

CELLTRACKS® CEC Profile 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® CEC Profile Kit is used to immunomagnetically capture CD146+ cells from whole blood. The kit is to be used with the CELLTRACKS® AUTOPREP® System for sample preparation.

Endothelial cells from the inner wall of blood vessels are shed into the blood stream during formation and destruction of blood vessels. Analysis of these cells may be useful to elucidate biological mechanisms of various diseases. The CELLTRACKS® AUTOPREP® System was designed to standardize and optimize the sample preparation protocol for use with the CELLTRACKS® CEC Profile Kit.

The CELLTRACKS® CEC Profile Kit contains a ferrofluid-based capture reagent, which consists of particles with a magnetic core surrounded by a polymeric layer coated with antibodies targeting the CD146 antigen to capture circulating endothelial cells (CECs) and other CD146+ cells. The CELLTRACKS® AUTOPREP® System precisely dispenses reagents and performs magnetic incubation steps.

WARNINGS AND PRECAUTIONS

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

CAUTION: For downstream cellular analysis applications, collect blood into a CellSave Preservative Tube only. For downstream molecular applications, use EDTA tubes because higher quality nucleic acid is generally recovered from EDTA tubes.

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 bottles of Dilution Buffer, which are 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 blood samples or control cells.

INSTRUMENTATION

The CELLTRACKS® CEC Profile Kit is designed for use with the CELLTRACKS® AUTOPREP® System.

REAGENT STORAGE AND HANDLING

- Reagents are supplied ready for use. Store 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 bottles, which are 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.
- Bring to room temperature (15–30 °C or 59–86 °F) before use.
- Protect reagents from exposure to direct 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 0.012% magnetic particles conjugated to a mouse monoclonal antibody that is specific for a cell surface marker present on endothelial cells in a buffer containing 0.3% bovine serum albumin (BSA) and 0.05% ProClin® 300 preservative (brown 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 Biotin/PBS:** Contains PBS, 0.62% biotin and 0.1% sodium azide. (red cap).
- **3 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**

MATERIALS REQUIRED, NOT PROVIDED

- CellSave Preservative Tubes (Catalog #7900005) for samples intended for downstream cellular analysis
- 10 mL EDTA evacuated blood collection tubes
- CELLTRACKS® AUTOPREP® System (Catalog #9541)
- 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

QUALITY CONTROL

The CELLSEARCH® CEC/CMC Cell Control Kit (Catalog #9572V) can be used with the CELLTRACKS® CEC Profile Kit if desired. Since the CELLTRACKS® CEC Profile Kit only captures cells and does not stain them, the use of the control cells will need to be optimized for each RUO application. The control cells are pre-labeled with dyes that show up in wavelengths associated with FITC (High level) and APC (Low Level). There are no control reference ranges for use with the CELLTRACKS® CEC Profile Kit.

TESTING PROCEDURE

Specimen Collection and Preparation

For Cellular Analysis

Use CellSave Preservative Tubes if subsequent analysis of the endothelial cells is for cellular analysis.

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 72 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.

For Molecular Analysis

Use EDTA tubes if samples will be processed for isolation of RNA or DNA. Follow the manufacturer's instructions. Process samples as soon as possible to maximize nucleic acid integrity and recovery. A 2-3 fold loss of mRNA from endothelial cells may result if processing is delayed up to 36 hours when compared to immediate processing as measured by real-time quantitative RT-PCR. Blood samples may be stored or transported in EDTA tubes for up to 36 hours at room temperature (15–30 °C or 59–86 °F) prior to processing. Do not refrigerate or freeze.

CAUTION: Examine each sample for clotting before processing on CELLTRACKS® AUTOPREP® System. Clotted samples should be discarded, as blood clots interfere with the performance of the assay.

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 4.0 mL of blood from the CellSave Preservative Tube into a correspondingly labeled 15 mL CELLSEARCH® Conical Centrifuge Tube provided with the CELLSEARCH® Kit.
3. Using a new pipette, add 10.0 mL of Dilution Buffer.
4. Cap the 15 mL CELLSEARCH® Conical Centrifuge 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 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 *CEC Profile Kit*.
8. See the *CELLSEARCH® Research Use Only User's Guide* for processing steps.

RESULTS












Not applicable.

INTERFERING SUBSTANCES

- Human Umbilical Vein Endothelial Cells (HUVEC) 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.
- Samples were lysed to simulate total hemolysis.
- Bilirubin at 7.4 mg/dL and hematocrit from 30-60% were studied.
- Lipemia, hemolysis, icterus and a broad range of hematocrit values do not interfere with the CELLTRACKS® CEC Profile 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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INSERT CEC PROFILE KIT

Article Number: MSB00503

Format Name: 3P_Sigma/1/8inch5x11inch/V1 (215.9x279.4mm)

Pointsize: 8 pt

File Name: MSB00503.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	MSB00503	<ul style="list-style-type: none"> • Rolled tailcode; address changed to HV
2017-08-18	MSB00502	<ul style="list-style-type: none"> • Rolled tailcode; replaced "JDX" with "MSB" • Updated to Menarini Silicon Biosystems business attributes:
2017-01-19	JDX00501	<p>Technically equivalent to 631500191 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 and S28 DSD Risk and Safety Phrases - Removed ProClin® 300 symptoms of overexposure statement - Added GHS Hazard Pictogram and signal word "Warning" underneath ProClin® 300 "WARNING" - Added GHS "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.