

CELLSEARCH® Epithelial 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

Cancer metastasis occurs when cells are shed from the primary tumor, enter the circulation and begin to grow in distant locations in the body. Malignant carcinomas are derived from epithelial cells (Cancer Biology, 3rd edition, Ray Ruddon 1995) that are not normally found in circulation. The CELLSEARCH® Epithelial Cell Kit (CELLSEARCH® Kit) contains reagents and supplies for immunomagnetic selection of rare circulating tumor cells (CTCs) from whole blood. The CELLTRACKS® AUTOPREP® System is an automated sample preparation instrument that utilizes a software protocol for the immunomagnetic selection and staining of CTCs using the CELLSEARCH® Kit. Analysis of CTCs is performed using the CELLTRACKS ANALYZER II®, a semi-automated fluorescence microscope, which is used to identify and enumerate CTCs.

The CELLSEARCH® 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 Epithelial Cell Adhesion Molecule (EpCAM) antigen for capturing CTCs. After immunomagnetic capture and enrichment, fluorescent staining reagents are added for identification and enumeration of CTCs. Anti-CKPE is specific for the intracellular protein cytokeratin (specific for epithelial cells), DAPI stains the cell nucleus, and anti-CD45-APC is specific for leukocytes.

The reagent/sample mixture is dispensed by the CELLTRACKS® AUTOPREP® System into a MAGNEST® Cartridge Holder, a fixture of two magnets held together by steel. The strong magnetic field of the MAGNEST® Cartridge Holder causes the magnetically-labeled epithelial cells to move to the surface of the cartridge. The CELLTRACKS ANALYZER II® acquires images and displays any events to the user where cytokeratin-PE and DAPI are co-located in the Cartridge. Images are presented to the user in a gallery format for final cell classification. The user classifies an event as a tumor cell based on morphology and correct phenotype, i.e., EpCAM+, CK-PE+, DAPI+ and CD45-APC-.

WARNINGS AND PRECAUTIONS

Please read the full package insert before testing 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 CTC count sample can affect samples subsequently processed on the CELLTRACKS® AUTOPREP® System, including the subsequent batch. For more detailed carryover information refer to the *CELLTRACKS® AUTOPREP® System User's Guide*.

LIMITATIONS OF THE PROCEDURE

- For Research Use Only. Not for use in diagnostic procedures. Results should not be used for patient management.
- CTC that do not express EpCAM will not be detected by the CELLSEARCH® test.
- CTC that express EpCAM but not cytokeratins 8, 18, and 19 will not be detected by the CELLSEARCH® test.
- Draw samples prior to intravenous chemotherapy. In addition if the patient is on doxorubicin therapy, allow at least 7 days following completion of therapy session before drawing blood.
- 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® Epithelial 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 matching its unique colored cap with the colors indicated on the label. 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 Anti-EpCAM Ferrofluid:** Contains a suspension of 0.022% magnetic particles conjugated to a mouse monoclonal antibody specific for the cell surface marker EpCAM present on epithelial cells in a buffer containing 0.03% bovine serum albumin (BSA) and 0.05% ProClin® 300 preservative. (brown cap)
- 3.0 mL Staining Reagent:** Contains 0.0006% mouse monoclonal antibodies specific to cytokeratins conjugated to phycoerythrin (PE); 0.0012% mouse anti-CD45 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 Capture Enhancement Reagent:** Contains 0.02% proprietary reagent for controlled ferrofluid aggregation, 0.5% BSA, and 0.1% sodium azide in buffer. (clear cap)
- 3.0 mL Permeabilization Reagent:** Contains 0.011% proprietary permeabilization reagent and 0.1% sodium azide in buffer. (green cap)
- 3.0 mL Cell Fixative:** Contains 25% proprietary fixative ingredients, 0.1% BSA, and 0.1% sodium azide in buffer. (red cap)
- 2 × 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® Epithelial Cell Control Kit (Catalog #7900002)
- 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-pipettes and tips

QUALITY CONTROL

The CELLSEARCH® Epithelial Cell Control Kit (Catalog #7900002) checks the overall system performance, including instrument, reagents and operator technique. A CELLSEARCH® Epithelial Cell Control should be run each day of patient testing or when using a new lot of the CELLSEARCH® Epithelial Cell Kit. Please refer to the CELLSEARCH® Epithelial Cell Control Kit Instructions for Use and expected values.

TESTING PROCEDURE

Specimen Collection and Preparation

Collection of whole blood into CellSave Preservative Tubes

1. Draw initial samples prior to initiation of a therapy regimen. Subsequent samples can be drawn after the start of a therapy regimen, usually at 3 to 4 week intervals, to follow CTC levels during therapy. If the patient is on doxorubicin therapy, allow at least 7 days following administration of a dose of doxorubicin before blood draw.
2. Collect whole blood aseptically by venipuncture or from a venous port into a CellSave Preservative Tube only.
3. 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.
4. 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 CELLSEARCH® Conical Centrifuge Tube provided with the CELLSEARCH® Kit.
3. Using a new pipette, add 6.5 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 *CellSearch® CTC Kit*.
8. See the *CELLSEARCH® Research Use Only User's Guide* for processing steps.

Analysis Using the CELLTRACKS ANALYZER II®

Process the sample on the CELLTRACKS® AUTOPREP® System dispenses the final 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 see 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 CTCs per 7.5 mL of blood.

INTERFERING SUBSTANCES

- SKBR-3 cells spiked into blood samples were exposed to potential interfering substances and compared to untreated controls. Toxic levels (five times therapeutic index) of the following cancer drugs, over-the-counter drugs, and other exogenous substances were tested: cyclophosphamide, Mitomycin C®, Procrit®, biotin, 5-fluorouracil, methotrexate, tamoxifen citrate, paclitaxel, Arimidex®, acetaminophen, acetylsalicylic acid, caffeine, dextromethorphan, Aredia®, Human Anti-Mouse Antibody (HAMA) type 1, HAMA type 2, Herceptin®, and ibuprofen. No significant differences in SKBR-3 cell numbers were detected, indicating that these substances do not interfere with the CELLSEARCH® kit.
- Samples spiked with toxic levels of doxorubicin resulted in aberrant staining of leukocytes as cytokeatin and CD45 dual positive cells, due to the doxorubicin being a fluorescent compound that is incorporated into nucleated cells. If seen, the staining

pattern of all cells being CD45 positive and cytokeatin positive is obvious and easily identified by the operator as a known interference staining profile. If blood is drawn after the recommended 7-day washout period, following doxorubicin infusion, this interference is unlikely to be observed in clinical practice given controlled therapeutic levels and rapid drug clearance.

- Potential interference from lipemia was studied by adding Intralipid to samples at 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, HAMA 1/HAMA 2 and hematocrit from 18-60% were studied.
- Lipemia, hemolysis, icterus and a broad range of hematocrit values do not interfere with the CELLSEARCH® test. HAMA 1 and HAMA 2 also do not interfere, indicating that individuals receiving mouse Ig by parenteral routes can be tested successfully with the CELLSEARCH® 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	

REVISION HISTORY

Date of Revision	Component Code	Description of Technical Changes
2017-01-19	JDX04002	Patent information has been updated.
12-08-2015	JDX04001	Technically equivalent to 631500071 with the following changes: <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

*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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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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