LEVICELL EOS BEAD TEST

A. Prepare Reagents

- **1.** Vortex bead tubes thoroughly immediately before pipetting to avoid sedimentation.
- 2. In 1.5 mL tubes, prepare the appropriate volumes of beads and buffer as indicated in Tables 1 and 2 below per the number of lanes being loaded.

	# of Lanes to run Volume (µL)			
Reagent/	1 Ln	2 Ln	3 Ln	4 Ln
LeviCell [®] Install Bead Mix 1	15	30	45	60
LeviCell Install Bead Mix 2	30	60	90	120
Total	45	90	135	180

Table 1. Bead Mixture

	# of Lanes to run Volume (µL)			
Reagent/	1 Ln	2 Ln	3 Ln	4 Ln
1 M Levitation Agent	31	63	94	125
LeviCell Install Buffer	219	437	656	875
Total	250	500	750	1000

Table 2. 125mM Levitation Buffer preparation for 1-4 lanes

B. Prepare Bead Sample for Loading

- 1. Pellet Bead Mixture at 300 RCF for 3 min, ambient temperature.
- 2. Remove supernatant for the Bead Mixture and discard.
- **3.** Resuspend in the full volume prepared in Table 2.
- 4. Mix sample thoroughly by pipetting up and down 10 times.
- If counting, immediately after mixing, set aside 20-30 μL1 of the input beads.
 ¹Please 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 your EOS module.
- 4. Select Bead Test 4 min 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.
- **6.** In the "Select and specify samples to run" screen, check the box labeled "Same Levitation Agent concentration or fluorescence stains for all samples", and fill in the details per table below.

Sample name2	LA (mM)	Green fluorescence	Red fluorescence
Bd1	125	Green Stain	Red Stain
Bd2	125	Green Stain	Red Stain
Bd3	125	Green Stain	Red Stain
Bd4	125	Green Stain	Red Stain

 Table 3. Levitation Agent concentration/fluorescent stains

 ²Name does not have to be unique and can be changed per user preference.

7. 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 220uL per lane into the cartridge.

- **8.** "Split line value" is 0 for the bead test. Once the timer on the instrument has concluded, click on **Start Collection**.
- **9.** Follow prompts to finish the run.
- Retrieve your 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 your samples for further analysis if desired.
 The Bead Mix 1 beads are in the top (T1-T4) wells. Bead Mix 2 beads are in the bottom (B1-B4) wells.

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



Figure 1: EOS-4 outlet wells and gripping points

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

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