

Stem Cell Instrumentation Foundry

Bio-Rad ZE5 SOP
Date of last revision: 9/1/26
Date established: 1/13/21
Author: David Gravano

General tips for success with the ZE5

- Start with voltages pre-determined by the SCIF and adjust from there as needed – list of starting voltages is next to the monitor
- Perform single stains with comp beads. These will be listed as “setup” wells. Often using cells for single stains gives poor results if you have a rare positive population or any autofluorescence in your sample.
- Ideally, have single stained cells to check/adjust voltages and verify comp bead results – list them as “sample” wells, not “setup” wells.
- Have setup wells as the first wells in your plate map/tube rack layout. Don’t intersperse “samples” between “setup” wells.
- Place a pause after the final compensation single stain well so you can apply compensation and view compensated data for the sample tubes

Start-up Procedure

1. Turn on the power to the Sheath (diH₂O) and Waste carts under the instrument. You should hear a pump start and the cart light should go from red to green.
2. Workstation remains on at all times. Wake up the screen and open “Everest” software link on the desktop
3. Log in using your UCM ID and password (password is of your choice)
4. Click the Startup button to start the fluidics. The instrument is always on standby so there is no physical switches to turn on.



Stem Cell Instrumentation Foundry

- a. Startup takes approximately 5 minutes.
- b. Note that once startup is completed, instrument will always be running sheath fluid unless the system is paused. Click "Pause System" if you will not be running samples within 15 minutes.



QC Successful 2021-01-11 14:03:33



5. **(Optional)** Quality Control (QC). This is performed regularly by staff, but if a user wants to perform it, follow these steps:
 - a. Find the QC beads on the door of the full-size fridge opposite the ZE5. The beads are on the door and have a blue cap. Alternatively, they may be in a foil bag with a yellow label and say "QC Beads". Use the bottle with the dot on the top.
 - b. Vortex the bottle for 10 seconds
 - c. Press the garage door button to open the sample loader
 - d. Remove the cap from the bottle and place in the bottle holder on the right side. Flip up the lid of the holder and place the bottle inside. Lid should close on top of the bottle.
 - e. Close the garage door
 - f. Press the QC button. The instrument will run QC by itself, which will take approximately 5 minutes

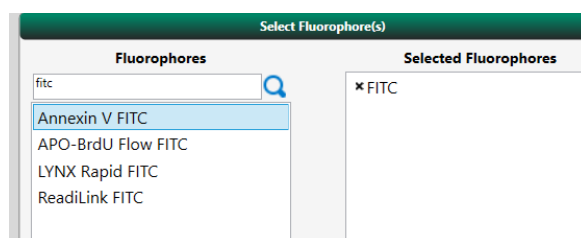


- g. Ensure that the instrument says "QC Successful"
 - i. If not, contact David
- h. Open the garage door, remove the beads, and put back in the fridge
- i. Click "QC Report" or "QC Trending" to see QC data
- j. Press the "Home" button to return to the main menu.

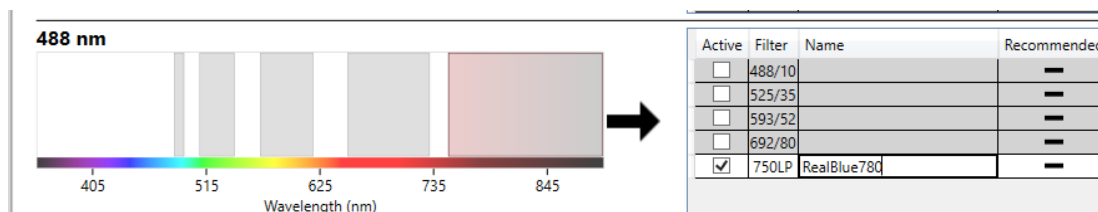
Stem Cell Instrumentation Foundry

Experimental Setup – New Experiment

1. From the Home screen, Click “New Experiment”
2. Select the format you have (eg. 5ml tubes, 96 well plate)
3. Give experiment a name. Recommend “Year_month_day_name”.
4. Add all fluorophores in your experiment by searching the list and double clicking the correct one



5. If you cant find your fluorophore, do the following
 - a. Click the check mark next to the appropriate channel



- b. Re-name the fluorophore to what you'd like
 - c. I recommend editing all the names to be as simple as possible. Example for APC, it defaults to “APC (Allophycocyanin)”. I would manually change this to “APC”
6. When all are entered, click the “Plate Setup” button. This will bring up a plate setup map.
 7. If you have unstained cells, click “Negative Control” to “On”
 8. If you have single stain comps or comp beads: click “Compensation Control” to “On”
 9. Click “Drag and Drop” to “On” if you need to reposition the unstained or control tubes on the plate map. Then they can be grabbed and dragged.
 10. To add samples, select the wells with sample and click on the green “Sample” button
 - a. In the “Sample naming” box, name your samples and controls
 11. Customization options:
 - a. Temperature – select the temperature button and set the temperature
 - b. Sample Line Flush – this is an extra flush that is optional. It already does a short flush between samples by default and I've found this is pretty sufficient
 - c. Shutdown when complete – Only recommended if you're running samples overnight
 - d. “Stop” – by default it will stop at 40ul of volume run. You can change this for all highlighted tubes to a fixed volume or a number of events in a particular gate

Stem Cell Instrumentation Foundry

- e. “Wash” – I recommend leaving this on. It’s a quick wash between samples
 - f. “Agitation” – This will give a good vortex to any highlighted samples/setup tubes. I recommend doing this every 3-4 samples. It will show a vortex symbol next to the selected tubes.
 - g. “High Throughput” – This will make plates run faster, but sample cannot be returned. I would only use this if experienced.
 - h. “Pause After” – This will pause the run and allow you to make adjustments. This is most useful after the last setup tube so you can perform auto-compensation prior to running your experimental samples.
 - i. “Volumetric Counting” – This will tell you how much sample was acquired. I recommend always turning it on. This will automatically turn on “Sample Return”
 - j. “Sample Return” – Returns any unused sample in the line to the well, approximately 40ul. I recommend always turning it on.
 - i. NOTE: turning this on will change the volume it will acquire, so you will have to go back and enter the appropriate volume
 - k. “Rate” – I recommend setting all to 0.1ul/second. This is approximately equivalent to low on the LSR. You can always turn up the rate later when the samples are acquiring. Keeping it low avoids taking too much sample during setup.
 - l. “Reagent” – This allows you to have a reagent in one of the wells that can be added to the sample immediately before running. You can specify how much to add and whether or not to mix.
12. Once ready to move on, click on “Plots and Gates”.
 13. On this next screen you can set voltages, draw gates and make new plots.
 - a. Set voltages to good starting values. For example of good starting values, see the chart on the wall.
 14. Click the bottom right double arrow to apply and you’re now ready to start acquiring data.

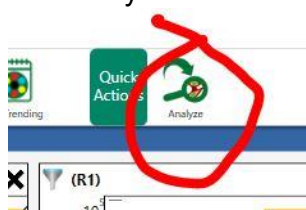
Running your Controls:

1. In setup mode I would recommend running each single stain and adjusting the appropriate voltage value (if first time running the panel). Note, this will not record any data. Click on the appropriate well on the plate map and press the play button.



Stem Cell Instrumentation Foundry

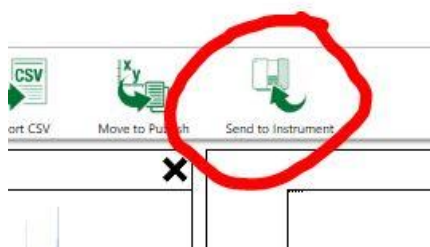
2. Once all voltages are set, change to “Acquisition” mode and click on the first sample and press play.
3. Run will pause after the last setup tube (if you put a pause step). At that point, click on the “Analyze” button. This will move the data into the “Analysis” module.



4. Click on the “Compensation” button
 - a. There is a dropdown for additional options, but if using comp beads the defaults should be fine.
 - b. After complete, there is a “view comp” button to visualize the matrix if desired.

Running your Samples:

1. Click “Send to Instrument”. This will go back to the acquisition mode and you can then resume running your samples in “acquisition” mode.



2. Sample can be advanced manually or the run stopped at any time.

Experimental Setup – Duplicating A Past Experiment

1. Click on an experiment in the “Recent experiment” window on the right side of the home screen
2. Click “Edit” next to a session of the experiment. This will open the experiment setup windows for modification of your past settings.

Cleaning and Shutdown:

1. After completed running samples return to the home screen
2. Select the “Stat tube” option
3. Insert a tube with at least 3ml of Cytometer Cleaner solution (2% Contrad) into the stat tube holder (metal holder next to where you load plates/tube rack)
 - a. Cytometer Cleaner is in a tube on the rack by the monitor

Stem Cell Instrumentation Foundry

4. Run cleaner at a rate of 2ul/sec for 5 minutes
5. Return to the home screen and click “Shutdown”
 - a. This process will take approximately 5 minutes
6. Turn off the sheath and waste carts by pressing the black buttons on each. The lights will turn red.

Transferring Data:

1. Click on the Export Data button
2. Uncheck the buttons for H and W for all the fluorescent parameters – this will make a very large number of parameters that are seldom used if you leave them checked, complicating downstream analysis
3. Click Export and save them to the default directory.

Troubleshooting a clog:

If no sample is being taken up by the ZE5, you may have a clog.

1. Go to the home screen and click on the “Stat Tube” button
2. Warm “Cytometer Cleaner” to 37C in a FACS tube by placing in the cell culture incubator for 5 minutes. The cleaner is found in the bottle next to the instrument
3. Place the cleaner in the metal single tube loader in the ZE5. Run the cleaner through the ZE5 for 1 minute on the highest rate (2.5ul/sec)
4. Stop the acquisition, go to the home screen, and run the “unclog” command one time.
5. Return to the Stat tube and restart the acquisition of the cleaner again for 1 minute.
6. Repeat the unclog and cleaner acquisition until volume is being taken up successfully by the instrument and resume your experiment.
7. If this does not resolve the clog, during business hours contact Bio-Rad tech support for advice. After hours, contact David for additional tips.