



REF 9594V
16 Test Kit

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. The ferrofluid reagent consists of particles with a magnetic core surrounded by a polymeric layer coated with antibodies targeting the CD146 antigen to capture CMCs. After immunomagnetic capture and enrichment, fluorescent reagents are added for identification and enumeration of CMCs. Anti-high molecular weight melanoma associated antigen (HMW-MAA-PE (MEL-PE)) is specific for melanoma cells, anti-CD34-APC for endothelial cells, anti-CD45-APC for leukocytes and DAPI for cell nucleus.

The reagent/sample mixture is dispensed by the CELLTRACKS® AUTOPREP® System into a cartridge that is inserted into a CELLTRACKS® MAGNEST® Cartridge Holder. The strong magnetic field of the MAGNEST® Cartridge Holder causes the magnetically labeled CD146+ cells to move to the surface of the cartridge. 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-.

WARNINGS AND PRECAUTIONS

Please read the full package insert before testing samples. Please read the *CELLTRACKS® AUTOPREP® System User's Guide* before processing samples. Please read the User's Guide for the analyzer for complete instructions before analyzing samples. Refer to the *CELLSEARCH® Research Use Only User's Guide* for more information.

- CAUTION:** Collect blood into a CellSave Preservative Tube only.
- CAUTION:** Samples must be transported and stored at temperatures of 15–30 °C (59–86 °F). Refrigerating samples prior to processing could adversely affect sample integrity.
- CAUTION:** All personnel should follow universal precautions and use laboratory safety equipment (i.e., safety glasses, laboratory coat, gloves).
- CAUTION:** Microbial contamination of reagents can cause erroneous results and should be avoided.
- CAUTION:** The bottle of Dilution Buffer, which is packaged separately from the reagent tray, must be equilibrated to room temperature (15–30 °C or 59–86 °F) before use.
- CAUTION:** Some of the reagents contain sodium azide preservative. If swallowed, seek medical advice immediately and provide the containers or labels. Keep out of reach of children. Keep away from food and drink. Wear suitable protective clothing. Contact with acids liberates very toxic gas. Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop.
- WARNING:** All biological specimens, cartridges and other materials coming into contact with the specimen(s) are considered biohazardous. Handle as if capable of transmitting infection. Treat and dispose of waste using proper precautions and in accordance with local, state, and federal regulations. Never pipette by mouth.

WARNING:



Following are the Hazard and Precautionary statements:

H317 May cause an allergic skin reaction

Prevention:

- P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
- P272 Contaminated work clothing should not be allowed out of the workplace.
- P280 Wear protective gloves.

Response:

- P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.
- P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

- P501 Dispose of contents/ container to an approved waste disposal plant.

Some of the reagents contain ProClin® 300 as a preservative.

"Proclin 300 (mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1))"

For additional information please refer to Safety Data Sheet on www.cellsearchruo.com

IMPORTANT: Carryover from a high count sample can affect samples subsequently processed on the CELLTRACKS® AUTOPREP® System, including the subsequent batch. Cell carryover levels have not been characterized for this reagent kit. Consider repeating samples in a run following a high sample. Perform daily cleaning procedures after each batch to prevent carrying over cells from one batch to another.

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®.

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, opened 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.

NOTE: After opening, the Dilution Buffer bottle, which is not a part of the reagent pack, must be stored at room temperature for no longer than 30 days.

- 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.



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); 0.0030% 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. (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)
- **2 × 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 (i.e. swing bucket) centrifuge capable of 800 × 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 Tubes

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 anticoagulant and 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 Centrifuge 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 × g for a full 10 minutes with the brake off using a horizontal swing out style rotor (i.e. 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.
6. Process on the CELLTRACKS® AUTOPREP® System **within 1 hour** of the above sample preparation. Refer to the *CELLTRACKS® AUTOPREP® System User's Guide* for full instructions.
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 allowed to incubate in

the dark for a minimum of 20 minutes and analyzed within 24 hours. 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.

	Use by or Expiration Date (Year-Month-Day)		Contains Sufficient for "n" Tests
	Lot Number		Temperature Limitation
	Serial Number		Consult Instructions for Use
	Catalog Number or Product Code		Biological Risk
	Caution		May cause an allergic skin reaction
	Manufacturer		

*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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This technology, including products and/or associated components thereof, and procedures and instrument systems described herein, are protected by United States patents and corresponding international patents and pending patent applications, including one or more of the following: US Patent Numbers 6,136,182; 6,551,843; 6,623,982; 6,790,366; 7,011,794 and 7,332,288.

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Menarini Silicon Biosystems Inc.
Artwork Specialist_jb

INSERT CELLTRACKS Circulating Melanoma Cell Kit IFU

Article Number: MSB03103

Format Name: 3P_Sigma/I/8inch5x11inch/V1 (215.9x279.4mm)

Pointsize: 8 pt

File Name: MSB03103.indd (CC2017-PC)

Current Revised Date: 11 October 2017

- Black
- Cyan
- Magenta
- Yellow

REVISION HISTORY

Date of Revision	Component Code	Description of Technical Changes
2017-10-11	MSB03103	<ul style="list-style-type: none"> • Rolled tailcode; address changed to HV
2017-08-16	MSB03102	<ul style="list-style-type: none"> • Rolled tailcode; replaced "JDX" with "MSB" • Updated to Menarini Silicon Biosystems business attributes:
2017-01-19	JDX03101	<p>Technically equivalent to 631500232 with the following changes:</p> <ul style="list-style-type: none"> • Assigned new part number • Updated Veridex name, address, website and phone number to reflect Janssen Diagnostics, LLC. • Updated Veridex trademark and patent information to reflect Janssen Diagnostics, LLC. • Updates to 'WARNINGS AND PRECAUTIONS' section: <ul style="list-style-type: none"> - Updated sodium azide paragraph from 'WARNING' to a "CAUTION" and moved to 'CAUTION' section - Removed R22, S28, R43, R52/53 and S37 DSD Risk and Safety Phrases - Removed ProClin® 300 symptoms of overexposure statement - Added "GHS compliant" Hazard Pictogram underneath ProCline® 300 "WARNING" - Added "Hazard and Precautionary statements" • In GLOSSARY OF SYMBOLS Section: <ul style="list-style-type: none"> - Deleted "Harmful" DSD Danger Symbol - DSD Danger Symbol "Irritant" replaced with GHS Hazard Pictogram and signal word "Warning", and added GHS Hazard Statement "May cause an allergic skin reaction" • Updated Address to align with ISO certificate • Patent information has been updated.