LEVICELL EOS
VIABLE CELL ENRICHMENT

A. Prepare Reagents

1. In a 1.5 mL tube, prepare the appropriate volume of 150 mM Levitation Buffer based on the number of lanes being run with each sample as shown in ***Table 1***

NOTE: Viable cell enrichment is compatible with different Levitation Agent concentrations

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

	# of Lanes to run[2] | Volume (µL)			
Reagent	**1 Ln**	**2 Ln**	**3 Ln**	**4 Ln**
1 M Levitation Agent	45	90	135	180
Media[1]	255	510	765	1020
Total	300	600	900	1200

Table 1: Levitation Buffer *preparation for 1-4 lanes.*

[1] *Recommended "Media" are either PBS + 0.5% BSA or RPMI 1640 + 10% FBS. If media have particulates/debris, pass through a 0.22 µm filter prior to use.*

[2] *Please refer to the User Manual for instructions on counting and yield evaluations.*

B. Prepare Cells

1. Aliquot 20,000 to 1,000,000 cells per lane into an appropriately-sized tube. If one sample type is being loaded across multiple cartridge lanes, the sample can be prepped in one tube.
2. Centrifuge sample at 300 RCF for 5 min.
3. Remove and discard supernatant. Retain cell pellet.
4. Resuspend sample with the appropriate volume of Levitation Buffer per ***Table 2***, pipetting up and down 10 times to mix thoroughly.

	# of Lanes to run[2] | Resuspension Volume (µL)			
Reagent	**1 Ln**	**2 Ln**	**3 Ln**	**4 Ln**
Same sample, replicate lane	270	490	730	970
Single lane sample	270 ea	n/a	n/a	n/a

Table 2: Resuspension volumes *for 1-4 lanes.*

[2] *Please refer to the User Manual for instructions on counting and yield evaluations.*

5. If counting, immediately after mixing set aside 20-30 µL[2] of the input sample.

C. LeviCell® EOS System Setup and Run

1. Start the EOS Manager on the Control PC and click **Start New Run**.
2. Scan cartridge barcode, then click **Next**.
3. Fill in run information and choose the EOS module to use.
4. Select the appropriate cell size protocol and **Ambient** run temperature, then click **Next** .
5. If fewer than 4 lanes are being run, de-select the lane numbers on the cartridge diagram so they are no longer highlighted in gold.
   a. Enter sample names and fluorescence stains accordingly [3,4]
   b. Levitation Agent concentration for all samples will be 150mM4[4]

   [3]*Green and Red fluorescence should be left blank if no stains/dyes are used. Green Stain and Red Stain are specifically for Beads Test protocol. Please refer to the User Guide to adjust or add stain preferences.*

   [4]*If Levitation Agent and fluorescence stains are different for any of the four lanes, uncheck the box above the run info to customize each field.*
6. Click **Next** to begin the run and follow prompts per instructions on the screen.

NOTE: When prompted to dispense samples, pipette mix sample well immediately before dispensing into the cartridge.

7. During levitation, but before collection, **Split line value** can be typed in or adjusted by dragging the split line on the screen (from -15 to +15). All four lanes will use the same split value. Once collection has started, the split line can no longer be adjusted.
8. Once the timer on the instrument has concluded, the user may wait to start collection if additional levitation time is desired.
9. Click on **Start Collection** to proceed and follow prompts to finish the run.
10. Retrieve the cartridge and place it on a flat surface. Hold the edges (Figure 1) of the cartridge down firmly and remove the outlet well covers to collect output samples. The viably enriched cells are in the top (**T1-T4**) wells. Waste is in the bottom (**B1-B4**) wells.

NOTE: Pipette mix each sample 3-5X before retrieval, aspirating only from the well.

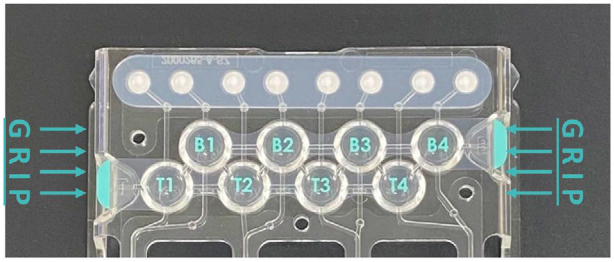

Figure 1: *EOS-4 outlet wells and grip points*

For more information, visit **levitasbio.com,** or contact **sales@levitasbio.com**.