

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.

Reagent	# of lanes being run Volume (μL)			
	1 Ln	2 Ln	3 Ln	4 Ln
1 M Levitation Agent	45	90	135	180
Media	255	510	765	1020
Total	300	600	900	1200

Table 1. Levitation Buffer preparation for 1-4 lanes. Please refer to the User Manual for instructions on counting and yield evaluations.



NOTE: Recommended Media: Use either PBS supplemented with 0.5% BSA or RPMI 1640 supplemented with 10% FBS. If the selected medium contains particulates or debris, filter it through a 0.22 μm filter before use.

B. Prepare Cells

1. Aliquot 20,000–1,000,000 cells per lane into an appropriately sized tube. If loading the same sample type into multiple cartridge lanes, prepare the total volume in a single 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.

Reagent	# of lanes being run resuspension volume (μL)			
	1 Ln	2 Ln	3 Ln	4 Ln
Same sample, replicate lane	270	490	730	900

Table 2. Resuspension volumes for 1-4 lanes.

5. If counting cells, set aside 20–30 μL of the input sample immediately after mixing. If counting cells, set aside 20–30 μL of the input sample immediately after mixing. Please refer to the User Manual for instructions on counting and yield evaluations.

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 running fewer than four lanes, de-select the unused lanes in the cartridge diagram so they are no longer highlighted in gold. Enter sample names and fluorescence stains as needed. Set the Levitation Agent concentration to 150 mM for all samples.



NOTE: Fluorescence Stains and Levitation Agent: Leave the Green and Red fluorescence fields blank if no stains or dyes are used. These stains are intended only for the Beads Test protocol. Refer to the User Guide for instructions on adjusting or adding stain preferences. If fluorescence stains or Levitation Agent concentrations differ across lanes, uncheck the box above the run information to customize each lane individually.

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, **the split line value** can be entered manually or adjusted by dragging the split line on the screen (range: -15 to +15). The same split value applies to all four lanes. Once collection begins, the split line can no longer be changed.
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 cartridge firmly at the designated grip points (see Figure 1), then remove the outlet well covers to collect the output samples. The viably enriched cells are located in the top wells (**T1–T4**), while waste is collected in the bottom wells (**B1–B4**).



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

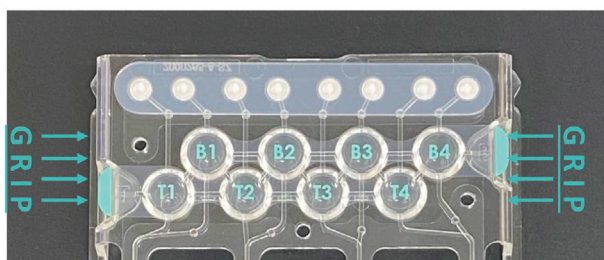


Figure 1: EOS-4 outlet wells and grip points

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

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