient's cells can be pooled, if necessary, to obtain sufficient cells from multiple tubes of blood which were collected and processed concurrently.
 - b) 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 10 years old and over: one lithium heparin 6 mL tube
 - Children 2-9 years old: one 4 mL tube
 - Children up to 2 years old: one 2 mL pediatric tube
2. Immediately prior to use with the T-SPOT.*TB* test, add 25µL of T-Cell *Xtend* reagent per mL of whole blood collected by removing the 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 room temperature (18-25°C).
5. Isolate the PBMC fraction using Ficoll density gradient centrifugation or alternative PBMC isolation method.
6. Prepare PBMCs for the T-SPOT.*TB* test following the manufacturer's instructions for use.

REAGENT PREPARATION

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

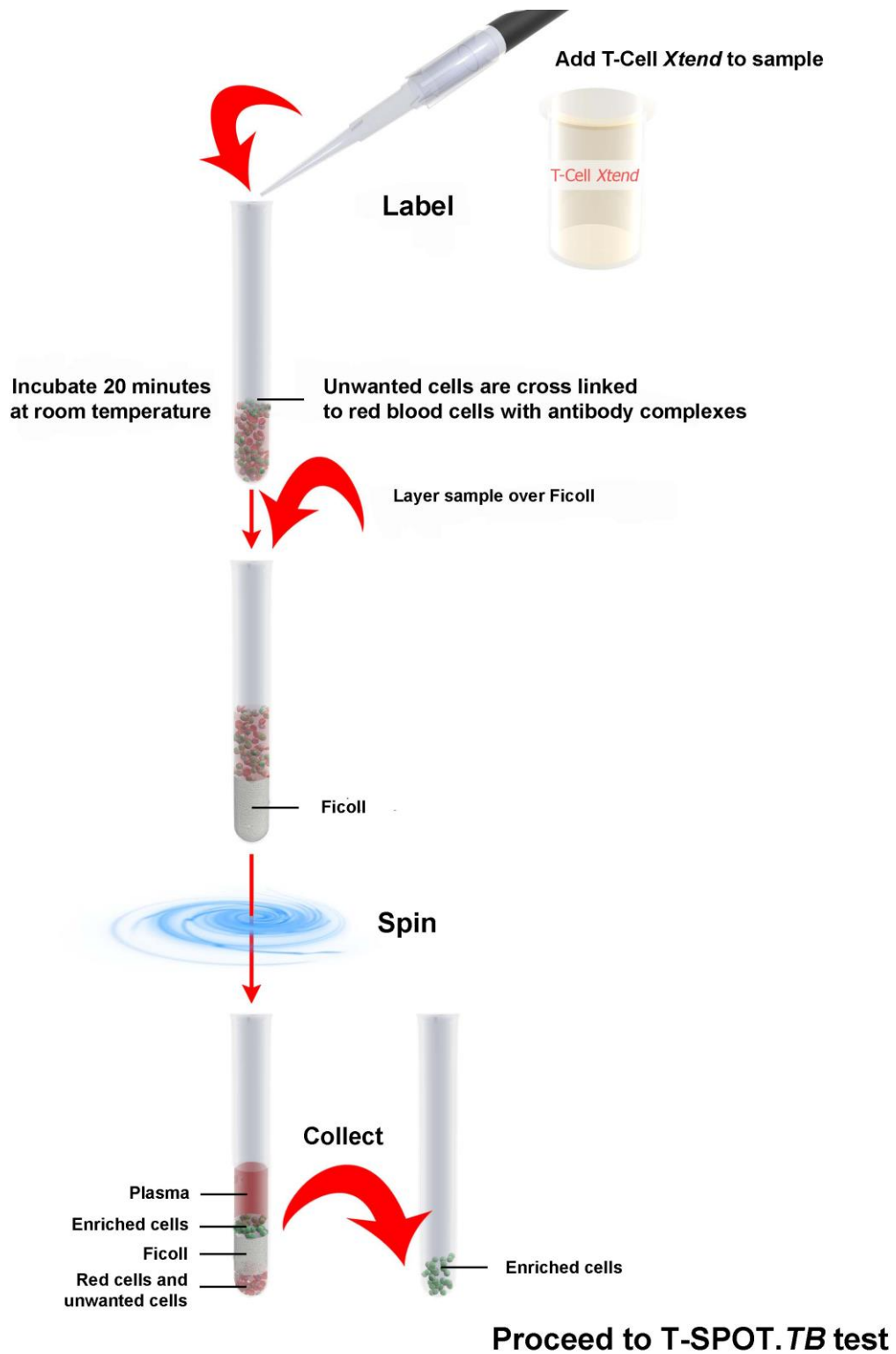


Figure 1: Diagram showing how the T-Cell *Xtend* reagent should be incorporated into the T-SPOT.TB test protocol for use with whole blood samples stored at room temperature (18-25°C) between 0 and 32 hours post-venipuncture.

LIMITATIONS

1. T-Cell *Xtend* reagent has not been evaluated for uses other than with the T-SPOT.*TB* test.
2. Do not allow blood samples to be exposed to temperatures above 25°C as this may result in increased background and other test anomalies.
3. Do not refrigerate or freeze whole blood samples. Store and transport blood samples to the laboratory between 18-25°C.
4. Any deviation from recommended procedures for pipetting, washing techniques, incubation times and/or temperatures may influence test results.
5. The use of T-Cell *Xtend* reagent with the T-SPOT.*TB* test has not been adequately evaluated with blood specimens from individuals younger than 17 years, pregnant women and in patients with hemophilia.

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-venipuncture with whole blood samples stored between 0 and 32 hours post venipuncture 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 assayed within the T-SPOT.*TB* test.

PERFORMANCE CHARACTERISTICS

Initial clinical studies were conducted in the U.S. and the European Union with and without T-Cell *Xtend* reagent added prior to cell separation for the processing of whole blood samples stored at room temperature (18-25°C) between 23 and 30 hours post venipuncture.

Overall agreement for the US study data (Sites 1 and 2) between the T-SPOT.*TB* test with and without the T-Cell *Xtend* reagent was 93.6% (190/203) [95%CI 89.3-96.5%].

Positive Agreement = 85.2% (46/54) [95%CI 72.9-93.4%]

Negative Agreement = 96.6% (144/149) [95%CI 92.3-98.9%]

Overall agreement for the EU study data (Sites 1, 2 and 3) 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%].

Positive Agreement = 96.9% (158/163) [95%CI 93.0-99.0%]

Negative Agreement = 96.3% (182/189) [95%CI 92.5-98.5%]

Additional clinical studies were conducted in the U.S. and South Africa with and without the addition of T-Cell *Xtend* reagent prior to cell separation for the processing of whole blood samples stored at room temperature (18-25°C) between 0 and 32 hours post venipuncture.

Overall agreement for the study data (Sites 1, 2, and 3) between the T-SPOT.*TB* test with and without the T-Cell *Xtend* reagent was 95.4% (288/302) [95%CI 92.3 – 97.4].

Positive Agreement = 93.3% (111/119) [95%CI 87.2 – 97.1]

Negative Agreement = 96.7% (177/183) [95%CI 93.0 – 98.8]

For detailed information refer to the T-SPOT.*TB* test package insert, Summary of Additional Clinical Data Using T-Cell *Xtend* Reagent.










LITERATURE REFERENCES

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

Troubleshooting Guidance in the Preparation of PBMCs for the T.SPOT.TB test

Problem	Potential Cause	Possible Solution
Low cell yield	<p>Leucopenia</p> <p>Incorrect blood collection</p> <p>Blood collection tube, prior to venipuncture, is not at ambient room temperature (18-25°C)</p> <p>Blood storage is not at 18-25°C</p> <p>Blood storage is over the recommended time</p>	<p>Add an additional blood collection tube</p> <p>Do not use Cell Preparation Tubes (CPT, Becton Dickinson) or blood collection tubes containing the anticoagulant EDTA</p> <p>Ensure blood collection tube has equilibrated to room temperature prior to sample collection.</p> <p>Collect another blood sample and repeat test.</p> <p>Collect another blood sample and repeat test.</p>
Red blood cell contamination	<p>Blood collection tube, prior to venipuncture, is not at ambient room temperature (18-25°C)</p> <p>Incorrect centrifugation</p>	<p>Ensure blood collection tube has equilibrated to room temperature prior to sample collection</p> <p>Increase centrifugation time to 30 minutes</p> <p>Check the centrifuge is refrigerated</p> <p>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</p>
No defined or distinct mononuclear layer	<p>Centrifuge is not correctly calibrated</p> <p>Centrifuge speed too low</p> <p>Centrifuge time too short</p> <p>Hyperlipidemic sample</p>	<p>Have centrifuge calibrated</p> <p>Increase centrifuge speed to produce 1500-1800 RCF</p> <p>Increase time of centrifugation to 30 minutes</p> <p>Collect fasting blood sample</p>
Invalid results	<p>Invalid results can be caused by a number of incorrect sample handling issues</p>	<p>Refer to the sections above</p>

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

BS EN ISO 15223-1:2016

The symbols used for the T-SPOT. *TB* test comply with the international standard ISO 15223-1:2016; ‘Medical devices – Symbols to be used with medical device labels, labelling and information to be supplied’.

T-SPOT and T-Cell *Xtend* are registered trademarks of Oxford Immunotec Ltd.
The Oxford Immunotec logo is a registered trademark of Oxford Immunotec Ltd.
CPT is a trademark of Becton Dickinson
Ficoll and Ficoll-Paque are trademarks of GE Healthcare

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

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