

CELLSEARCH® Tumor Phenotyping Reagent HER-2/neu 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 HER-2/neu may be used to determine Circulating Tumor Cells (CTC) with HER-2/neu over-expression using the CELLTRACKS ANALYZER II®.

HER-2/neu is the human homologue of the neu gene (also termed c-erbB2 or HER-2), which has been identified as being over-expressed in advanced human mammary carcinomas, and other human cancer of glandular origin. The neu oncogene encodes a protein of 185 kDa (p185), which shares extensive homology with the epidermal growth factor receptor. Like EGFR, the HER-2 receptor molecule has three principal domains: an extracellular receptor domain, a single transmembrane domain, and an intracellular domain with tyrosine kinase activity. It has been hypothesized that activation of this receptor by ligand binding results in the stimulation of cytoplasmic tyrosine kinase activity causing signal transduction in the cell.

The CELLSEARCH® Tumor Phenotyping Reagent HER-2/neu is a fluorescein conjugate. When used with the CELLSEARCH® Epithelial Cell Kit, the CELLSEARCH® Tumor Phenotyping Reagent HER-2/neu may be used to determine Circulating Tumor Cells (CTC) with HER-2/neu 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 HER-2/neu is added to identify those CTCs over-expressing HER-2/neu.

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 HER-2/neu when its morphological features are consistent with that of a tumor cell and it exhibits the phenotype EpCAM+, CK-PE+, DAPI+, CD45-APC - and HER-2/neu-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 HER-2/neu 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-HER-2/neu-FLU: Contains 0.0008% mouse monoclonal antibody specific for HER-2/neu 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

- 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.
- Collect whole blood aseptically by venipuncture or from a venous port into a CellSave Preservative Tube only.
- 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.
- 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

- Mix the blood in the CellSave Preservative Tube by manually inverting five times. Then remove the rubber stopper.
- Using a new pipette, transfer 7.5 mL of blood from the CellSave Preservative Tube into a correspondingly labeled 15 mL conical tube provided with the CELLSEARCH® Kit.
- Using a new pipette, add 6.5 mL of Dilution Buffer.
- Cap the conical tube and mix by inversion five times.
- 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.
- 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.
- Add HER-2/neu reagent to the Reagent Cup according to the number of samples that will be processed with the HER-2/neu reagent.

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

- Place the Reagent Cup in Position 1 on the reagent carrier.
- Continue to follow the instructions in the *CELLTRACKS® AUTOPREP® System User's Guide* or on-screen prompts.
- When prompted to select a kit, choose *CellSearch CTC Kit*.
- 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 Her-2/neu.











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 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	

REVISION HISTORY

Date of Revision	Component Code	Description of Technical Changes
2017-01-19	JDX01901	Technically equivalent to 631500181 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 "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.

*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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