

CELLSEARCH® Tumor Phenotyping Reagent EGFr

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 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® Epithelial Cell Kit. The CELLSEARCH® Tumor Phenotyping Reagent EGFr may be used to determine Circulating Tumor Cells (CTC) with EGFr over-expression using the CELLTRACKS ANALYZER II®.

The receptor protein for EGF, the epidermal growth factor receptor (EGFr), is a 170 kd transmembrane glycoprotein that comprises a cytoplasmic or tyrosine kinase domain, a transmembrane region and an extracellular domain or ectodomain, that contains the binding site for epidermal growth factor (EGF) and transforming growth factor alpha (TGF- α).^{1,2} Predominantly expressed in squamous cell carcinomas, increased numbers of EGF receptors are present in several types of human tumors, including gliomas and meningiomas, squamous carcinoma of the lungs, and ovarian, cervical and renal carcinomas.^{3,4}

The CELLSEARCH® Tumor Phenotyping Reagent EGFr is a fluorescein conjugate. When used with the CELLSEARCH® Epithelial Cell Kit, the CELLSEARCH® Tumor Phenotyping Reagent EGFr, may be used to determine Circulating Tumor Cells (CTC) with EGFr over-expression. The CELLSEARCH® Epithelial Cell Kit contains a ferrofluid-based capture reagent and immunofluorescent reagents. After immunomagnetic capture and enrichment, fluorescent reagents are added for identification and enumeration of CTC. The CELLSEARCH® Tumor Phenotyping Reagent EGFr is added to identify those CTCs over-expressing EGFr.

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 attracts the magnetically labeled epithelial cells to the surface of the cartridge. In Research Mode, the CELLTRACKS ANALYZER II® automatically scans the entire surface of the cartridge, acquires images and displays any event to the user where CK-PE and DAPI fluorescence are co-located. Images are presented to the user in a gallery format for final classification. An event is classified as a tumor cell expressing EGFr when its morphological features are consistent with that of a tumor cell and it exhibits the phenotype EpCAM+, CK-PE+, DAPI+, CD45-APC - and EGFr-FITC+.

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:** 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.

IMPORTANT: Refer to the specific Instructions for Use of the reagent kit being used with this TPR for information concerning cell carryover levels.

LIMITATIONS OF THE PROCEDURE

- For research use only. Not for use in diagnostic procedures. Results should not be used for patient management.
- Tumor Phenotyping Reagents (TPRs) cannot be run with Control cells.
- TPRs will need to be optimized for use on the CELLTRACKS ANALYZER II® prior to generating test results.

INSTRUMENTATION

The CELLSEARCH® Tumor Phenotyping Reagent EGFr is designed for use with the CELLSEARCH® Epithelial Cell Kit, the CELLTRACKS® AUTOPREP® System, and the CELLTRACKS ANALYZER II®.

REAGENT STORAGE AND HANDLING

- Store at 2–8 °C (36–46 °F).
- Bring to room temperature (15–30 °C or 59–86 °F) before use.
- Protect reagents from exposure to direct sunlight.
- When properly stored, reagent is stable until the expiration date printed on the reagent pack. Do not use expired reagents.
- Discard the Tumor Phenotyping Reagent Cup (Reagent Cup) after the sample run is completed.

MATERIALS PROVIDED

- **1 Package Insert**
- **20 Tumor Phenotyping Reagent Cups**
- **3.0 mL anti-EGFr-FLU:** Contains 0.0008% mouse monoclonal antibody specific for EGFr in PBS containing 0.5% BSA and 0.1% sodium azide. (brown cap)

MATERIALS REQUIRED, NOT PROVIDED

- CellSave Preservative Tubes (Catalog # 7900005)
- CELLTRACKS® AUTOPREP® System (Catalog # 9541)
- CELLTRACKS ANALYZER II® (Catalog # 9555)
- CELLSEARCH® Epithelial Cell Kit (Catalog # 7900000)
- 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

Liquid quality control reagents check the total system including instruments, reagents and operator technique. A CELLSEARCH® Epithelial Cell Control Kit (Catalog #7900002) should be run each day of testing. Please see the CELLSEARCH® Epithelial Cell Control Kit package insert for instructions 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. Add EGFr reagent to the Reagent Cup according to the number of samples that will be processed with the EGFr reagent.

# Samples in Batch to be processed with EGFr reagent	Reagent Volume (µL) to be added
1	450
2	600
3	750
4	900
5	1050
6	1200
7	1350
8	1500

8. Place the Reagent Cup in Position 1 on the reagent carrier.
9. When prompted to select a reagent kit, choose *CELLSEARCH® CTC Kit*.
10. See the *CELLSEARCH® Research Use Only User's Guide* for processing steps.

Analysis Using the CELLTRACKS ANALYZER II®

Refer to 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 blood and the number or percentage of those CTCs that are also positive for EGFr.

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 seven 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	
 Manufacturer	

REFERENCES

1. Marquandt, H., et al. Human Transforming Growth Factors. *J Biol Chem.* 1982;257:5220-5225.
2. Carpenter, G., et al. Antibodies to the epidermal growth factor receptor block the biological activities of sarcoma growth factor. *Proc Natl Acad Sci.* 1983;80:5627-5630.
3. Kawamoto, T., et al. Growth stimulation of A431 cells by epidermal growth factor; identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. *Proc Natl Acad Sci.* 1983;80:1337-1341.
4. Gill, G., et al. Monoclonal anti-epidermal growth factor receptor antibodies which are inhibitors of epidermal growth factor binding and antagonists of epidermal growth factor-stimulated tyrosine protein kinase activity. *J Biol Chem.* 1984;259:7755-7760.

*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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Menarini Silicon Biosystems Inc.
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INSERT CELLSEARCH TPR EGFr

Article Number: MSB02203

Format Name: 3P_Sigma/I/8inch5x11inch/V1 (215.9x279.4mm)

Pointsize: 8pt

File Name: MSB02203.indd (CC2017-PC)

Current Revised Date: 11 October 2017

- Black
- Cyan
- Magenta
- Yellow

REVISION HISTORY

Date of Revision	Component Code	Description of Technical Changes
2017-10-11	MSB02203	<ul style="list-style-type: none"> • Rolled tailcode; address change to HV
2017-08-21	MSB02202	<ul style="list-style-type: none"> • Rolled tailcode; replaced "JDX" with "MSB" • Updated to Menarini Silicon Biosystems business attributes:
2017-01-19	JDX02201	<p>Technically equivalent to 631500091 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 "WARNING" • Updated Results section • In GLOSSARY OF SYMBOLS Section: <ul style="list-style-type: none"> - Deleted both "Harmful" and "Irritant" DSD Danger Symbols • Updated Address to align with ISO certificate • Patent information has been updated