

# LeviCell System | Live Cell Enrichment

## A. Prepare Reagents

1. **Prepare Cells**
  - a. Aliquot  $50 \times 10^3$  to  $1 \times 10^6$  cells into a 2 mL Eppendorf tube.
  - b. Pellet cells by centrifuging tube at 300 RCF for 5 min.
  - c. Carefully remove supernatant using a P200 or P1000 pipet.
2. **Prepare Levitation Buffer**
  - a. In new 1.5 mL tube, prepare Levitation Buffer as shown in Table 1 (final conc. = 150 mM).
  - b. Vortex mixture well to completely mix the Levitation Buffer.
3. **Resuspend Sample in Levitation Buffer**
  - a. Resuspend cells in 240  $\mu$ L of Levitation Buffer.
  - b. Save 10  $\mu$ L for bead counting on hemocytometer (input cells).

Reagent	Volume
Cell Media	255 $\mu$ L
Levitation Agent	45 $\mu$ L
<b>TOTAL</b>	<b>300 <math>\mu</math>L</b>

TABLE 1

## B. Run LeviCell Instrument

1. Follow instructions in 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.
3. Pipette mix sample thoroughly. Add sample to cartridge and start run.
4. When levitation is finished, adjust the split setting to a value between -15 and 15 depending on your cells, click "Go" to start flow.
5. Transfer Top well output to a 1.5 mL tube. Measure the volume with pipette.

<b>Levitation Agent Concentration</b>	150 mM
<b>Split Settings</b>	Leave blank unless known
<b>Brightfield Exposure</b>	100-200 $\mu$ s
<b>Ex474/Em524 Exposure</b>	Depends on stain used
<b>Ex560/Em628 Exposure</b>	Depends on stain used
<b>Total flow rate (preset)</b>	100 $\mu$ L/min

TABLE 2

## C. Count Cells

1. Count 10  $\mu$ L aliquots of input cell sample and Top output well on a hemocytometer.