



T-Cell *Select*TM



PACKAGE INSERT

T-Cell *Select*
TSK.910 / TSK.960

For *In Vitro* Diagnostic Use
For use with the T-SPOT[®].*TB* test only

INTENDED USE

The T-Cell *Select*TM reagent kit is intended for use with the T-SPOT[®].*TB* test for the isolation of peripheral blood mononuclear cells from whole blood stored at room temperature (18 – 25 °C) using positive selection via a magnetic bead-based cell separation system.

SUMMARY & EXPLANATION

The T-SPOT.*TB* test has been evaluated for the processing of blood samples within 8 hours of venipuncture. With the T-Cell *Select* reagent kit, blood samples stored or maintained at room temperature (18 – 25 °C) for up to 54 (0 – 54) hours post venipuncture can be used with the T-SPOT.*TB* test. The T-Cell *Select* reagent may also enable automation of the cell isolation process and reduce sample separation time relative to preparation via the density gradient separation methodology. With the T-Cell *Select* reagent kit, blood samples may be shipped and/or stored overnight at room temperature (18 – 25 °C), up to 54 (0 – 54) hours post venipuncture.

PRINCIPLE OF METHOD

The T-Cell *Select* reagent kit has been designed with an associated positive selection methodology, which can be used as an alternative to density gradient isolation when preparing Peripheral Blood Mononuclear Cells (PBMC) for use in the T-SPOT.*TB* test. 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. Antibodies are added, which bind to PBMC in the sample. Addition of superparamagnetic beads result 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 and validated magnetic separation system to isolate the PBMC from the sample for subsequent use in the T-SPOT.*TB* test. Laboratories should validate the positive selection method on their own specific equipment, including appropriate blood volumes and number of cycles that the samples are exposed to the magnet.

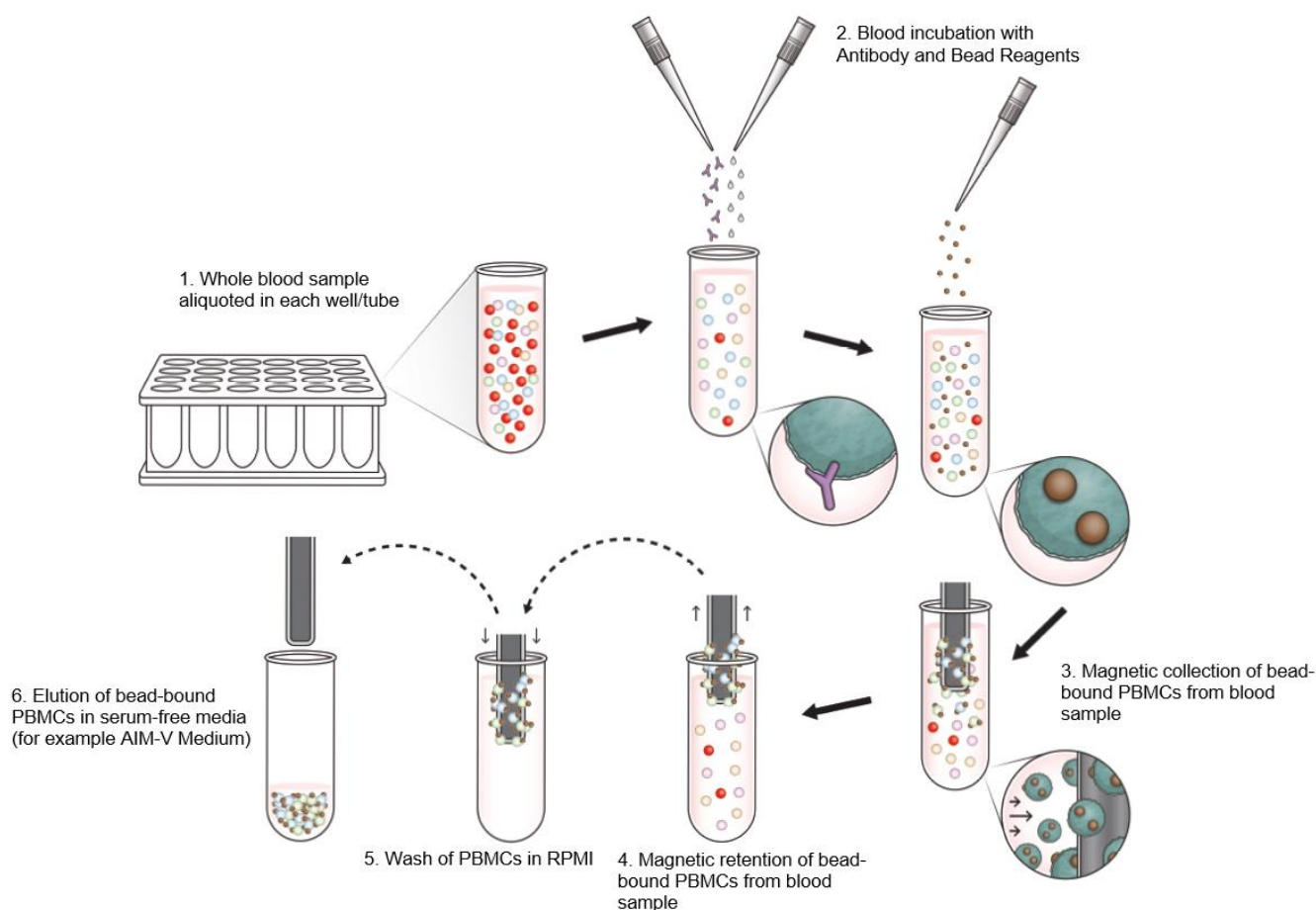


Figure 1: Diagram showing how the T-Cell *Select* reagent kit and positive selection methodology should be incorporated into the T-SPOT.*TB* test protocol for use with whole blood samples stored at room temperature (18 – 25 °C) between 0 and 54 hours post-venipuncture.

WARNINGS & PRECAUTIONS

1. The T-Cell *Select* reagent kit 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; operators must be trained in this procedure.
4. Blood samples should be considered potentially hazardous. Care should be taken when handling material of human origin.
5. 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.
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 at 18 – 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 before using the T-Cell *Select* reagent kit.
10. Although the T-Cell *Select* reagent kits are indicated for either 144 or 4608 tests, the number of tests each laboratory is able to perform with each kit may differ, based on variables including magnetic equipment used, starting volume of blood and number of cycles that samples are exposed to the magnet.
11. Only use single-use containers for venous blood sample collection.
12. Do not mix different reagents from different lots in a single patient sample.
13. Do not use beyond the expiration date.
14. Do not use with a whole blood sample that has been stored for more than 54 hours.
15. Use aseptic techniques when using this product.

MATERIALS PROVIDED

T-Cell *Select* is provided in two configurations. Each box contains:

TSK.910 (suitable for separation of up to 144 tests*)

Reagent	Quantity
Buffer Concentrate	1 x 50 mL
Antibody 1	3 x 2 mL
Antibody 2	3 x 2 mL
Bead Reagent	1 x 10 mL

TSK.960 (suitable for separation of up to 4608 tests*)

Reagent	Quantity
Buffer Concentrate	20 x 50 mL
Antibody 1	2 x 100 mL
Antibody 2	2 x 100 mL
Bead Reagent	3 x 100 mL

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

STORAGE & STABILITY

Store unopened T-Cell *Select* reagent kits at 2 – 8 °C until the expiration date shown on the box. Store opened and resealed vials of the T-Cell *Select* reagents at 2 – 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 & MATERIALS REQUIRED BUT NOT PROVIDED

1. Blood collection tubes, such as lithium heparin, sodium heparin, or sodium citrate tubes.
2. Distilled or deionized water.
3. Sterile serum free cell culture medium, such as Gibco® AIM-V® Media. Life Technologies; catalog number 31035-025
4. RPMI 1640 Medium. Such as Gibco RPMI Media. Life Technologies; catalog number 11875-093
5. Suitable magnetic particle purification equipment, either

- The ThermoFisher KingFisher™ Flex with 24-Deep-well head; catalog number 5400640, OR
 - The STEMCELL 'Big Easy' EasySep™; catalog number 18001
6. Tubes and containers compatible with the laboratory's magnetic particle purification equipment are required.
 7. Pipettes and sterile pipette tips.
 8. Timer.
 9. T-SPOT. TB test kit

REAGENT PREPARATION

The Antibody 1, Antibody 2 and Bead Reagent are supplied ready to use.

The Buffer Concentrate requires dilution in distilled or deionized water to achieve a working concentration. Use a ratio of 2:3, Buffer Concentrate:deionized or distilled water.

Note: For example, if 10 mL of the working concentration of buffer is required, 4 mL of the Buffer Concentrate should be diluted in 6 mL of deionized or distilled water.

PROCEDURE

This procedure demonstrates the T-Cell *Select* isolation method for the ThermoFisher KingFisher Flex or the STEMCELL 'Big Easy' EasySep magnet.

Equipment, protocols and Instructions for Use have been documented in equipment specific Setup Guides and User Guides, as detailed below;

- The KingFisher Flex instrument, manufactured by ThermoFisher, can be used for magnetic field exposure and mixing stages of the T-Cell *Select* isolation method. An optimized isolation method can be inputted onto the instrument using the parameters provided in Oxford Immunotec's Setup Guide for the KingFisher Flex (TS-US-TG-NA-0001). Instructions for Use and materials required for use of the KingFisher Flex instrument with the T-Cell *Select* isolation method can be found in Oxford Immunotec's User Guide for the use of the KingFisher Flex with this reagent (TS-US-UG-NA-0001).
- The 'Big Easy' EasySep magnet, manufactured by STEMCELL Technologies Inc., can be used for magnetic field exposure stages of the T-Cell *Select* isolation method. The optimized Instructions for Use and materials required for use of the 'Big Easy' EasySep magnet with the T-Cell *Select* isolation method can be found in Oxford Immunotec's User Guide for the use of the EasySep with this reagent (TS-US-UG-NA-0002).

The equipment-specific protocols can be obtained from www.oxfordimmunotec.com.

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

1. Whole blood samples should be maintained between 18 °C and 25 °C until processed.
2. Collect a blood sample according to the instructions supplied with the collection device. The tube contents must be inverted (8-10 times) to ensure that the whole blood is mixed thoroughly with the anticoagulant. Store collected blood at room temperature (18-25 °C). **Do not refrigerate or freeze.**
3. Typically, for an immunocompetent patient, sufficient PBMC 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 sodium citrate, or one sodium heparin, or one lithium heparin 4 or 6 mL tube.
 - Children less than 10 years old: one 4 mL tube.
 - A patient's cells can be pooled, if necessary to obtain sufficient cells from multiple tubes of blood which were collected and processed concurrently.
4. Thoroughly mix blood sample prior to use.
5. Aliquot blood into a container compatible with your laboratory's magnetic separation system.

Note: Blood volume should be defined and validated for the equipment used, ensuring a consistent PBMC quantity ($\geq 1 \times 10^6$ cells per sample) are isolated for use. The T-SPOT. TB test requires $250,000 \pm 50,000$ PBMC per well. Each well requires 250,000 cells. Four wells are required for each sample (2 controls and 2 antigens), therefore

$\geq 1 \times 10^6$ cells per sample are required per patient. The number of T cells in the specimen is normalized to a fixed number of PBMC.

6. Dilute T-Cell *Select* Buffer Concentrate in distilled or deionized water in the ratio 2:3, Buffer Concentrate:distilled or deionized water.
7. Add diluted T-Cell *Select* Buffer to whole blood in the ratio 1:7, buffer:blood.
8. Add 10 μ L of Antibody 1 per mL of the combined volume of whole blood and buffer.
9. Add 10 μ L of Antibody 2 per mL of the combined volume of whole blood and buffer.
10. Mix sample thoroughly.
11. Incubate sample for 15 minutes at room temperature.
12. Thoroughly mix the Bead Reagent for 30 to 60 seconds immediately before use. Add 15 μ L of Bead Reagent per mL of the combined volume of whole blood and buffer.
13. Mix sample gently.
14. Incubate sample for 15 minutes at room temperature, mixing at least every 5 minutes.
15. Expose sample to either the ThermoFisher KingFisher Flex **OR** the STEMCELL 'Big Easy' EasySep magnet. Please refer to the specific protocol as detailed within Oxford Immunotec's User Guides for the use of:
 - a. The KingFisher Flex (TS-US-UG-NA-0001), OR
 - b. The STEMCELL 'Big Easy' EasySep (TS-US-UG-NA-0002).

Note: Laboratories must validate their procedures for collection and separation of PBMC to obtain sufficient numbers when using either of the above, which includes validating any other equipment used for the cell isolation procedure. The following is recommended:

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

LIMITATIONS

1. The T-Cell *Select* reagent kit has not been evaluated for uses other than with the T-SPOT.*TB* test.
2. Do not refrigerate or freeze whole blood samples. Store and transport blood samples to the laboratory between 18 – 25 °C.
3. Do not allow blood samples to be exposed to temperatures above 25 °C, as this may result in increased background and other test anomalies.
4. Any deviation from recommended procedures for pipetting, washing techniques, incubation times and/or temperatures may influence test results.
5. The use of the T-Cell *Select* reagent kit with the T-SPOT.*TB* test has not been adequately evaluated with blood specimens from individuals younger than 2 years, pregnant women and in patients with hemophilia.
6. Although the T-Cell *Select* reagent kits are indicated either for 144 or 4608 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.
7. Excess biotin in whole blood samples has the potential to interfere with assays that utilize biotin-streptavidin chemical interactions, including the T-Cell *Select* reagent. Analytical interference studies have shown that whole blood biotin concentrations of 100 ng/mL or greater may result in insufficient PBMC recovery for T-SPOT.*TB* testing when using the T-Cell *Select* reagent.

QUALITY CONTROL

As part of an individual laboratory's quality control activity, magnetic separation and cell counting methods should be validated to ensure that sufficient mononuclear immune cells can be obtained for the T-SPOT. *TB* test. In addition, quality control activities should employ the use of positive and negative controls to ensure the expected performance of the T cells assayed with the T-SPOT. *TB* test.

PERFORMANCE CHARACTERISTICS

Clinical studies were conducted in the U.S. and South Africa comparing the density gradient cell isolation methodology with the T-Cell *Select* reagent kit and isolation methodology for processing of whole blood samples stored at room temperature (18-25 °C) from 0 to 54 hours post venipuncture. Blood storage time brackets examined for the test arms were: 0 – 8, 18 – 32, 39 – 46 and 48 – 55 hours. Subjects' serum biotin levels were not established during this study.

Summary of Study Sites and Data

Study site 1 was located in South Africa. Study sites 2, 4 and 5 were in Texas, Ohio and Massachusetts, respectively. Study site 3 was closed prior to enrollment. A flow chart is shown below in figure 9 for the study sites' recruitment. It shows the number of study participants who were excluded, those with invalid results and the number of participants with results that were used to calculate study agreements for the test arm against the control arm.

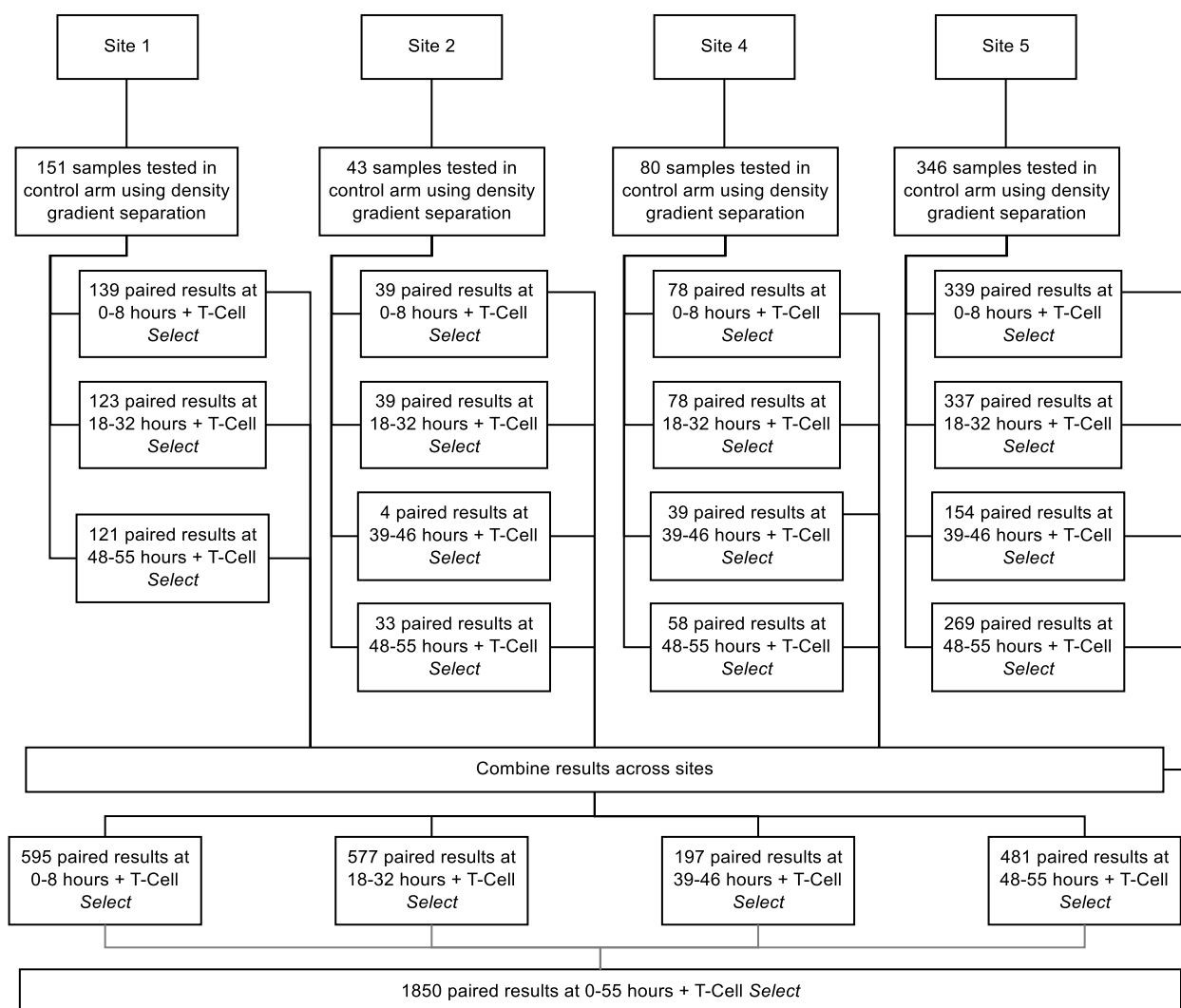


Figure 2: Flow chart of study site recruitment showing numbers of participants tested in the control arm (using T-SPOT. *TB* and density gradient separation) and paired results (where corresponding samples have been tested using T-SPOT. *TB* and T-Cell *Select*) included in data analysis

The following study outcomes are illustrated in Table 1 and 2, and include:

- A 5 x 5 table for the control arm (0 - 8 hours storage using T-SPOT.TB and the density gradient separation cell isolation method) compared with the test arm (0 – 55 hours storage using T-SPOT.TB and the T-Cell *Select* reagent kit and cell isolation method).
- Study agreements calculated using the 6 spot cutoff for the test

T-Cell <i>Select</i> reagent kit All study results 0-55 hours		T-SPOT.TB + T-Cell <i>Select</i> 0-55 Hours (Test Arm)					Total
		Positive ≥8 spots	Borderline			Negative ≤4 spots	
			7 spots	6 spots	5 spots		
T-SPOT.TB (Control Arm)	Positive	375	2	3	3	8	391
	Borderline	7 spots	5	0	0	0	5
		6 spots	9	1	2	0	18
		5 spots	3	1	0	1	15
	Negative	18	5	14	36	1348	1421
Total	410	9	19	40	1372	1850	

Table 1: All study data comparing test arms (with T-Cell *Select* reagent kit and methodology) and control arm (density gradient separation cell isolation methodology) for 1850 T-SPOT.TB test results.











Based on the cut-off of ≥6 spots, agreement results are as detailed in Table 2 as follows:

Data analysis set	Overall agreement with [95 % CI]	Positive agreement with [95 % CI]	Negative agreement with [95 % CI]
All Study Results	96.9 % (1792/1850) [96.0-97.6]	95.9 % (397/414) [93.5-97.6]	97.1 % (1395/1436) [96.1-97.9]
Site 1 results	94.8 % (363/383) [92.1-96.8]	98.8 % (335/339) [97.0-99.7]	63.6 % (28/44) [47.8-77.6]
Site 2 results	88.7 % (102/115) [81.4-93.8]	84.9 % (45/53) [72.4-93.3]	91.9 % (57/62) [82.2-97.3]
Site 4 results	98.0 % (248/253) [95.4-99.4]	100 % (6/6) [54.1-100.0]	98.0 % (242/247) [95.3-99.3]
Site 5 results	98.2 % (1079/1099) [97.2-98.9]	68.8 % (11/16) [41.3-89.0]	98.6 % (1068/1083) [97.7-99.2]
0-8 Hours (all sites)	96.5 % (574/595) [94.7-97.8]	98.0 % (145/148) [94.2-99.6]	96.0 % (429/447) [93.7-97.6]
18-32 Hours (all sites)	96.4 % (556/577) [94.5-97.7]	94.9 % (129/136) [89.7-97.9]	96.8 % (427/441) [94.7-98.3]

39-46 Hours (all sites)	98.0 % (193/197) [94.9-99.4]	50.0 % (2/4) [6.8-93.2]	99.0 % (191/193) [96.3-99.9]
48-55 Hours (all sites)	97.5 % (469/481) [95.7-98.7]	96.0 % (121/126) [91.0-98.7]	98.0 % (348/355) [96.0-99.2]

Table 2: Summary clinical agreements for 0-55 hours for each study site based on a cut off of ≥ 6 spots.

GLOSSARY OF SYMBOLS

	Use by/Expiration date (Year-Month-Day)
	Lot number
	Catalog 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
	Keep away from sunlight

BS EN ISO 15223-1:2021

The symbols used for the T-SPOT.*TB* test and T-Cell *Select* reagent kit comply with the international standard ISO 15223-1:2021; ‘Medical devices – Symbols to be used with medical device labels, labeling and information to be supplied’.

T-SPOT and the Oxford Immunotec logo are registered trademarks of Oxford Immunotec Ltd.

T-Cell *Select* is a trademark of Oxford Immunotec Ltd.

KingFisher is a trademark of Thermo Fisher Scientific.

EasySep is a trademark of STEMCELL Technologies Inc.

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The use of the T-Cell *Select* reagent kit is protected by the following patents: US9090871; EP2084508; AU2007303994; CA2665205; CN101529221; IN289117; JP5992393

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