

CELLSEARCH® Circulating Endothelial 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 CELLSEARCH® Circulating Endothelial Cell Kit is used to immunomagnetically capture and fluorescently label 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 CD146+ sub-populations.

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 cancer. The CELLTRACKS® AUTOPREP® System was designed to standardize and optimize the sample preparation protocol for use with the CELLSEARCH® Circulating Endothelial Cell Kit. Analysis and enumeration of circulating endothelial cells (CECs) is performed using the CELLTRACKS ANALYZER II®.

The CELLSEARCH® Circulating Endothelial Cell Kit contains a ferrofluid-based capture reagent and immunofluorescent 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 CECs. After immunomagnetic capture and enrichment, fluorescent reagents are added for identification and enumeration of CECs. The fluorescent reagents include the following: anti-CD105-PE / anti-CD45-APC (anti-CD105 is specific for the protein endoglin and is expressed by endothelial cells; activated monocytes, stromal cells and pre-B cells, and expression of anti-CD45 is restricted to leukocytes); and DAPI which stains the cell nucleus.

The reagent/sample mixture is dispensed by the CELLTRACKS® AUTOPREP® System into a cartridge that is inserted into a 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 CD105-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 CEC when its morphological features are consistent with

that of a cell and it exhibits the correct phenotypes, i.e., CD146+, CD105-PE+, DAPI+ 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 *CELLTRACKS ANALYZER II® User's Guide* 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 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:

H317

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 CELLSEARCH® Circulating Endothelial 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 at 2–8 °C (36–46 °F).

- After opening, reagents in the reagent pack should be stored up to 30 days at 2–8 °C (36–46 °F) with caps on.

NOTE: After opening, the Dilution Buffer bottles that are not a part of the reagent pack, must be stored at room temperature for up to 30 days.

- Bring the reagent kit pack to room temperature (15–30 °C or 59–86 °F) before use.
- Protect reagents from exposure to direct sunlight.
- When properly stored, unopened reagents are stable until the expiration date printed on the reagent pack. 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 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 vial anti-CD105-PE/anti-CD45-APC (Staining Reagent):** Contains <0.0006% mouse monoclonal antibodies specific to CD105 conjugated to phycoerythrin (PE); <0.0013% mouse anti-CD45 monoclonal antibody conjugated to allophycocyanin (APC) in phosphate buffered saline (PBS) 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 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 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 in buffer. (red cap)
- **3 x 110 mL bottle Dilution Buffer:** Contains buffer with 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 CELLSEARCH® Circulating Endothelial 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 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 a 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.

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 × 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 *Endothelial 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 CECs per 4.0 mL of blood.

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 to not interfere with the CELLSEARCH® Circulating Endothelial Cell Kit.

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	

REVISION HISTORY

Date of Revision	Component Code	Description of Technical Changes
01-19-2017	JDX03701	<p>Technically equivalent to 631500081 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.

*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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