LEVICELL SYSTEM | LIVE CELL ENRICHMENT

A. Prepare Reagents

1. Prepare Levitation Buffer
   a. In new 1.5 mL tube, prepare Levitation Buffer as shown in Table 1 (final conc. = 150 mM).

TABLE 1

Reagent	Volume
Cell Media	255 µL
1M Levitation Agent	45 µL
TOTAL	**300 µL**

   b. Vortex mixture well to completely mix the Levitation Buffer.

2. Prepare Cells
   a. Aliquot $50x10^3$ to $1x10^6$ cells into a 2 mL low bind microfuge tube.
   b. Pellet cells at 300 RCF for 5 min.
   c. Carefully remove supernatant, without disturbing cell pellet.

3. Resuspend Sample in Levitation Buffer
   a. Resuspend cell pellet in 270 µL of Levitation Buffer. Mix cells and buffer thoroughly by pipetting up and down 10X.
   b. Immediately after mixing, set aside 2 x 15 µL aliquots for cell counting. These two replicates are for the input cell counts.

B. Run LeviCell™ Instrument

1. Follow instructions in LeviCell User Interface for Live Cell Enrichment, selecting the option that reflects the estimated cell size in your sample (Small, Standard, Large).

2. Use run parameters as shown in Table 2.

TABLE 2

Levitation Agent Concentration	150 mM
Split Settings	Leave blank unless known
Brightfield Exposure	100-200 µs
Ex474/Em524 Exposure	Depends if fluorescent stain is used
Ex560/Em628 Exposure	Depends if fluorescent stain is used

3. When ready to start run, mix sample thoroughly by pipetting up and down gently 5X and immediately load 220 µL into input well.

4. The default split line is set at “0”. When levitation is finished, the split line setting can be adjusted as necessary (from -15 to +15) to better separate live cell band from dead cells/ debris.

5. Harvest your cells from Top well output into a 1.5 mL low-bind tube. Ensure all liquid from the output well and serpentine channel is collected.

6. Measure the final output volume using a pipette. When split line is set to 0, typical recovery is between 70-100 µL.

7. Set aside 15 µL for cell counting in step C1.

C. Count Cells

1. Perform cell counts using the 15 µL aliquots of sample input and output collected in steps A3 and B7. LevitasBio recommends the Nexcelom™ cell counter along with a live/dead stain such as AO/PI for cell counting.

** For more details on how to set your split line, please see our full technical note, 90-00013 Parameters to Adjust When Using LeviCell for Viable Cell Enrichment*