

LeviCell System Installation Test

A. Prepare Reagents

1. **Prepare Bead Mixture**
 - a. Vortex bead tubes thoroughly immediately before pipetting to avoid sedimentation.
 - b. In new 1.5 mL tube, prepare bead mixture as shown in Table 1.
 - c. Pellet the beads by centrifuging tube at 300 RCF for 3 min.
 - d. Carefully remove supernatant using a P200 set to 45 μ L.
2. **Prepare Levitation Buffer**
 - a. In new 1.5 mL tube, prepare Levitation Buffer as shown in Table 2 (final conc. = 125 mM).
 - b. Vortex mixture well to completely mix the Levitation Buffer.
3. **Resuspend Beads in Levitation Buffer**
 - a. Resuspend beads in 240 μ L of Levitation Buffer.
 - b. Save 10 μ L for bead counting on hemocytometer (input beads).

Reagent	Volume
LeviCell Install Bead Mix 1	15 μ L
LeviCell Install Bead Mix 2	30 μ L
TOTAL	45 μL

TABLE 1

Reagent	Volume
LeviCell Install Buffer	262.5 μ L
Levitation Agent	37.5 μ L
TOTAL	300 μL

TABLE 2

B. Run LeviCell Instrument

1. Follow instructions in User Interface under System Tools, Install Test.
2. Use run parameters as shown in Table 3.
3. Pipette mix sample thoroughly. Add sample to cartridge and start run.
4. Transfer Top and Bottom well outputs to separate 1.5 mL tubes. Measure each output volume with pipette.

Levitation Agent Concentration	125 mM
Split Settings	0
Brightfield Exposure	100-200 μ s
Ex474/Em524 Exposure	1,000-2,000 μ s
Ex560/Em628 Exposure	5,000-10,000 μ s
Total flow rate (preset)	100 μ L/min

TABLE 3

C. Count Beads

1. Count 10 μ L aliquots of input beads and Top and Bottom output wells on a hemocytometer.