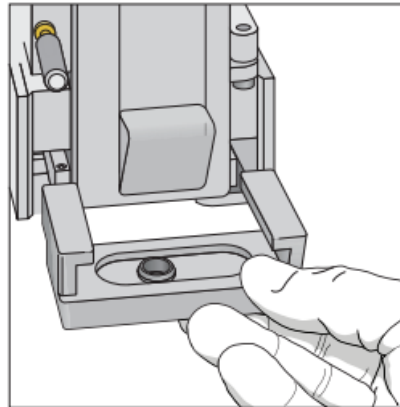


Fusion Sansa – Setting Up for Plate Sorting

To calibrate the plate stage for plate sorting, we recommend using the Auto Drop Delay Experiment Template and Accudrop Beads.

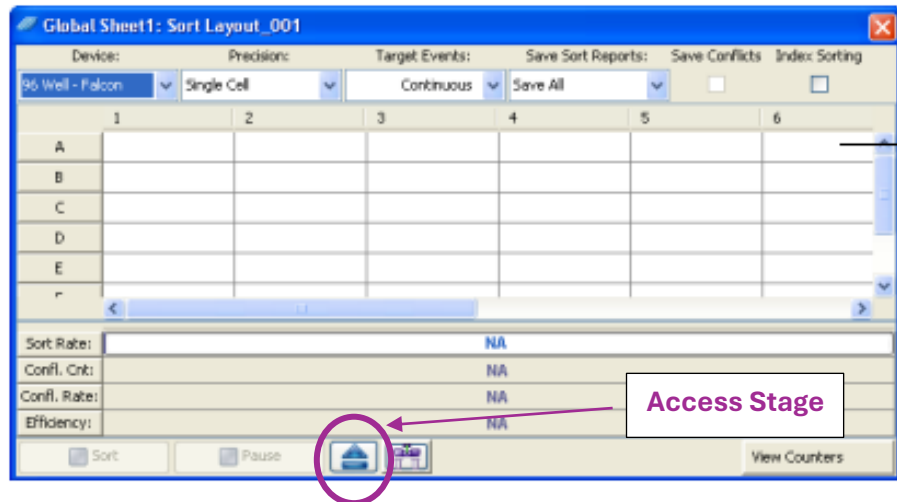
When using Fusion Sansa to sort in plates, do not calibrate the plate stage using the standard 384 and 96 well Falcon plate format. Use instead the 384 and 96 well custom devices. The following document guides you step-by-step on how to calibrate the plate stage using a custom device.

1. Install the Splash Shield by sliding into the fitting beneath the sort aspirator drawer.



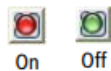
2. In the Side Stream Window, change the guide to **ACDU**.
If sorting with the 100 μ m nozzle, set the **far-left voltage slider** to a value of **18^a**, all the remaining 3 voltage sliders should be kept at 0.
3. Open the Accudrop Experiment.
4. Create a new Sort Layout under the Accudrop Experiment Tube:
 - a. If sorting in a 384 well plate, in the Device drop-down menu select **384 well armadillo plate – New**
 - b. If sorting in a 96 well plate, in the Device drop-down menu select **96 well plate Costar – New**.
5. In the **Target Event** field box, set the number of events to be sorted to 50.
Add Population P1 to the 4 wells at each corner of the plate and to 4 wells in the center of the plate.
6. Click on the **Access Stage** button on the Sort Layout to bring the plate stage to the front of the Collection Chamber.
Connect the plate stage to the refrigerated circuit if you wish to keep your plate and collected samples at 4°C.

a) The value of 18 is optimised only for Fusion Sansa with the 100 μ m configuration. Other configurations/sorters will have different optimized values for the % charge applied to the side stream.



7. Insert the plate (with lid or film on) on the plate stage. A1 well should be placed in the front left position.
8. Select **Sort > Custom Device**
9. **First Check: Side Stream is passing the Splash Shield.**
In the side stream window, turn the **Voltage** button ON, and OPEN the **Waste Drawer**.

Voltage button



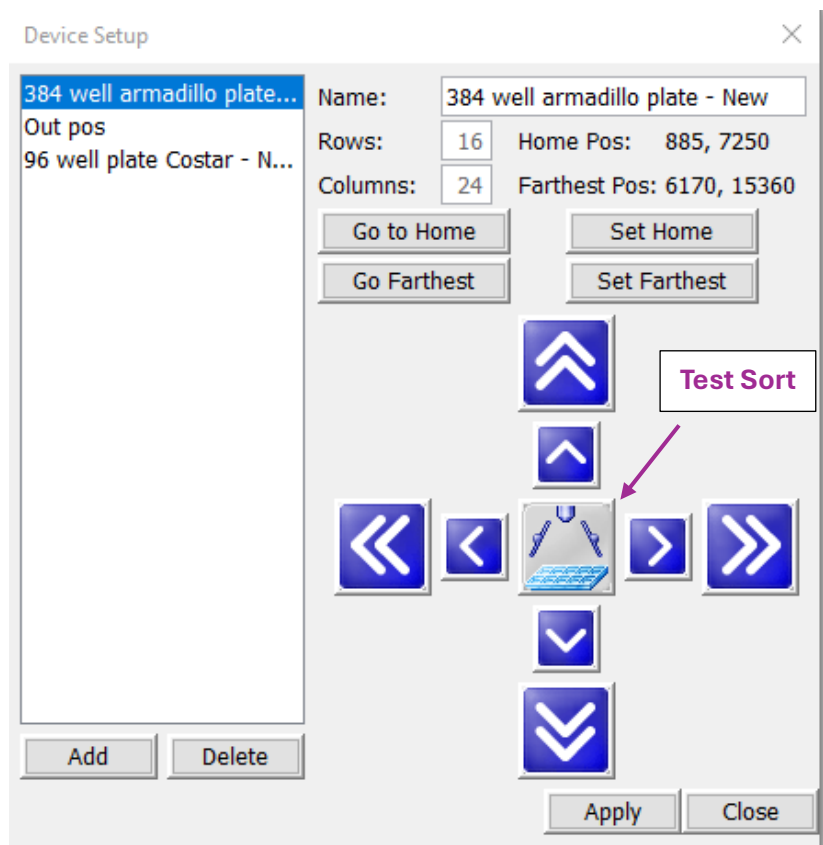
Waste Drawer button



In the Device Setup Window, select the custom device you are using (*384 well armadillo plate – New* or *96 well plate Costar – New*) and press the **Test Sort** button.

Inspect visually the plate and the side stream window to check that the side stream is passing the Splash Shield. If it so, stop the Test Sort and move to Step 10.

If you don't see any liquid passing the Splash Shield, or if you see a "splash" line in the side stream window, correct the voltage slider and repeat Step 9.



10. **Second Check: Set the Home Position.**

In the Device Setup Window, click on Go To Home button to send the plate stage to the current Home Position (Well A1) coordinates.

In the side stream window, turn the **Voltage** button ON, and OPEN the **Waste Drawer**. In the Device Setup Window, press the **Test Sort** button ON & OFF quickly to deposit a drop in the Home Position.

Inspect visually the plate to check if the Home Position corresponds to the center of A1 well.

- a. If so, move to Step 11.
- b. If the Home Position is not centered on well A1, adjust its coordinates by pressing the appropriate arrow button (large arrow = 5 steps, small arrow = 1 step) to move the plate stage. After any change, click on **Set Home** to save the new coordinates. Repeat Step 10 until calibration is correct.

11. **Third Check: Set the Farthest Position.**

In the Device Setup Window, click on **Go Farthest** button to send the plate stage to the current Farthest Position (Well H12 in 96 well plate, Well P24 in 384 well plate) coordinates.

In the side stream window, turn the **Voltage** button ON, and OPEN the **Waste Drawer**. In the Device Setup Window, press ON and OFF quickly on the **Test Sort** button to deposit a drop in the Farthest Position.

Inspect visually the plate to check if the Farthest Position corresponds to the center of last well of the plate.

- a. If so, click **Apply** in the Device Setup Window and move to Step 12.

- b. If the Farthest Position is not centered on the last well, adjust its coordinates by pressing the appropriate arrow button (large arrow = 5 steps, small arrow = 1 step) to move the plate stage. After any change, click on **Set Farthest**. Repeat Step 11 until calibration is correct.

12. **Last Check: Confirm plate stage calibration sorting Accudrop Beads.**

In the Device Setup Window, click on **Go to Home** button.

Load a tube with Accudrop beads and select the appropriate sort layout (see Step 4).

Start the sort.

When the sort is completed, inspect visually the plate.

- a. If the plate stage alignment is correct, you can proceed with sorting your sample.
- b. If there is any misalignment, go back to the Device Setup Window, click on **Go to Home** and then make further adjustments in the coordinates for the Home Position as described in Step 10. Click **Go Farthest** if you need to adjust the Farthest Position as described in Step 11. Repeat Step 12 until calibration is correct.

13. Create an experiment for your sort and prepare to sort your sample.

Please remember before starting a plate sort (& even between plates) to always click on **Go to Home on the Device Setup Window, otherwise the plate stage will lose its alignment.**