liquidbio

Instructions for Use

Rare Cell Enrichment Kit - IgG

Applies to: 910-0092

Updated: 1-10-24

Rev. B

INTENDED USE

The intended use for the IsoFlux™ Rare Cell Enrichment Kit (IgG) (RCE Kit) is as a general-purpose laboratory reagent for the enrichment of rare cells in the blood circulation. The kit is used with the IsoFlux System, a benchtop instrument for semi-automated cell isolation. The kit contains an immunomagnetic bead reagent functionalized with a monoclonal human anti-mouse IgG. It will bind all mouse IgG subclasses via Fc-region. The RCE kit is for Research Use Only.

SUMMARY AND EXPLANATION

Many rare cell types of scientific and clinical interest exist in the peripheral blood circulation. These include circulating tumor cells (CTCs), cancer stem cells, immune cells, and circulating endothelial cells. In many cases, these cells can be preferentially enriched from the background cells using differentially expressed cell-surface antigens.

The IsoFlux RCE Kit is designed to standardize and automate the enrichment of rare cells from biological samples using the IsoFlux System. Cells are positively isolated from the sample using an immunomagnetic capture reagent while the sample flows through a microfluidic cartridge designed for cell isolation. The kit produces an enriched cell pellet that can subsequently be used for further testing.

PRINCIPLES OF THE PROCEDURE

The IsoFlux RCE Kit contains immunomagnetic pan-mouse capture beads (RCE beads), microfluidic cartridges, and additional reagents required for performing rare cell enrichments. The RCE beads consist of micro-scale particles with a magnetic core surrounded by a polymeric layer coated with a monoclonal human anti-mouse IgG antibody. RCE beads in combination with primary mouse IgG antibodies (selected by the user, based on specific application) are ideal for enrichment of rare cells, depending on the specificity of the primary antibody. Rare cells can be isolated from mononuclear cell suspensions of whole blood or other similar cell samples. The primary mouse IgG antibodies are either pre-coated onto the pan-mouse beads (direct method, most recommended) or added to the cell sample (indirect method). RCE 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 RCE 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 rare cells are ready for further analysis.

MATERIALS PROVIDED

- Instructions for Use
- 8 sterile microfluidic cartridges (includes 8 low-volume recovery holders, 8 microfuge tubes for cell recovery)
- 500µL RCE (pan mouse IgG) 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. • RCE 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. 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)
- Swing bucket centrifuge capable of 1500xg (with brake settings) • Test tube racks
- Calibrated micro-pipettors and tips
- Serological pipettes and pipettor
- 2mL microfuge tubes (preferably low retention)
- Microfuge tube rotator
- 50mL Leucosep® tubes (with frit) (Greiner, Catalog No. 227290) • Phosphate Buffer Saline without Ca^{2+} Mg^{2+} (PBS-CMF)
- 50mL conical tubes
- Ficoll-Paque™ Plus (GE Healthcare, Catalog No. 17-1440-02)
- Permanent magnets (accessory parts included with IsoFlux instrument, large round and small cylindrical magnet)
- 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 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.
- 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.

1. Specimen collection and preparation

1.1. Collect biological samples aseptically into an appropriate sample collection tube.

1.2. 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 with cold (not frozen) gel packs as a buffer to temperature fluctuations.

1.3. Depending on the sample type, a pre-processing step might be required such as density centrifugation or red blood cell lysis.

1.4. 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.

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

2.1. A 7 to 10mL peripheral blood sample should be drawn into anti-coagulant K_2EDTA coated blood collection tube and kept at room temperature. Whole blood sample should be processed within 36 hours of collection.

2.2. For each sample to be processed, coat a 2mL tube with 1mL of Binding Buffer and rotate at 4°C until use.

2.3. Prepare the 50mL Leucosep (with frit) tube by adding 15mL of Ficoll-Paque PLUS and centrifuging the tube at 1000xg for 30 seconds with the brake setting to ON.

2.4. Only when ready to process the blood sample, gently add 5mL of PBS-CMF to the Leucosep tube.

2.5. Immediately add blood sample to Leucosep tube by first decanting it from the blood collection tube into the Leucosep tube. Then, gently wash down the walls of the blood collection

tube twice, each time with 10mL of PBS-CMF.

2.6. Immediately centrifuge the tubes at 800xg for 15 minutes with the brake setting to OFF.

2.7. Decant about 20mL of the supernatant in Leucosep tube into a new 50mL conical tube. 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.*

2.8. Centrifuge at 280xg for 10 minutes with the brake setting to ON.

2.9. Use a 25 or 50mL serological pipette to gently aspirate off the supernatant as much as possible without disturbing the pellet. Use a 5mL pipette to remove the remaining supernatant closer to the bottom of the tube and avoid disturbing the pellet (up to ~500µL buffer may be left remaining). Keep the pellet on ice. *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 to minimize cell aggregation due to cell lysis. *Benzonase Nuclease should be avoided if performing nuclease sensitive down-stream analysis.*

2.10. 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.

2.11. Remove and discard the Binding Buffer from the tube prepared in Step 2.2. Transfer cell suspension into the 2mL tube. Rinse the residual cells in the 50mL conical tube with Binding Buffer and transfer to the same 2mL tube. The final volume should be approximately 1mL. Keep cell suspension on ice until use.

3. Coupling reaction of beads and cell sample:

Coupling method (direct or indirect) selection: Direct method usually gives more favorable results for rare cell isolation. It is recommended to try direct method first before trying indirect method. If the affinities of mouse primary antibodies are low, the cells express low number of target antigens, or a cocktail of many primary mouse antibodies are used, indirect method may yield better results.

3.1. Coupling reaction – direct method (coat RCE beads with mouse antibody) This preparation suffices for 8 samples. Scale up or down as appropriate. 3.1.1. Use 0.5–1.5µg of mouse IgG primary antibody per 400µL of washed beads. It is recommended to titrate the primary antibody to optimize the amount used.

3.1.2. Resuspend the RCE beads stock with a micropipette. Dispense 400µL of beads stock into a microfuge tube containing 1 mL of Binding Buffer. Place the tube on the large magnet for 1 minute and discard the supernatant. Remove the tube from the magnet and resuspend the washed beads in 400µL Binding Buffer.

3.1.3. Add the mouse IgG primary antibody to the 2mL microfuge tube containing 400µL pre-washed beads.

3.1.4. Incubate for ≥30 minutes at 4°C with gentle tilting and rotation.

3.1.5. Place the tube on the large magnet for 1 minute and discard the supernatant.

3.1.6. Remove the tube from the magnet and resuspend the beads in 2mL of Binding Buffer.

3.1.7. Place the tube on the large magnet for 1 minute and discard the supernatant. Resuspend the beads in 400µL of Binding Buffer. Keep on ice until use.

3.1.8. Add 40µL of Fc Blocking Reagent to the 1mL cell sample (prepared in Section 2). Mix gently by inverting the tubes a few times and incubate on ice for 5 minutes.

3.1.9. Add 40µL of primary mouse antibody coated beads (Step 3.1.7) to the cell sample. Incubate for 2 hours at 4°C with gentle tilting and rotation. The cell sample is ready for rare cell enrichment by IsoFlux System.

3.2. Coupling reaction – indirect method (add mouse antibody to cell sample) Use approximately 10µg of mouse IgG primary antibody (for multiple capture antibodies, use approximately 10µg of each mouse IgG) per 1mL of cell sample (e.g. mononuclear cells preparation from 7-10 mL of whole blood or ~2 x 10^7 cells). Titrate the primary antibody to optimize the amount used.

3.2.1. Add 40µL of Fc Blocking Reagent to the 1mL cell sample (prepared in Section 2). Mix gently by inverting the tubes a few times and incubate on ice for 5 minutes.

3.2.2. Add the mouse IgG primary antibody to the cell sample. Incubate for ≥20 minutes at 4°C with gentle tilting and rotation.

3.2.3. Centrifuge at 280×g for 10 minutes. Discard the supernatant. 3.2.4. Wash the cells by adding 1.8mL Binding Buffer and centrifuge at 280×g for 10 minutes. Discard the supernatant.

3.2.5. Resuspend the cells in 1mL of Binding Buffer.

3.2.6. Resuspend the RCE beads stock with a micropipette. Dispense 400µL of beads stock into a microfuge tube containing 1 mL of Binding Buffer. Place the tube on the large magnet for 1 minute and discard the supernatant. Remove the tube from the magnet and resuspend the washed beads in 400µL Binding Buffer (This preparation suffices for 8 samples. Scale up or down as appropriate).

3.2.7. Add 40µL washed RCE beads to the cell suspension.

3.2.8. Incubate for 2 hours at 4°C with gentle tilting and rotation. The cell sample is ready for rare cell enrichment by the IsoFlux System.

Note: If any visible cell aggregates are observed in the cell samples, filter through a 40-micron nylon mesh cell strainer before loading the cell samples onto the IsoFlux cartridges.

4. Rare cell enrichment with IsoFlux System

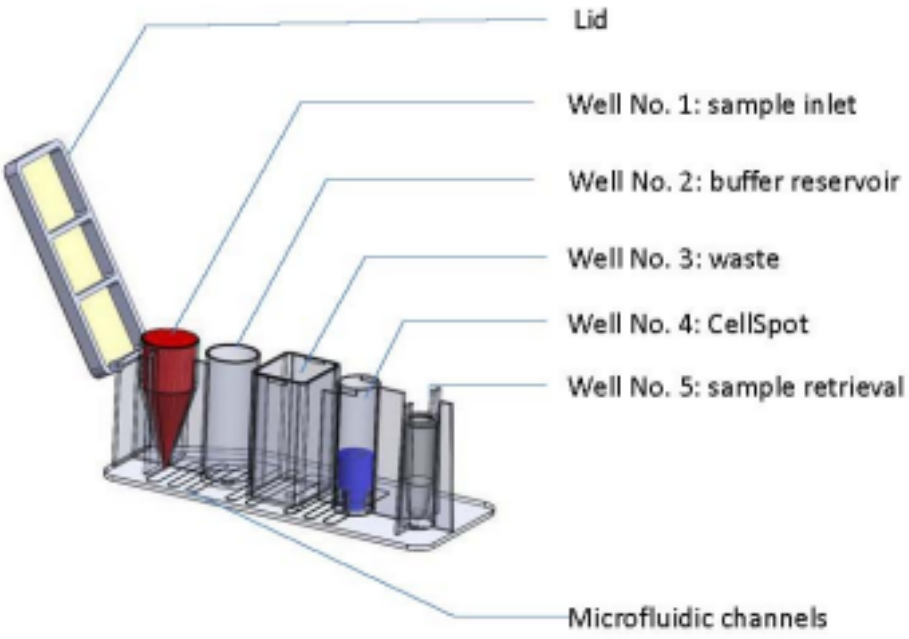
4.1. Refer to the IsoFlux System Instructions for Use and on-screen commands for full instructions to process samples for cell enrichment.

4.2. Power on the IsoFlux instrument. The touch screen panel will light up; the instrument will initialize and perform automatic routine system check. The touch screen will display “Run Protocol” and “Select Protocol” icons when the instrument is ready for use.

4.3. Press “Run Protocol” to run the most recent protocol used (shown at the bottom of the touch screen). Press “Select Protocol” to select the appropriate protocol and then press “Run Protocol”.

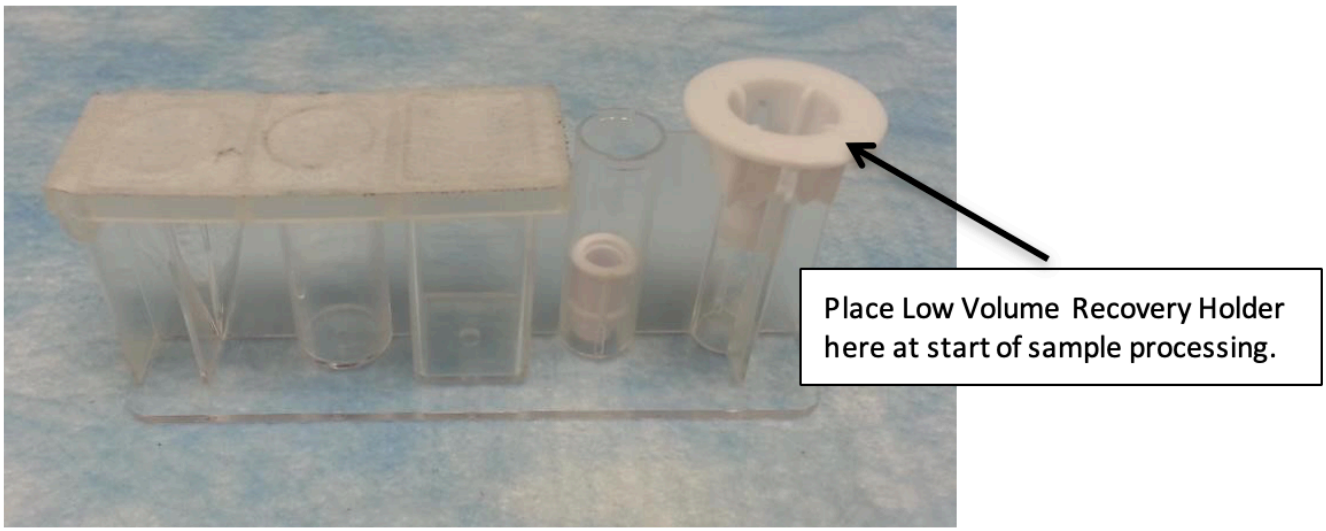
4.4. Select the Number of Samples to Run. Cartridge loading carriage(s) will slide out automatically. A total 4 samples can be processed simultaneously. Positions No. 1 and 2 are on the left carriage. Positions No. 3 and 4 are on the right carriage. Samples should be loaded from left to right sequentially from Position No. 1 to 4.

4.5. Remove the microfluidic cartridge from the pouch and position it upright on a flat surface (see drawing below). The plastic retainer helps to keep the removable CellSpot (well No. 4) and sample retrieval Microfuge Tube (well No. 5) in place. The Low-Volume Recovery Holder is attached to the retainer. Hold the base of the cartridge down and carefully remove the retainer (taking care to keep the CellSpot and Microfuge Tube in place). Remove and keep the Low-Volume Recovery Holder to be used in Step 4.6.1.

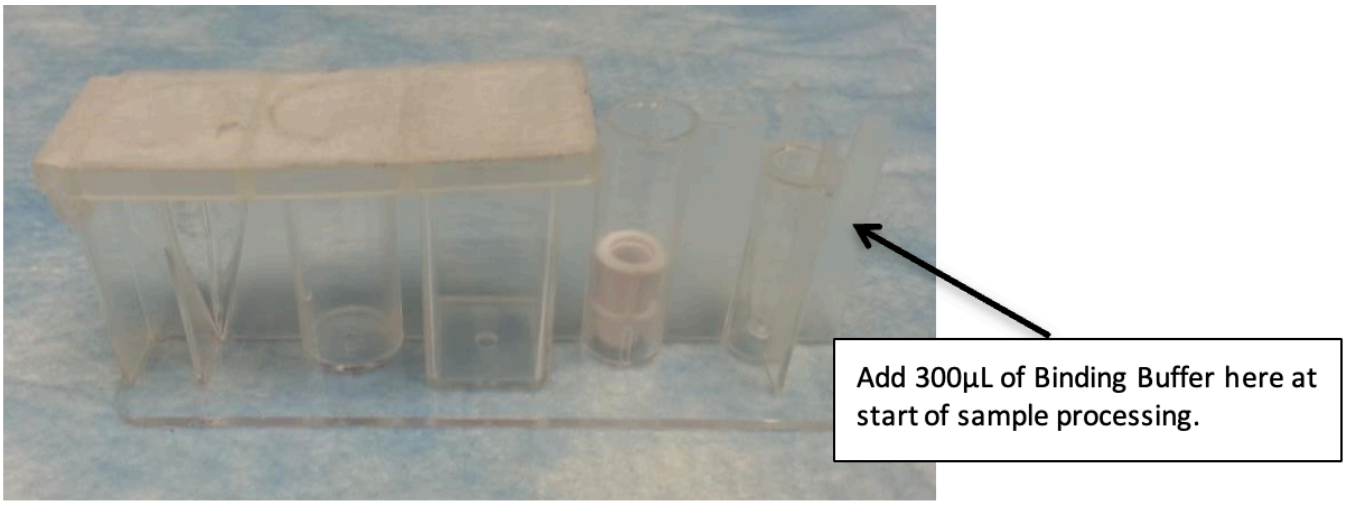


4.6. Decide if the enriched cells will be recovered with Low-Volume Recovery Holder or in the Microfuge Tube.

4.6.1. If using Low-Volume Recovery Holder – Remove the Microfuge Tube in well No. 5 and insert the Low Volume Recovery Holder as shown below.



4.6.2. If using Microfuge Tube – Add 300µL of Binding Buffer (or other desired recovery buffer) in the Microfuge Tube in the sample retrieval position on the cartridge (well No. 5) as showing below.



4.7. Carefully open the cartridge lid, add 3mL of Binding Buffer to the buffer reservoir (well No. 2) of each cartridge. Carefully snap close the cartridge lid.

4.8. Load cartridge(s) onto the carriage(s). Press Prime. Cartridge loading carriage(s) will slide in automatically. Machine will prime for about 6 minutes.

4.9. After priming is complete, touch screen will show Ready to Load Sample. Press Ready to Load Sample. Left carriage will slide out automatically.

Carefully open cartridge lids. Add cell samples (with beads coupled) from Step 3.1.9 or 3.2.8 to the Sample well (well No. 1), avoid forming any bubbles. Carefully snap close the cartridge lid and load onto the carriage.

4.10. 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.

4.11. Cell isolation typically takes about 45 minutes, but it may vary for different samples.

4.12. After cell isolation is completed, touch screen will show Extract Sample. Press Extract Sample. Carriage(s) will slide out automatically.

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

5. Cell retrieval

Immediately recover cells (well No. 5) with one of the two modes:

5.1. Cell retrieval if using the Low Volume Recovery Holder:

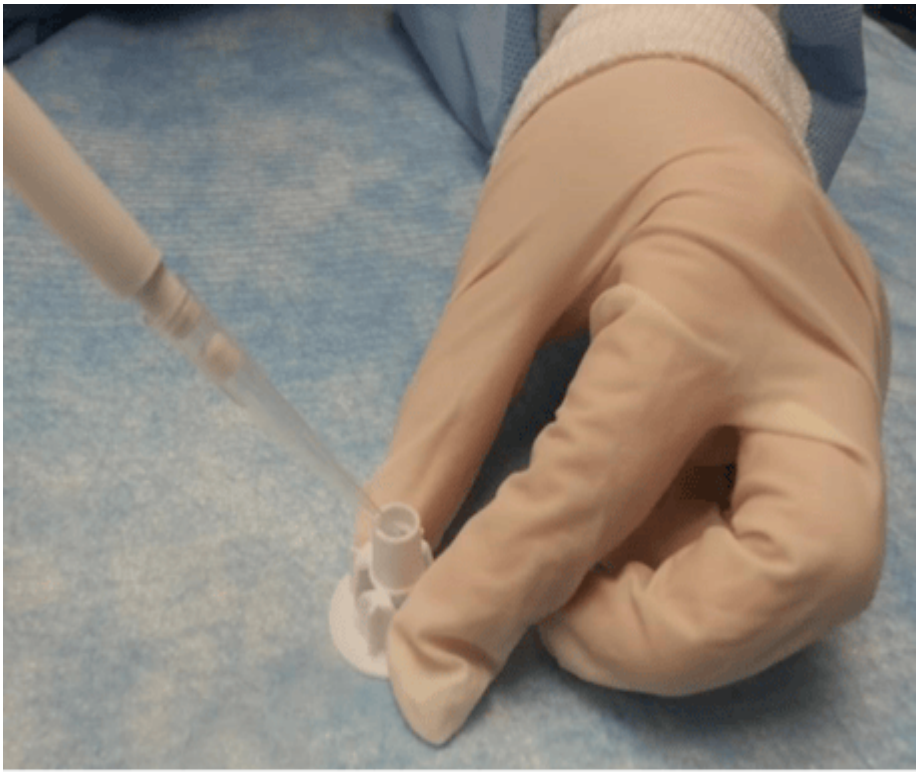
5.1.1. Remove and invert the holders such that the enriched cells are facing up. Immediately add 20µL of Binding Buffer to all the CellSpots to prevent cells from drying.

5.1.2. Place the CellSpot over the small cylindrical magnet for 5 seconds to center the cells/beads pellet.

5.1.3. Rinse the micro-pipette tip with Binding Buffer to avoid cell sticking. Remove the CellSpot from the magnet. Gently aspirate the cells/beads into the pipette tip. Dispense the collected cells/beads into a new microfuge tube (not provided).

5.1.4. Place the microfuge tube on the large magnet. Aspirate most of the supernatant and rinse

the CellSpot to collect any residual cells/beads. Repeat the previous two steps until no visible cells/beads are observed on the CellSpot.



5.2. Cell retrieval if using the Microfuge Tube:

5.2.1. Gently invert the tube 2-3 times until all the cells/beads are suspended in the Binding Buffer at the bottom of the tube.

5.2.2. Centrifuge the microfuge tubes briefly to collect all cells.

5.3. Enriched cells are now ready for further testing.

5.4. Remove and discard all used cartridges. Press Done. Carriage(s) will slide in automatically.