**CELLTRACKS® Circulating Melanoma Cell Kit**

**INSTRUCTIONS FOR USE**

For Research Use Only. Not for use in diagnostic procedures. The performance characteristics and safety and effectiveness have not been established and are not cleared or approved by the FDA.

**PRODUCT OVERVIEW**

The CELLTRACKS® Circulating Melanoma Cell Kit is used to immunomagnetically capture and fluorescently label CD146 positive (CD146+) cells from whole blood. The kit is to be used with the CELLTRACKS® AUTOPREP® System for sample preparation and the CELLTRACKS ANALYZER II® for enumeration and identification of circulating melanoma cells (CMC).

The CELLTRACKS® AUTOPREP® System was designed to standardize and optimize the sample preparation protocol for use with the CELLTRACKS® Circulating Melanoma Cell Kit. Analysis and enumeration of CMCs is performed using the CELLTRACKS ANALYZER II®.

The CELLTRACKS® Circulating Melanoma Cell Kit contains a ferrofluid-based capture reagent and immunofluorescent staining reagents. Reagents are designed to enumerate specific cell surface markers on melanoma cells. The CELLTRACKS ANALYZER II® automatically scans the entire surface of the cartridge, acquires images and displays any event to the user where MEL-PE and DAPI are co-located. Images are presented to the user in a gallery format for final classification of the magnetically captured cell. An event is classified as a CMC when its morphological features are consistent with that of a cell and it exhibits the correct phenotypes, i.e. CD146+, MEL-PE+, DAPI+, CD34/APC-, and CD45/APC-. The kit contains PBS, 25% proprietary ingredients, 0.1% BSA and sodium azide (white cap), PBS, 0.5% BSA, 0.02% dithiothreitol (DTT) and Propylene Glycol (PG) (brown cap), PBS, 0.02% dithiothreitol (DTT) and 0.02% sodium azide (300 as a preservative). (brown cap) for use with the CELLTRACKS® AUTOPREP® System and the CELLTRACKS ANALYZER II®.

**MATERIALS PROVIDED**

- 1 Package Insert
- 3.0 mL vial Anti-CD146-Ferrofluid: Contains a suspension of 0.022% magnetic particles conjugated to a mouse monoclonal antibody that is specific for a cell surface marker present on melanoma cells in a buffer containing 0.3% bovine serum albumin (BSA) and 0.05% ProClin® 300 preservative. (brown cap)
- 3.0 mL Staining Reagent: Contains 0.0006% mouse monoclonal antibody specific to HMW-MAA conjugated to phycoerythrin (PE); 0.003% mouse monoclonal antibodies specific to anti-CD45 monoclonal antibody and anti-CD34 monoclonal antibody conjugated to allophycocyanin (APC) in buffer containing 0.5% BSA and 0.1% sodium azide. (white cap)
- 3.0 mL Nucleic Acid Dye: Contains 0.005% 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI) and 0.05% ProClin® 300 preservative. (blue cap)
- 3.0 mL bottle Capture Enhancement Reagent: Contains PBS, 0.5% BSA, 0.02% proprietary reagent for controlled ferrofluid aggregation and 0.1% sodium azide. (clear cap)
- 3.0 mL Permeabilization Reagent: Contains PBS, 0.011% proprietary permeabilization reagent and 0.1% sodium azide. (green cap)
- 3.0 mL Cell Fixative: Contains PBS, 25% proprietary ingredients, 0.1% BSA and 0.1% sodium azide. (red cap)

**REAGENT STORAGE AND HANDLING**

- Reagents are supplied ready for use. Store unopened at 2–8 °C (36–46 °F).
- After opening, reagents in the reagent pack should be stored for no longer than 30 days at 2–8 °C (36–46 °F). For storage, open reagents must be recapped with their unique colored caps using the colors indicated on the reagent tray labels as a guide. This is to ensure cross-contamination of reagents does not occur.

**WARNING:**

- Protect reagents from heat in excess of 35 °C (95 °F). Do not freeze.
- Visually inspect the reagent pack for the proper placement of the reagents. Verify that each reagent is in the proper location by comparing the reagent carousel contents with the picture to the right. If reagents are found to be incorrectly placed or if duplicate bottles are present, do not use the reagent pack and notify Customer Technical Services to arrange for a replacement.
- Protect reagents from exposure to sunlight.
- When properly stored, reagents are stable until the expiration date printed on the reagent container or kit box. Do not use expired reagents.
- The kit components are manufactured and tested as a master lot. Do not mix and match reagents from different kits.

**LIMITATIONS OF THE PROCEDURE**

- For research use only. Not for use in diagnostic procedures. Results should not be used for patient management.
- User-defined reagents cannot be run with Control Cells.
- User-defined reagents must be conjugated with a Fluorescein isothiocyanate-type fluorochrome.
- User-defined reagents will need to be optimized for use on the CELLTRACKS ANALYZER II® prior to generating test results.

**INSTRUMENTATION**

The CELLTRACKS® Circulating Melanoma Cell Kit is designed for use with the CELLTRACKS® AUTOPREP® System and the CELLTRACKS ANALYZER II®.
• 2 x 110 mL bottle Dilution Buffer: Contains PBS, 0.5% BSA, 0.6% other animal protein, and 0.1% sodium azide.
• 16 CELLSEARCH® Conical Centrifuge Tubes (15 mL) and Conical Tube Caps
• 16 Cartridges and Cartridge Plugs

MATERIALS REQUIRED, NOT PROVIDED
• CellSave Preservative Tubes (Catalog #7900005)
• CELLTRACKS® AUTOPREP® System (Catalog #9541)
• CELLTRACKS ANALYZER II® (Catalog #9555)
• CELLSEARCH® CEC/CMC Cell Control Kit (Catalog #9572V)
• CELLTRACKS® AUTOPREP® Instrument Buffer (Catalog #7901003)
• Horizontal swing out style rotor (swinging bucket) centrifuge capable of 800 x g
• Test tube racks
• Calibrated micro-pipettors and tips

QUALITY CONTROL
The CELLSEARCH® CEC/CMC Cell Control Kit (Catalog #9572V) checks the overall system performance, including instrument, reagents and operator technique. The CELLSEARCH® CEC/CMC Cell Control should be run each day of patient testing or when using a new lot of the CELLTRACKS® Melanoma Cell Kit. Please refer to the CELLSEARCH® CEC/CMC Cell Kit Instructions for Use and expected values.

TESTING PROCEDURE
Specimen Collection and Preparation
Collection of whole blood into CellSave Preservative Tube
1. Collect whole blood aseptically by venipuncture or from a venous port into a CellSave Preservative Tube only.
2. Fill the tube until blood flow stops to ensure the correct ratio of sample to preservative. Immediately mix by gently inverting the tube eight times. Tube inversion prevents clotting. Inadequate or delayed mixing may result in inaccurate test results.
3. Blood samples may be stored or transported in CellSave Preservative Tube. Process samples within 96 hours of collection. Please refer to the CellSave Preservative Tube Instructions for Use for process, storage and handling instructions. Do not refrigerate samples.

CAUTION: Visually inspect each sample for clotting before processing on the CELLTRACKS® AUTOPREP® System. Clotted samples should be discarded.

Processing with the CELLTRACKS® AUTOPREP® System
1. Mix the blood in the CellSave Preservative Tube by manually inverting five times. Then remove the rubber stopper.
2. Using a new pipette, transfer 7.5 mL of blood from the CellSave Preservative Tube into a correspondingly labeled 15 mL conical tube provided with the CELLTRACKS® Circulating Melanoma Cell Kit.
3. Using a new pipette, add 6.5 mL of Dilution Buffer.
4. Cap the conical tube and mix by inversion five times.
5. Centrifuge the sample at 800 x g for a full 10 minutes with the brake off using a swing bucket centrifuge. The 10 minute centrifugation time does not take into account the time required to reach 800 x g. Set the centrifuge brake to "off" or if your centrifuge provides a variable braking feature, set the brake to the lowest brake setting. Centrifuge at room temperature using a room temperature capable centrifuge. Following sample centrifugation, visually inspect each sample tube for separation of plasma and red blood cells.
7. When prompted to select a reagent kit, choose CELLTRACKS® CMC Kit.
8. See the CELLSEARCH® Research Use Only User’s Guide for processing steps.

Analysis Using the CELLTRACKS ANALYZER II®
The CELLTRACKS® AUTOPREP® System dispenses the processed sample into a cartridge ready for analysis using the CELLTRACKS ANALYZER II®. The filled cartridge within the MAGNEST® Cartridge Holder should be placed into the MAGNET®, ensuring that the sample is submerged in the MAGNET® Buffer. The cartridge should be processed within 24 hours of filling. Please refer to the CELLTRACKS ANALYZER II® User’s Guide and the CELLSEARCH® Research Use Only User’s Guide for instructions on sample analysis and data review.

RESULTS
Results are reported as the number of CMCs per 7.5 mL of blood.

INTERFERING SUBSTANCES
• Human tissue culture tumor cells spiked into blood samples were exposed to potential interfering substances and compared to untreated controls.
• Potential interference from lipemia was studied by adding Intralipid to samples to a concentration of 2.6%, which corresponds to greater than 1000 mg/dL triglyceride.
• Bilirubin at 7.4 mg/dL and hematocrit from 30-60% were studied.
• Lipemia, icterus and a broad range of hematocrit values do not interfere with the CELLTRACKS® Circulating Melanoma Cell test.

GLOSSARY OF SYMBOLS
The following symbols may have been used in the labeling of this product.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
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<tbody>
<tr>
<td><img src="image" alt="Chemical Symbol" /></td>
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<tr>
<td><img src="image" alt="Chemical Symbol" /></td>
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</tr>
<tr>
<td><img src="image" alt="Chemical Symbol" /></td>
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<tr>
<td><img src="image" alt="Chemical Symbol" /></td>
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REVISION HISTORY

Date of Revision | Component Code | Description of Technical Changes
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2014-03-04 | 631500232 | • PRODUCT OVERVIEW
– updated CD146+, MEL-PE, DAPI+, CD34-PE-, and CD45/PE- to CD146+, MEL-PE, DAPI+, CD34/APC-, and CD45/APC-
• Specimen Collection and Preparation
– deleted reference to therapy regimen, formerly #1
• Processing with the CELLTRACKS® AUTOPREP® System
 – in Step #2, updated 4.0 mL to 7.5 mL and updated kit name
 – in Step #3, updated 10 mL to 6.5 mL
 – in Step #4, updated kit name
• RESULTS
 – updated 4.0 mL to 7.5 mL

*The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

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