The CELLSEARCH® CXC Kit contains a ferrofluid-based capture reagent and fluorescence microscope, which is used to identify and enumerate CTCs. The CTC is inserted into a MAGNEST® Cartridge Holder, a fixture of two magnets held magnetically to move to the surface of the cartridge. The CELLTRACKS Analysis of CTCs is performed using the CELLTRACKS ANALYZER II®, a semi-automated microscope. For more information, please refer to the full package insert before testing samples.

INSTRUMENTATION

The CELLSEARCH® CXC 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, open reagents must be recapped with their unique colored caps using the colors indicated on the reagent tray labels. This is to ensure cross-contamination of reagents does not occur.

MATERIALS PROVIDED

• 1 Package Insert
• 3.0 mL Anti-EP-CAM Ferrofluid: Contains a suspension of 0.02% 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 0.0008% mouse monoclonal antibodies specific to cytookeratins conjugated to fluorescein (FLU); 0.0013% 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 0.01% proprietary permeabilization reagent and 0.1% sodium azide in buffer. (red cap)
• 7900017 16 Tests

CAUTION:

• 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.

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

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

LIMITATIONS OF THE PROCEDURE

• For Research Use Only. Not for use in diagnostic procedures. Results should not be used for patient management.
• CTCs 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 Phycoerythrin (PE).
• User-Defined reagents will need to be optimized for use on the CELLTRACKS ANALYZER II® prior to generating test results.
**MATERIALS REQUIRED, NOT PROVIDED**
- CellSave Preservative Tubes (Catalog #7900005)
- CELLTRACKS® AUTOPREP® System (Catalog #9541)
- CELLTRACKS ANALYZER II® (Catalog #9555)
- CELLSEARCH® CXC Control Cell Kit (Catalog #7900018)
- CELLTRACKS® AUTOPREP® Instrument Buffer (Catalog #7901003)
- CELLSEARCH® Epithelial Cell Control Kit (Catalog #7900002)
- Horizontal swing out style rotor (i.e. swing bucket) centrifuge capable of 800 × g
- Test tube racks
- Calibrated micro-pipettors and tips
- Vortex mixer

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

**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 × 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 × g. Set the centrifuge brake to “off” or if your centrifuge provides a variable braking feature, centrifugation time does not take into account the time required to reach 800 × 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.
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®**
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 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 IntraApidil 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.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
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<tr>
<td>LOT</td>
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</tr>
<tr>
<td>SN</td>
<td>SN Number</td>
</tr>
<tr>
<td>REF</td>
<td>Catalog Number or Product Code</td>
</tr>
<tr>
<td>CAUTION</td>
<td>May cause an allergic skin reaction</td>
</tr>
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</table>

*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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