The reagent/sample mixture is dispensed by the CELLTRACKS® AUTOPREP® System into at least 16 CTCs. Anti-CKPE is specific for the intracellular protein cytokeratin (specific for epithelial cell enrichment, fluorescent staining reagents are added for identification and enumeration of EpCAM antigen for capturing CTCs. After immunomagnetic capture, the CTCs are surrounded by a polymeric layer coated with antibodies targeting the Epithelial Cell Adhesion Molecule (EpCAM) antigen for capturing CTCs. A 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 an anti-EpCAM antibody. Handle with care. The 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 leucocytes.

The reagent/sample mixture is dispensed by the CELLTRACKS® AUTOPREP® System into a Cartridge Holder, a mixture of two magnets held together by steel. The strong magnetic field of the CARTRIDGE HOLDER causes the magnetically-labeled epithelial cells to move to the surface of the cartridge. The CELLSEARCH ANALYZER II® acquires images and displays any events to the user where cytokeratin-PE and DAPI are co-localized 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-.

The CELLSEARCH® Kit is designed for use with the CELLTRACKS® AUTOPREP® System and the CELLSEARCH ANALYZER II® above the water’s surface. The product label (i.e., EpCAM+, CK-PE+, DAPI+ and CD45-APC-) provides 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 0.006% mouse monoclonal antibodies specific to cytokeratins conjugated to phycocerythrin (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 0.05% 4’,6-diamidino-2-phenylindole, dihydrochloride (DAPI) and 0.05% ProClin® 300 preservative. (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. (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 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 #955)
- CELLSEARCH® Epithelial Cell Control Kit (Catalog #7900002)
- CELLTRACKS® AUTOPREP® Instrument Buffer (Catalog #791003)
- Horizontal swing out style rotor (i.e. swing bucket) centrifuge capable of 800 x 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 2 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. Process on the CELLTRACKS® AUTOPREP® System. Clotted samples should be discarded.

5. Process 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.


   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 MAGNET® 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, dextranmethorphan, Aredia®, Human Anti-Mouse Antibody (HAMAMA type 1, HAMA type 2, Herceptin®, and ibuprofen. No significant differences in SKBR-3 cells 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.

- Interference from aspirin was determined to be IgG and IgM antibodies against aspirin. Doxetra®, ibuprofen, and aspirin were tested and found to interfere at therapeutic levels and rapid drug clearance.

- The incidence of HAMA 1 and HAMA 2 was 1.3% and 1.5%, respectively.

- Acetaminophen, acetylsalicylic acid, caffeine, dextranmethorphan, Aredia®, Human Anti-Mouse Antibody (HAMA) type 1, HAMA type 2, Herceptin®, and ibuprofen. No significant differences in SKBR-3 cells numbers were detected, indicating that these substances do not interfere with the CELLSEARCH® kit.

- Interference from aspirin was determined to be IgG and IgM antibodies against aspirin. Doxetra®, ibuprofen, and aspirin were tested and found to interfere at therapeutic levels and rapid drug clearance.

- The incidence of HAMA 1 and HAMA 2 was 1.3% and 1.5%, respectively.

- The following symbols may have been used in the labeling of this product.