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## Guava Flow Cytometry

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Dr. Steven Wilhelm, Alyssa Alsante

The Aquatic Microbial E...



Steven W Wilhelm

The University of Tennessee, Knoxville

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## Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.ed) for additional information regarding this protocol.

## Troubleshooting

## Cleaning the machine

- 1 Click CLEANING from the main menu

### Note

Always clean the machine before and after use

- 2 Click START CLEAN

- 3 Load the following:
  - \*dH<sub>2</sub>O in W1,W2, W3, W4, W5, W6, and position 1
  - \*100 µL bleach in position 2
  - \*GUAVA ICF in position 3 and 4
  - \*dH<sub>2</sub>O in capillary shutdown (position 9)

## GUAVA InCyte Acquisition Workflow

- 4 To ensure optimal performance, allow the machine to warm up for at least 10 min prior to acquiring samples.
- 5 Select InCyte from the main menu
- 6 Click the EDIT WORKLIST button
- 7 Define the worklist parameters (e.g., wells to analyze, events to acquire, time to acquire, dilution factor, original volume, replicates, etc)
- 8 Click RUN WORKLIST
- 9 Select the folder where you want to save the data file and click SAVE
- 10 Select the Analysis Method that you want to use for your specific sample set. To open a saved method, click RETRIEVE on the dialog box, then OPEN.

**Note**

If you want to create a new Analysis Method, click NEW, then OK and set the parameters.

- 11 Place the microtiter plate in the tray
- 12 Load the following cleaning solutions:
  - \*dH<sub>2</sub>O in W2, W4, W5
  - \*Empty tubes in W5, W6
  - \*100 µL bleach in W1
  - \*ICF in position 10
  - \*dH<sub>2</sub>O in position 9 (capillary shutdown)
- 13 Click OK to load
- 14 Select the sample for ADJUST SETTINGS in the dialog box
- 15 Select the type of plots and parameters you wish to display
- 16 Use the GAIN CONTROLS to select the Threshold parameter from the drop-down menu and use to adjust the gains (FSC, SSC, GRN, YLW, RED, NIR, RED2, or NIR2)
- 17 To adjust COMPENSATION, click NEXT STEP, then ADJUST SETTINGS
- 18 When you are finished adjusting the settings, click NEXT STEP.
- 19 Click RESUME WORKLIST and it will begin acquiring samples