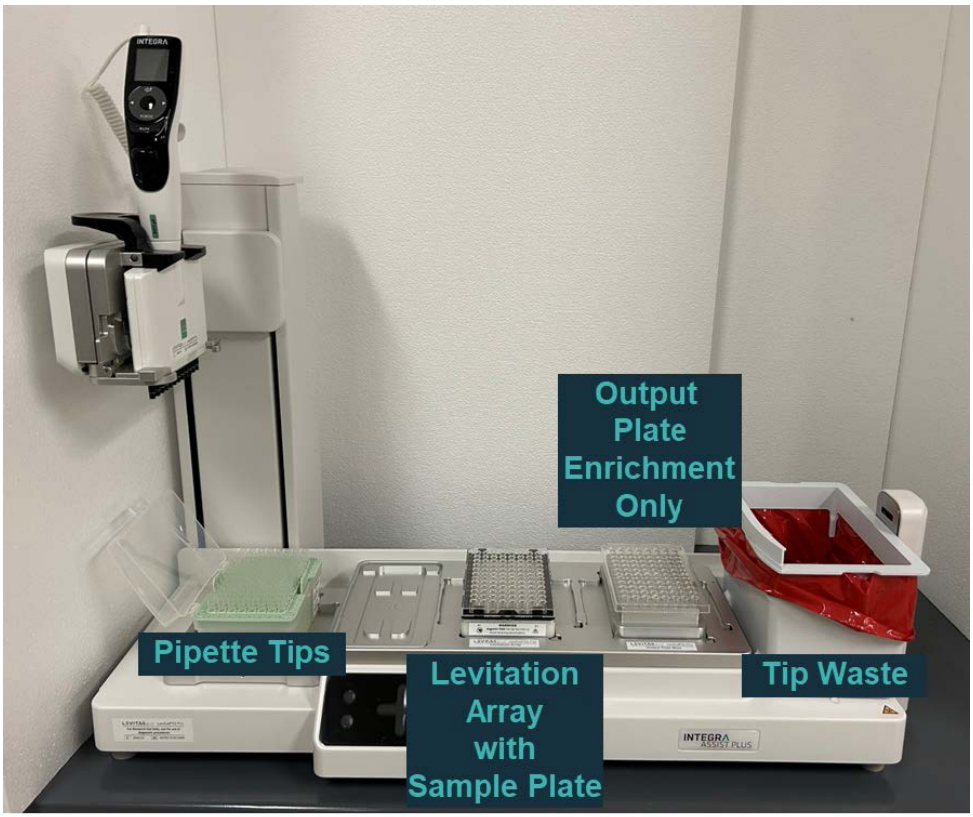
LEVICELL 96 | CELL WASHING

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 “3WASH24_*” 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.



B. PREPARE REAGENTS, CELLS AND CONSUMABLES

1. Prepare Cells
    a. Prepare up to 24 sample tests with 450,000 - 600,000 stained cells each in 4uL of staining buffer.
        i. Stain cells according to user defined protocol.
2. If staining at room temperature, staining can be done during levitation without an additional incubation step on the bench. Contact Technical Support as needed for guidance.
    a. Spike in 6uL of Levitation Agent for a total of 60uL per test.

Reagent	Part Number	Volume (µL) for 1 sample well with overage	Volume (µL) for 24 sample wells
450k-600k Stained Cells in Staining Buffer		54	1296
1 M Levitation Agent	1003006	6	144
Total		60	1440

3. Prepare Wash Buffer

Reagent	Part Number	Volume (µL) for 1 sample well with overage	Volume (µL) for 24 sample wells
Diluent Buffer	Recommended: PBS + 2%FBS +/- 2 mM EDTA or as appropriate for staining protocol used	54	2592
1 M Levitation Agent	1003006	6	288
Total		60	2880

4. Prepare Output Buffer

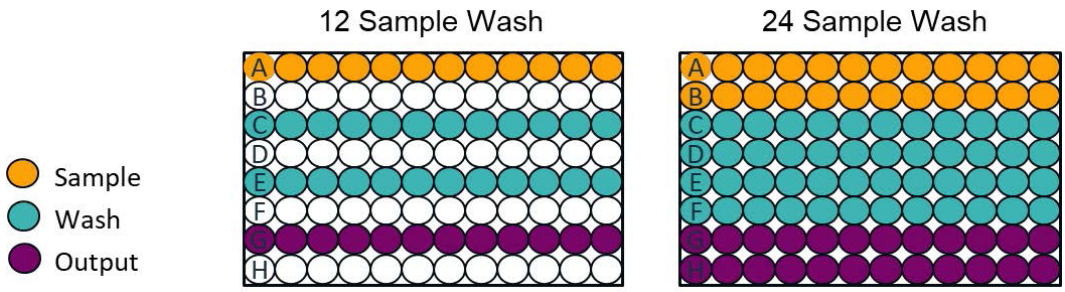
Reagent	Part Number	Volume (µL) for 1 sample well with overage	Volume (µL) for 24 sample wells
Diluent Buffer	Recommended: PBS + 2%FBS +/- 2 mM EDTA (with or without live/dead stain) or as appropriate for staining protocol used	110	2640
Total		110	2640

5. Prepare Sample Plate

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

a. Pipette exactly 50 µL of cell mix (containing 100mM Levitation Agent) into each well of row A (for 1-12 samples) and row B (for 13-24 samples) of the sample plate as shown below.
   i. 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. Load wash rows (C, E (for 1-12 samples) and D, F (for 13-24 samples)) of sample plate with 50 µL buffer, free of dyes, containing 100 mM Levitation agent (Wash Buffer). Dispense to the bottom of the well, to the first pipettor stop to avoid introducing air bubbles.
c. Load output rows (G and/or H) of sample plate with 100 µL of Output Buffer (buffer free of any dye or levitation agent).
d. Seal plate with the film provided.

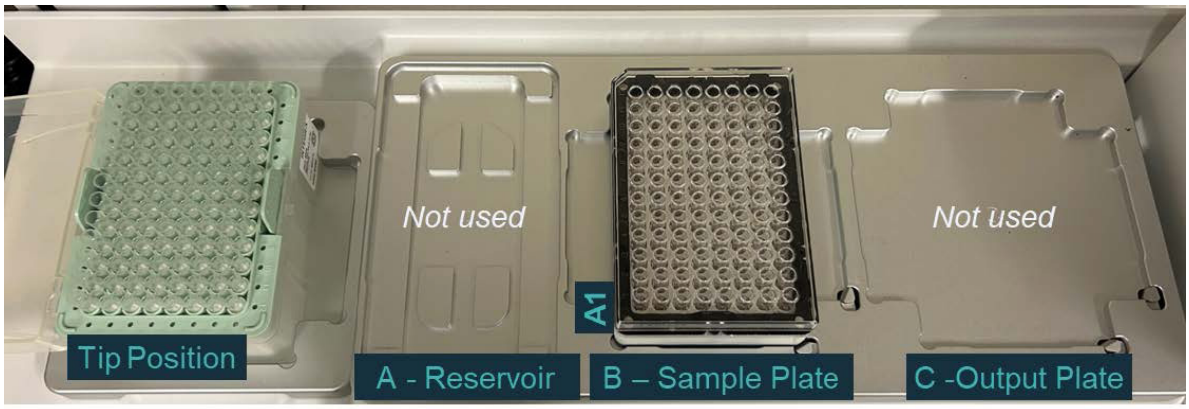
Note: *If bubbles are present in sample or wash wells, a gentle brief centrifugation step may be employed to aid in bubble removal.*



Configuration of the sample plate for 12 or 24 sample inputs

C. RUN THE LEVICELL 96 SYSTEM

1. Insert sample plate into Levitation Array on the robot deck as shown, ensuring that the plate is fully seated in the array.



2. Levitate for 60 minutes to separate live cells from dead cells and debris.
    a. 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 wash and output wells are filled, and pipette tips and the waste container are positioned correctly.
4. Press Run on the pipettor to start the “3WASH24_*” 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), scroll to starting row position (A) and select.
    ii. You will be prompted to remove the plate film, do so carefully so as not to disturb the plate or Levitation Array.
    iii. The robot will transfer the levitated sample(s) and move them to wash wells, dwell in the wash wells, and ultimately dispense the washed samples to the output wells.
    iv. The pipettor screen will indicate when the protocol is complete.

5. Add a new film to the plate before removing the plate from the Levitation Array. The sample(s) in the output wells are ready for 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.