THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

BECKMAN COULTER GALLIOS

QUICK GUIDE: START UP, QUALITY CONTROL, AND SHUT DOWN

PROCEDURE

1.0 Start Up

1.1. Check that the sheath cube is full and the waste cube is empty. If the waste cube is full replace it with an empty waste cube and follow the disposal instructions listed on the cube.

1.2. Turn on the computer. Double click the “Cytometer On” icon on the desktop to turn on the instrument.

1.3. Once the Status display on the instrument has turned green, double click the “Kaluza for Gallios” icon to open the instrument acquisition software.

2.0 Quality Control

2.1. Open “Startup QC” from the Acquisition Worklists folder. Set the output to the current month within the Gallios QC folder.

2.2. Load a carousel with tubes in the following order: Tube 1) 10% bleach; tube 2) diH_2O; tube 3) diH_2O; tube 4) diH_2O; tube 5) Flow Set Pro beads; tube 6) Flow Check Pro beads. The beads are used undiluted.

2.3. Place the carousel into the carousel loader, close the lid, and select “Acquire”.

2.4. After the Flow Set and Flow Check beads are collected, click the red icon in the Worklist window to view the QC files in the Kaluza Analysis software and verify that all fluorescent populations are visible and within the expected range. Levy Jennings plots may be created using the batch file in the QC folder. Delete cleaning files prior to initiating the batch program.

3.0 Sample Acquisition

3.1. Select an Acquisition protocol from the list. Modify if needed to add or delete parameters.

3.2. Select the appropriate data storage location according to the User’s PI folder. Create a new folder using the User’s name and date in the format YYYYMMDD. A brief description of the experiment may also be included.

3.3. Edit the Carousel list entering the PI name and User name in fields one and two. Add tubes to the carousel by duplicating the selected protocol until the required number of tubes are created. Individual tube ID information may be added in field three as desired. Tubes will automatically be named with a suffix of the carousel tube location 001, 002 etc.

3.4. If this is a repeat of an existing experiment with no major changes expected, place the carousel into the carousel loader, close the lid, and select “Acquire”. Make adjustments as needed to gates, settings and stop criteria while in the blue Set Up mode. Exit this mode and the header will return to grey and data will accumulate and be saved.

3.5. If this is a new experiment, click on a fully stained tube and click “Acquire Single”. Ensure that the speed is on “Low” to conserve sample. Adjust scatter and PMT voltages as needed so that positive and negative populations are on scale. Exit Set Up mode and record a small amount of

data. Save the changes to the template by clicking the “Save” icon at the top of the screen or select “Save As” from the menu to save the protocol with a new name.

3.6. Return to Carousel position 1 and run all samples. Click the red icon in the Worklist window to view the data files in the Kaluza Analysis software and adjust gates, analysis regions and compensation as needed. You may use an existing analysis protocol from the list to create a report sheet for each sample which can be exported as a PDF.

4.0 Compensation

4.1. Load unstained, single color and fully stained tube into Kaluza. Use Detector controls to associate the files. Click “Add Selected to new Compensation”

4.2. Click “Detect Controls” and assign each control accordingly: Autofluorescence for unstained, Verification for All Stained and appropriate channels for each single color. Choose the method for compensation: positive only, positive with autofluorescence subtraction or positive and negative.

4.3. Click “Generate Compensation and adjust gates as needed.

4.4. Evaluate the verification tube and adjust compensation as needed with sliders and save compensation matrix.

5.0 Shut Down

5.1. Open “Shutdown” from the Acquisition Worklists folder. Set the output to the current month within the Gallios QC folder.

5.2. Load a carousel with tubes in the following order: Tube 1) 10% bleach; tube 2) diH_2O; tube 3) diH_2O; tube 4) diH_2O.

5.3. Place the carousel into the carousel loader, close the lid, and select “Acquire”.

5.4. Following acquisition of all four tubes, select “Clean” to perform cleaning with Coulter Clenz. When complete, the system will depressurize then exit Kaluza for Gallios. Once the Status display on the instrument has turned red, double click the Cytometer Off icon, and shut down the computer.

RELATED PROCEDURES

This handout is related to ACSF SOP IN004. Please see the full SOP for further information.