

Yeti User Guide

Startup

- 1) Check the fluids. If a waste tank is full, take it out and empty it into the sink. Fill the waste tank with bleach up to the mark before putting it back in.
- 2) Open Evo Software
- 3) Click Start up button underneath the picture of the instrument (Sun button)
- 4) Log in with Kerberos ID and password (set to be the same as your KID until you change it)
- 5) Create new experiment (or reuse old one/import runlist/use STAT tube)

Creating New Experiment:

- 1) Enter Experiment Name
- 2) Select colors
 - a. Search for fluors and double click to select
 - b. Verify colors are mapped to correct filters
- 3) Select media type and read direction
- 4) Select instrument temperature
- 5) Specify auto-shutdown (not recommended)
- 6) Indicate if there is a Negative Control
- 7) Indicate if there are Compensation Controls
 - a. Check box to use the 'Negative Control' as a universal negative for compensation. Only do this if the Negative control is the same sample type as the positive single color controls (ie Used cells for single color controls and the SAME cells for the negative).
- 8) Create Sample wells
 - a. Select wells with samples and click the light green 'Sample' box. To rearrange the positions of samples in wells, click Drag and Drop toggle. Unclick it once done rearranging.
- 9) Assign settings for all tubes (each tube can have different settings!):
 - a. Sample volume - amount to run from each sample/well.
 - b. Event limit - Amount of events the Yeti will acquire (unless volume limit is reached)
 - c. Flow rate
 - d. Wash volumes
 - e. Agitation
 - f. High Throughput mode - allows to run multiple samples much faster than in regular mode by having several samples in the aspirator at the same time; allows for lower than 40 uL minimum volumes of sample. Does not have the "return sample" function. Does not allow to set a minimum stop gate number of events.
 - g. Pause after tube - useful to add a pause to the last of the comp controls, so compensation can be done before running the samples.
 - h. Return sample - puts the part of the sample that is inside the tubing back into the tube/well instead of discarding it.

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- i. Reagent - can be used to add a specified amount of a reagent to specified wells. Does not allow for incubation.

NOTE: Most options above will increase time necessary to run one plate - time estimate is displayed on the left side of the screen.

NOTE: Adding these options generates the Run List on the right side of the screen, which will display the actual settings for each tube. It is important to double check this list as sometimes the settings displayed on the GUI are not correct.

10) Name tubes

- a) Can be done manually
- b) To automatically name them:
 - i) Select wells to be named and click Names tab on Run List
 - ii) Select options at the top and/or enter a custom prefix
 - iii) Click 'Set selected'

11) Designate any tubes that will need a multi-worksheet instead of a global worksheet (bottom right). Multi-worksheets are only used for setup tubes.

12) Confirm settings were added to the Run List by expanding with the button on the top right.

13) Click Next.

14) Create plots and gates for tubes using global worksheet

- a) Plots can be created using the second button and selecting combinations of measurements/scaling that you would like to view. If you want to see compensated plots, you must select "Comp" when creating the plot.
- b) Can select tubes you wish to add a gate limit to and apply.
 - i) Acquisition will stop when the gate limit, event limit or volume limit is reached.

15) Click Apply (or export Run List if in Experiment Builder)

Acquiring Samples

- 1) Confirm software is in 'Setup Mode'
- 2) Run unstained and positive stained cells to check/set voltages
- 3) Change to acquisition mode, select first well and click the run/play button.

Compensation

- 1) After Comp Controls are run, Run List can be paused
- 2) Click Quick Action 'Analyze Current Run List'
- 3) Click Auto-Comp button
- 4) Send Run List back to Local Instrument and click local instrument tab.

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NOTE: Auto-comp is still rudimentary and will only work with very good controls. It is recommended to do actual compensation in off-line analysis software. Auto-comp should only be used to get a quick idea of your staining.

Exporting Data



- 1) To export .fcs files for analysis, click the Export icon in the middle of the task bar.
- 2) Save your files to the Exported User Data folder on the desktop, and from there either take data with you on a USB hard drive or send data over network. User data will be cleared from computer on a regular basis.

Shutdown and Cleaning

- 1) From the Home screen, click Load Experiment and navigate to the Post Run Cleaning runlist from the desktop. Open it, switch to the Acquisition mode. Load the rack with the 4 cleaning tubes. Run the experiment. The instrument will automatically shut down after the cleaning finishes.

Troubleshooting

Air trapped in sample line

- If a tube/well is run dry, air may become trapped in the sample line. This will negatively impact your successive samples. This can be prevented by monitoring volumes during setup tubes and by ensuring the correct volume is in your well and entered into the software correctly.
 - To remove air from the line, click the double sided expansion arrow at the top right of the software. Then click the **Remove Clog** (check this!!!) button.