

CellSearch®

CELLSEARCH® 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 CELLSEARCH® Profile Kit is for the isolation of circulating tumor cells (CTC) of epithelial origin in whole blood in conjunction with the CELLTRACKS® AUTOPREP® System. Cancer metastasis occurs when cells 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 that are not normally found in circulation (Cancer Biology, 3rd edition, Ray Ruddon 1995). The CELLTRACKS® AUTOPREP® System was designed to standardize and optimize the sample preparation protocol using the CELLSEARCH® Profile Kit. Analysis of CTC may be performed using a variety of analytical methods, including flow cytometry and molecular techniques.

The CELLSEARCH® 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 Epithelial Cell Adhesion Molecule (EpCAM) antigen for capturing CTC. The CELLTRACKS® AUTOPREP® System precisely dispenses reagents and performs magnetic incubation steps.

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:	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 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.
- 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 blood samples or control cells.

INSTRUMENTATION

The CELLSEARCH® 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 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.
- 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 Anti-EpCAM Ferrofluid:** Contains a suspension of 0.022% magnetic particles conjugated to a mouse monoclonal antibody that recognizes a cell surface marker 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 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% proprietary reagents 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**

MATERIALS REQUIRED, NOT PROVIDED

- CellSave Preservative Tubes (Catalog #7900005)
- EDTA Tubes (molecular analysis)
- CELLTRACKS® AUTOPREP® System (Catalog #9541)
- 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
- CELLSEARCH® Epithelial Cell Control Kit (Catalog #7900002; Not Required)

QUALITY CONTROL

The CELLSEARCH® Epithelial Control kit (Catalog #7900002) can be used with the CELLSEARCH® Profile reagent kit if desired. Since the CELLSEARCH® 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 CELLSEARCH® Profile reagent kit.

TESTING PROCEDURE

Specimen Collection and Preparation

For Cellular Analysis

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

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.

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 epithelial 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.

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 Profile Kit*.
8. See the *CELLSEARCH® Research Use Only User's Guide* for processing steps.

RESULTS

Not applicable.












INTERFERING SUBSTANCES

- SKBR-3 cells spiked into blood samples were exposed to potential interfering substances and compared to untreated controls. Toxic levels (5 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 cytokeratin 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 cytokeratin 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		

*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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