liquidbio

Procedure

IsoFlux CTC Enrichment Kit

Applies to: 910-0091

Updated: 2023-03-28
Revision: E

INTENDED USE

The intended use for the IsoFlux™ Circulating Tumor Cell Enrichment Kit (CTC Kit) is as a general-purpose laboratory reagent for dilution of blood samples to enrich for circulating tumor cells (CTCs). The kit is used with the IsoFlux System, a bench-top instrument for semi automated cell isolation. The kit contains an immunomagnetic bead reagent targeted towards cells of epithelial origin. The CTC kit is for Research Use Only.

SUMMARY AND EXPLANATION

Circulating tumor cells (CTCs) are cancer cells that shed from a primary or metastatic tumor and enter the peripheral circulation. Carcinomas are cancers of epithelial origin and include breast, prostate, lung, and colorectal cancers. These tumors shed CTCs that are of epithelial origin. CTs are distinct from other blood cells since cells of epithelial origin are not normally found in the circulation.
The IsoFlux CTC Enrichment Kit is designed to standardize and automate the enrichment of CTs from biological samples using the IsoFlux System. CTCs are enriched from the sample using an immunomagnetic capture reagent while the sample flows through a microfluidic cartridge designed for cellular isolation. The kit produces an enriched cell pellet that can subsequently be used for further testing.

PRINCIPLES OF THE PROCEDURE

The IsoFlux CTC Kit contains immunomagnetic capture beads (CTC beads), microfluidic cartridges, and additional reagents required for performing CTC enrichments. The CTC beads consist of micro-scale particles with a magnetic core surrounded by a polymeric layer coated with antibodies targeting the EpCAM antigen. CTs can be isolated from mononuclear cell suspensions of whole blood or other similar cell samples. CTC beads are then mixed with the cell sample to bind to the target cells during a period of incubation.

The cell sample and beads mixture is loaded onto the microfluidic cartridge and processed with the IsoFlux instrument, where the cells pass through the fluidic channel of the cartridge. Midway through the fluidic channel is a cell isolation zone that is exposed to an external magnetic field inside the instrument. The target cells having CTC beads attached are attracted towards the magnetic field. The target cells are collected on a removable disk that forms the roof of the isolation zone. After the sample is processed, the enriched cells are transferred inside the instrument to low volume recovery holder or a microfuge tube. The enriched CTCs are ready for further analysis.

MATERIALS PROVIDED

- 8 sterile microfluidic cartridges (includes 8 low-volume recovery holders, 8 microfuge tubes for cell recovery)
- 500µL CTC beads*
- 500µL Fc blocker reagent*
- 4x 12mL sterile preservative free Binding Buffer

*Contains 0.02% sodium azide as a preservative.

REAGENT STORAGE AND HANDLING

- Microfluidic cartridges should be stored unopened at room temperature. CTC beads and Fc blocker reagents should be stored at 2° to 8°C and used within 60 days after opening.
- Binding Buffer should be stored unopened at 2° to 8°C and used. After opening, unused buffer may be stored frozen at -20°C, thawed and used once within 60 days. Protect reagents from heat in excess of 35°C. Do not freeze.
- Protect reagents from exposure to light.
- When properly stored, reagents are stable until the expiration date printed on the reagent container or kit box. Do not use expired reagents.
- Do not mix and match reagents from different kits.

MATERIALS REQUIRED, NOT PROVIDED

- IsoFlux Instrument (Catalog No. 950-0100)
- Permanent magnets (accessory parts included with IsoFlux instrument: large round and small cylindrical magnets)
- Swing bucket centrifuge capable of 1500xg (with brake settings) Test tube racks Calibrated micro-pipettors and tips
- Serological pipettes and pipettor
- 1.5 or 2mL microfuge tubes (preferably low retention)
- Microfuge tube rotator
- 50mL Leucosep® tubes (with frit) (Greiner, Catalog No. 227290)
- Phosphate Buffer Saline without Ca2+ Mg2+ (PBS-CMF)
- 50mL conical tubes
- Ficoll-Paque™ Plus (GE Healthcare, Catalog No. 17-1440-02)

- Optional: CTL-Wash™ Supplement (CTL, Catalog No. CTLW-010)
- Optional: Benzonase® Nuclease (Sigma, Catalog No. E8263)
- Optional: Nylon Mesh Cell Strainer, 40 Micron (BD, Catalog No. 352340)

WARNINGS AND PRECAUTIONS

- For Research Use Only
- Please read the entire contents of the Instructions for Use before processing samples. **Caution:** Care should be taken to collect and transfer blood samples before processing. Cells are fragile and can be damaged or lost if not handled properly. **Caution:** All personnel should follow universal precautions for biological sample handling and use personal protective equipment (i.e., safety glasses, laboratory coat, gloves, etc.).
- **Caution:** Microbial contamination of reagents can cause erroneous results and should be avoided.
- **Warning:** All biological specimens, cartridges and other materials coming into contact with the specimen(s) are considered bio-hazardous. 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. **Warning:** Some of the reagents contain sodium Azide as a preservative. If swallowed, seek medical advice immediately. 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. Operator training is required to perform the test procedure.

ENRICHMENT PROCEDURE

Specimen collection and preparation

1. Collect biological samples aseptically into an appropriate sample collection tube.
2. If samples are being shipped or transported, pack the samples accordingly to control exposure to excessive temperatures or agitation. Typically, samples can be shipped in an insulated Styrofoam shipping container. Cold (not frozen) or warm gel packs may be used in the outer foam box to minimize temperature fluctuations.
3. Depending on the sample type, a pre-processing step might be required such as density centrifugation or red blood cell lysis.
4. A typical pre-processing procedure for human whole blood (mononuclear cell fraction preparation) is provided in Section 2. Please consult the manufacturer's instructions for pre processing procedure for other cell sample types.

Pre-processing of whole blood sample (mononuclear cell (MNC) fraction preparation)

1. A 7 to 10mL of peripheral blood sample should be drawn into anti-coagulant K2EDTA

coated blood collection tube and kept at room temperature. Whole blood sample should be processed within 36 hours of collection.

2. For each sample to be processed coat a microfuge tube with 1mL of Binding Buffer and rotate at 4°C (or room temp) until use. Alternatively if using Protein LoBind tubes (Eppendorf Cat. No. 022431081), coating is not required.
3. Prepare the 50mL Leucosep® (with frit) tube by adding 15.2mL of Ficoll- Paque™ PLUS and centrifuging the tube at 1000xg for 30 seconds with the brake setting to ON.
4. Only when ready to process the blood sample, gently add 5mL of PBS-CMF to the Leucosep® tube.
5. Immediately decant the blood from the blood collection tube into the Leucosep® tube. Gently rinse down the wall of the blood collection tube with 10mL of PBS-CMF. Re-cap and gently invert the blood collection tube several times to mix. Add the rinse to the same Leucosep® tube. Repeat the rinse once more.
6. Immediately centrifuge the tubes at 800xg for 15 minutes with the brake setting to OFF. Alternatively spinning at 1000xg for 15 minutes with brake setting to OFF may be used
7. Decant the supernatant from Leucosep® a tube into a new 50mL conical tube, leaving about 5 to 10mL remaining. Gently swirl the remaining supernatant to dislodge any cells that may be stuck to the wall of the Leucosep® tube and then decant it into the same conical tube. Rinse the wall of the Leucosep® tube with 5mL of PBS-CMF and add that to the same 50mL conical tube. Be careful not to suction the Ficoll-Paque™ PLUS through the frit; avoid pressing the pipette against the frit. Optional: CTL-Wash™ Supplement may be added to improve cell viability.
8. Centrifuge at 280xg for 10 minutes with the brake setting to ON.
9. Use a 25 or 50mL serological pipette to gently aspirate off the supernatant as much as possible without disturbing the pellet. Use a smaller pipette to remove the remaining supernatant closer to the bottom of the tube with up to ~500µL buffer may be left remaining. a Alternatively, a vacuum set-up may be used to suction off the supernatant. Keep the pellet on ice. This is considered MNC. We recommend that you do not decant the supernatant, because the pellets might be very loose in clinical samples. Optional: Benzonase® Nuclease (up to 1000 Units per sample) may be added. Addition of nucleases is required for sample that have been stored for ≥24 hours or severely lysed to minimize cell aggregation due to cell lysis.
10. Add 40µL of Fc Blocking Reagent to the MNC sample in the 50mL conical tube above.
11. Gently tap the tube on the bench a few times to loosen pellet. Tap patiently until the pellet is completely resuspended. If necessary, add up to 300µL of Binding Buffer to the tube. All cell clumps must be dispersed as they may clog the micro-channel during isolation. Gently resuspend the cells with P1000 pipette.
12. Remove the Binding Buffer from the microfuge tube prepared in Step 2.2. Transfer cell suspension into the microfuge tube. Rinse the residual cells in the 50mL conical tube with Binding Buffer and transfer to the same microfuge tube. The final volume should be no more than 1mL. Keep cell suspension on ice until use.

Caution: maximum sample volume is 1mL when loading onto the cartridge. Try to prepare

the sample at this step so that the volume is ~800µL to allow for additional rinsing when loading onto the cartridge. We recommend using a 200µL micropipette with wide-bored tip when transferring to minimize shear force, and allow for estimating the sample volume.

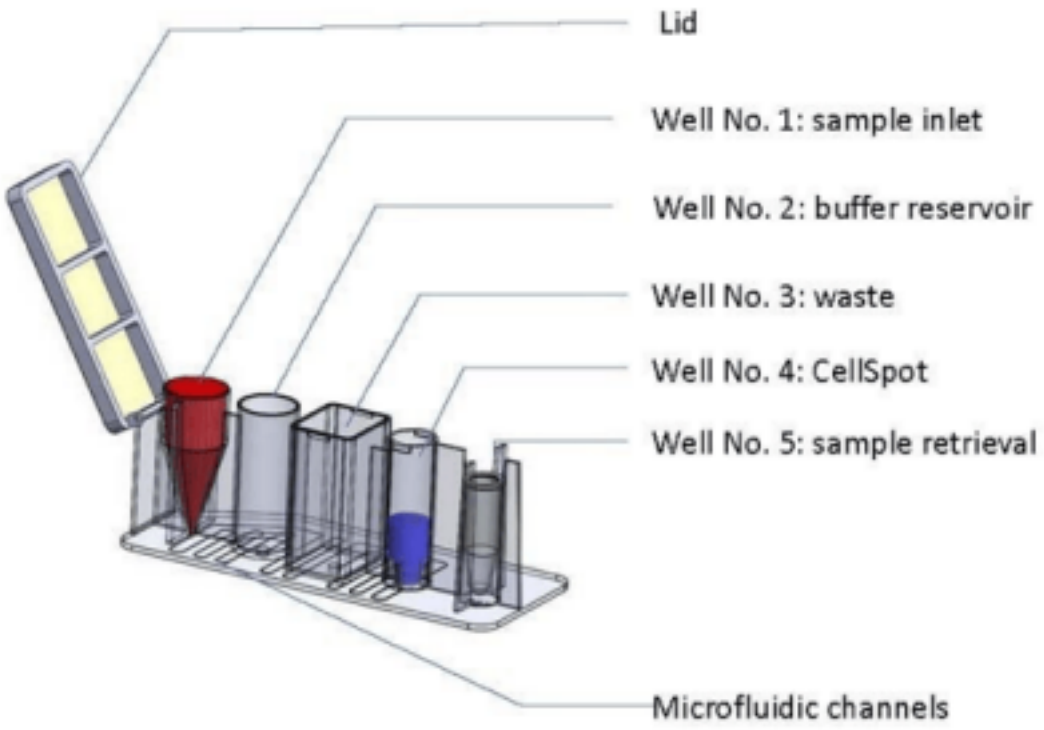
Coupling reaction of beads and cell sample:

This preparation suffices for 8 samples. Scale up or down as appropriate.

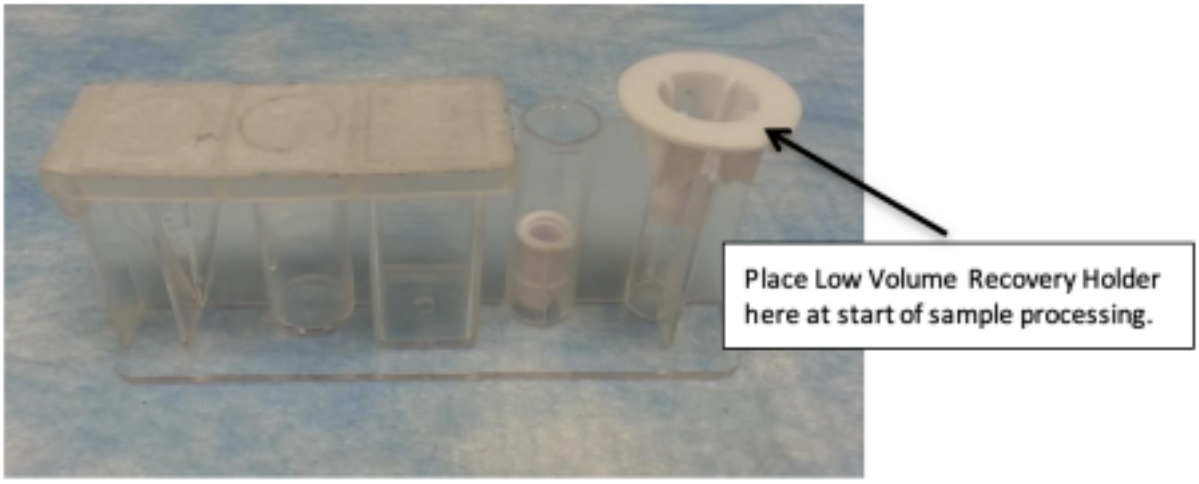
1. Resuspend the CTC beads stock with a micropipette. Dispense 400µL of beads stock into a microfuge tube containing 1mL of Binding Buffer.
2. Place the tube on the 1 large magnet for 1 minute and discard the supernatant.
3. Remove the tube from the magnet and resuspend the beads in 400µL of Binding Buffer.
4. Add 40µL of washed CTC beads to the cell suspension.
5. Incubate for 1.5 hours at 4°C with gentle tilting and rotation. The cell sample is now ready for CTC enrichment with the IsoFlux System. Optimum incubation time is 2 hours. At 1.5 hours after incubation, the preparation for enrichment (section below) can begin.

CTC enrichment with IsoFlux System

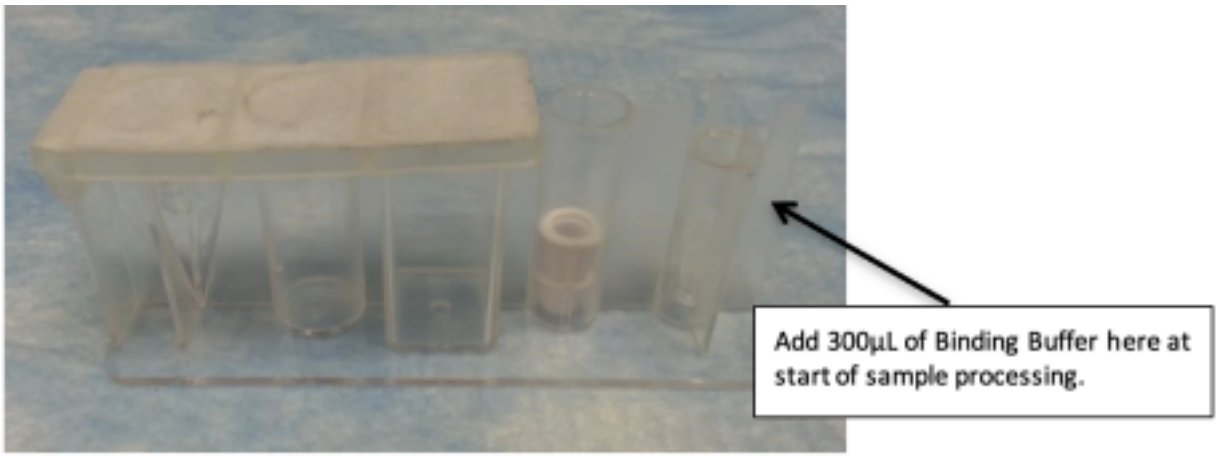
1. Refer to the IsoFlux System **Instructions for Use** and on-screen commands for full instructions to process samples for cell enrichment.
2. Power on the IsoFlux instrument. The touch screen panel will light up; the instrument will initialize and perform automatic routine system check.
3. The touch screen will display “**Run Protocol**” and “**Select Protocol**” icons when the instrument is ready for use. Choose one the options below:
    1. “**Run Protocol**” to run the most recent protocol used (shown at the bottom of the touch screen).
    2. “**Select Protocol**” to select the appropriate protocol and then press “**Run Protocol**”.
4. Select the **Number of Samples to Run**. Cartridge loading carriage(s) will slide out automatically. A total 4 samples can be processed simultaneously. Positions No. 1 and 2 are on the left carriage. Positions No. 3 and 4 are on the right carriage. Samples should be loaded from left to right sequentially from Position No. 1 to 4.
5. Remove the microfluidic cartridge from the pouch and position it upright on a flat surface (see drawing below).



6. Sample retrieval Microfuge Tube is in well No. 5.
7. The Low-Volume Recovery Holder is in well No. 3.
8. Remove and keep the Low-Volume Recovery Holder to be used in the next step.
9. Decide if the enriched cells will be recovered with Low-Volume Recovery Holder or in the Microfuge Tube.
10. If the enriched cells will be recovered with Low-Volume Recovery Holder, remove the Microfuge Tube in well No. 5 and insert the Low Volume Recovery Holder as shown below:



11. If using the Microfuge tube, add 300µL of Binding Buffer to the Microfuge tube in the sample retrieval position on the cartridge (well No. 5) as showing below.



12. Carefully open the cartridge lid and add 3mL Binding Buffer to the buffer reservoir (well No. 2) of each cartridge. Carefully snap close the cartridge lid.
13. Load cartridge(s) onto the carriage(s). Press **Prime**. Cartridge loading carriage(s) will slide in automatically. Machine will prime for about 6 minutes.
14. After priming is completed, touch screen will show **Ready to Load Sample**. Press **Ready to Load Sample**. Left carriage will slide out automatically. Carefully open cartridge lids.
15. Gently add the beads-coupled cell samples from Step 4 of the ***Coupling reaction of beads and cell sample*** section to the Sample well (well No. 1) and avoid forming bubbles. Carefully snap close the cartridge lid and load onto the carriage.
16. After all cell samples are loaded for the left carriage, press **Load**. Left carriage will slide in automatically. If running one or two samples, cell isolation will start at this point. If running more than two samples, right carriage will slide out automatically. Load the rest of samples and press **Load**, right carriage will slide in automatically. Cell isolation will start.
17. Cell isolation typically takes about 45 minutes but it may vary for different samples. 18. After cell isolation is completed, touch screen will show **Extract Sample**. Press **Extract Sample**. Carriage(s) will slide out automatically.
19. The instrument will place the CellSpot into either the Low-Volume Recovery Holder or the Microfuge Tube.

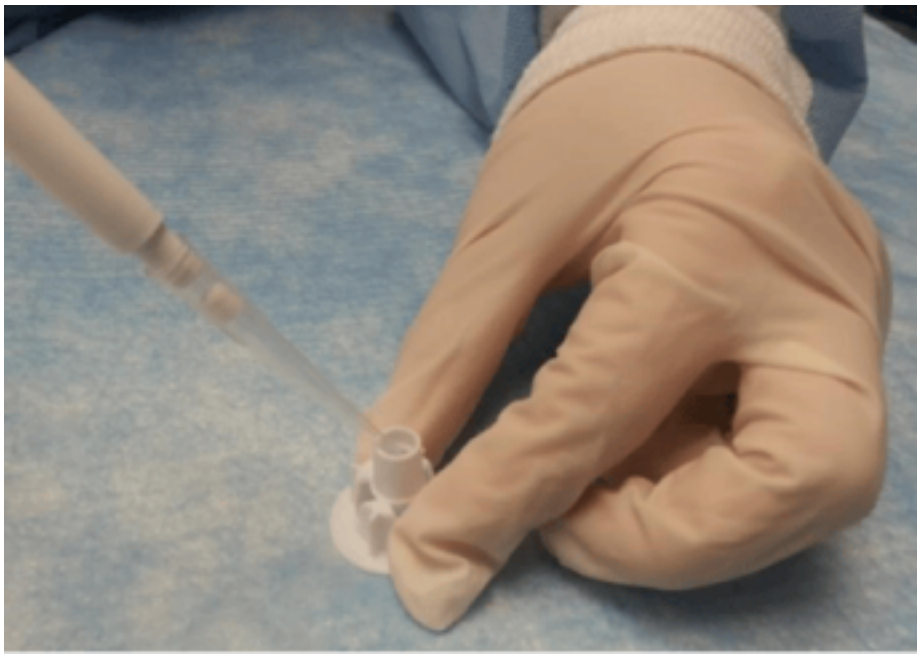
Warning: Recover the sample within 5 minutes after the isolation is finished.
DO NOT ALLOW SAMPLE TO DRY

Cell retrieval (if using Low-Volume Recovery Holder)

1. Remove and invert the holder such that the enriched cells are facing up. 2. Immediately add 20µL (a drop) of Binding Buffer to the CellSpot to prevent cells from drying.
3. If necessary, place the CellSpot over the small cylindrical magnet for 5 seconds to center the cells/beads pellet. Remove the CellSpot from the magnet.
4. Rinse the micropipette tip with Binding buffer to minimize cell sticking to the tip. Gently aspirate the cells/beads into the pipette tip. Dispense the collected cells/beads into a

new microfuge tube (not provided). *We recommend doing the liquid-to-liquid transfer (i.e. the microfuge tube should also contain a small volume of Binding Buffer).*

5. Place the microfuge tube on the large magnet.
6. Aspirate most of the supernatant and rinse the CellSpot to collect any residual cells/beads. Repeat the previous steps 4-6 until no visible cells/beads are observed on the CellSpot.



Cell retrieval (if using Microfuge Tube)

1. Gently invert the tube 2-3 times until all the cells/beads are suspended in the Binding Buffer at the bottom of the tube.
2. Centrifuge the microfuge tubes briefly to collect all cells. Enriched CTCs are now ready for further testing.