

Community-Provided Qualified Antibody Method Requirements for Posting to the DCTD Biomarkers Web Site

SCOPE

Antibody Qualification and Laboratory Proficiency Testing methods for additional biomarkers to be assessed in circulating tumor, or similar, cells using the CellSearch System can be provided by the scientific community for posting to the DCTD Biomarkers Web site (<http://dctd.cancer.gov/ResearchResources/biomarkers/CTCs.htm>). Requirements for community-provided antibody qualification and preparation workflow submissions are based on DCTD-validated SOPs, but will not be validated by DCTD.

REQUIREMENTS

- Must be an analytically validated method for the enumeration of biomarker-positive cells using the CellSearch System.
- Assay **must be** for evaluation of **increases** in biomarker response to an agent; the current CellSearch platform is not optimized to measure decreases in biomarker levels within labeled CTCs.
- Antibody qualification methods for use with LHTP003.8.1 (Immunofluorescence Assay for Circulating Tumor Cells Using the CellSearch System) must use an antibody-FITC (or similar) conjugate, not separate primary and secondary antibodies.
- Antibody Qualification and Laboratory Proficiency Testing should:
 - 1) Be performed for the biomarker in a manner similar to that in LHTP003.8.1.1 and LHTP003.8.1.2 (<http://dctd.cancer.gov/ResearchResources/biomarkers/CTCs.htm>) and the generic workflow diagram provided (page 3), **and**
 - 2) Meet the following criteria:
 - Antibody Qualification should have been completed on a minimum of two lots of antibody conjugate using the recommended cytospin method and meet the following minimum requirements:
 - The negative control cell line should have < 3% biomarker-positive cells. A value of zero is acceptable.
 - The positive control cell line should have \geq 10% biomarker-positive cells.
 - Laboratory Proficiency Testing should be completed on a minimum of two lots of antibody conjugate using the CellSearch System and meet the following minimum requirements:
 - Enumeration of negative control cell line-spiked blood samples should have < 3% biomarker-positive cells. A value of zero is acceptable.
 - Enumeration of the positive control cell line-spiked blood samples should have \geq 10% biomarker-positive cells.

SUBMISSION PACKAGE FOR POSTING TO THE DCTD BIOMARKERS WEB SITE

- 1. Workflow diagram**
 - A. Similar to that in LHTP003.8.1.1 and LHTP003.8.1.2 (<http://dctd.cancer.gov/ResearchResources/biomarkers/CTCs.htm>) and the generic workflow diagram provided (page 3).
- 2. Description of positive and negative control cell line(s):**
 - A. Vendor and catalog number or other source of cell line(s).
 - B. Description of recommended culture conditions, media, maximum passage number, and other pertinent information related to the cell line(s).
 - C. If drug treatment is used to generate positive-control cells: vendor and catalog number or other source of drug treatment, recommended preparation and storage of drug, concentration and duration of drug treatment, recommended confluence of cells at the initiation of treatment, and any other information pertinent to producing positive-control cells.
 - D. Recommended confluence of cells and any other pertinent information needed to produce negative-control cells.
- 3. Description of antibody-FITC (or similar) conjugate:**
 - A. Vendor and catalog number, or other, of purchased antibody conjugate and concentration and recommended storage conditions for stock antibody conjugate.
 - a. If the antibody was not purchased commercially, describe method of antibody production and purification.
 - b. If the antibody conjugation was performed internally or contracted to a reagent service provider, describe method of conjugation and re-purification. *If a commercial service was used for generating the conjugate, please list contact information for the contractor.*
 - B. Recommended staining conditions:
 - a. Time and temperature of cell fixation and permeabilization.
 - b. Antibody conjugate concentration, volume, and buffer.
 - c. Time and temperature of antibody conjugate incubation if performed offline.
- 4. Does the recommended fixation and permeabilization method differ from that described in SOP LHTP003.8.1.1? If so, provide details.**
- 5. What volume and cell number are spiked into healthy donor blood during Laboratory Proficiency Testing?**
- 6. Submit a minimum of three images each for recovered positive and negative control cells from spiked blood samples analyzed on the CellTracks Analyzer.**

WORKFLOW DIAGRAM FOR ANTIBODY QUALIFICATION AND LABORATORY PROFICIENCY TESTING

