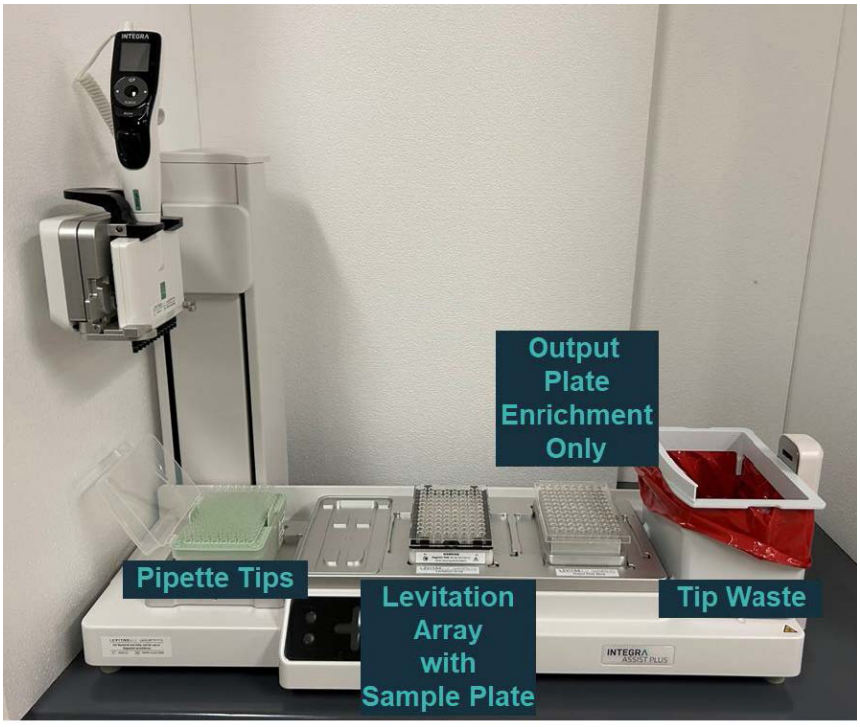
LEVICELL 96 | VIABLE CELL ENRICHMENT

A. PREPARE THE LEVICELL® 96 SYSTEM

1. Power on the robot at the main switch and push the flashing play button on the front to initialize.
2. Press and release Run on the pipettor to turn it on.
3. Connect the pipettor to the robot.
    a. Press and release the back arrow button on the pipettor until you reach the Main Menu.
    b. Scroll and select “Assist Plus” at the top of the menu to establish communication (press center OK button on pipettor to select).
4. Scroll to and select “Custom Programs”, then select the “VCE96_*” protocol.
5. Insert a new box of 300 µL wide bore tips on the robot deck as shown below.
6. Insert a new waste bag as needed for used tip collection (lining the waste bin at the far right).
7. Position the Levitation Array in the Nest B position on the robot deck in portrait orientation, with the A1/H1 label at the front.
8. Position the Output Plate Block in the Nest C position on the robot deck.



B. PREPARE REAGENTS, CELLS AND CONSUMABLES

1. Prepare 100mM Levitation Buffer for 96-sample input plate.

Reagent	Part Number	Volume (µL) for 1 sample well with overage	Volume (µL) for 96 sample wells
Diluent Buffer	Recommended: PBS + 0.5% BSA or RPMI 1640 + 10% FBS	63	6048
1 M Levitation Agent	1003006	7	672
Total		**70**	**6720**

2. Prepare Cells
    a. Aliquot 50,000 to 250,000 cells per well into low bind tubes or a plate for sample preparation. If a single sample is to be loaded across multiple wells, the sample can be prepared in one tube.
    b. Pellet sample(s) at 300 RCF for 5 minutes - or as appropriate for your sample type.
3. Resuspend sample(s) in 60uL Levitation Buffer per sample.
4. Prepare Sample Input Plate

Note: *Avoid introducing and transferring bubbles in all pipetting steps.*

    a. Pipette exactly 50 µL of cell mix to each well in the designated row of Sample Input Plate.
        i. If processing less than 96 samples, samples should be loaded in rows starting with row A→H.
        ii. Pipette mix sample to resuspend before transfer and dispense to the bottom of the well, to the first pipettor stop to avoid introducing air bubbles.
    b. Seal plate with the film provided.

Note: *If bubbles are present in sample or wash wells, a gentle brief centrifugation step may be employed to aid in bubble removal.*

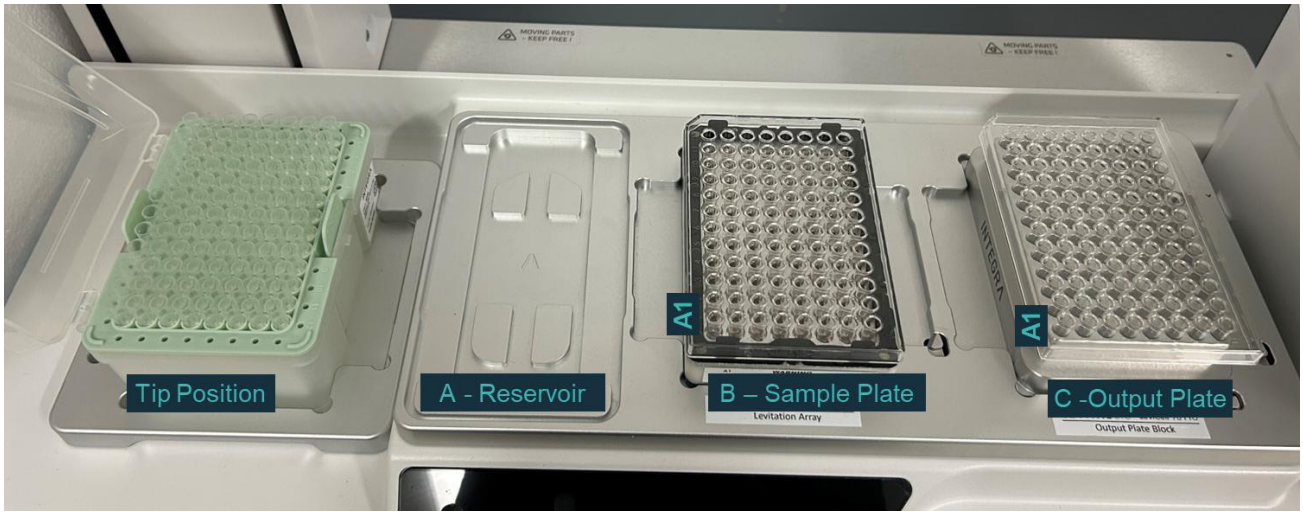
5. Prepare Sample Output Plate

Note: *To save time, this plate can be prepared during levitation.*

    a. Load output plate with 100 µL cell culture media or appropriate buffer per well.
    b. Seal plate with the film provided.

C. RUN THE LEVICELL 96 SYSTEM

1. Insert the Sample Input Plate into the Levitation Array and the Sample Output Plate into the Output Plate Block on the robot deck as shown (plate notch is at A12), ensuring that the plates are fully seated.



2. Levitate for 60 minutes to separate live cells from dead cells and debris. i. If the sample particle diameter is larger than 20 µm, a shorter levitation time may be feasible. Contact Technical Support for guidance.
3. Ensure that pipette tips, output plate, and waste container are positioned correctly.
4. Run the “VCE96_*” program on the robot.

Note: *If the robot has entered sleep mode, follow steps in section A to reconnect.*

   i. Press OK to confirm the tip box orientation (portrait) and scroll to starting row position (A) and select.
   ii. You will be prompted to remove the input and output plate films, do so carefully so as not to disturb the input plate or Levitation Array.
   iii. The robot will transfer the levitated sample(s) and move them to the output plate.
   iv. The pipettor screen will indicate when the protocol is complete.

5. Add a new film to the output plate before removing the plate from the Output Plate Block. The sample(s) are ready for counting (optional) and your downstream application.
6. To turn off the pipettor, press the back button until you are in the main menu, then hold the back button until the pipettor screen is black. Turn off the robot from the main switch.