QC Reagent Tube Format (RTF) Assay Kit –Instructions For Use

Intended use

The Accellix QC assay includes microfluidic cartridges and a series of fluorescent particles intended for routine verification of the Accellix Instrument. The fluorescent particles have a defined fluorescence intensity across all channels which are utilized to assess parameters such as optical alignment, sensitivity, linearity, and calibration of the Accellix instrument.

Assay Kit Contents

1. Assay reagent tube
2. Microfluidic cartridge*

Equipment/Materials Required

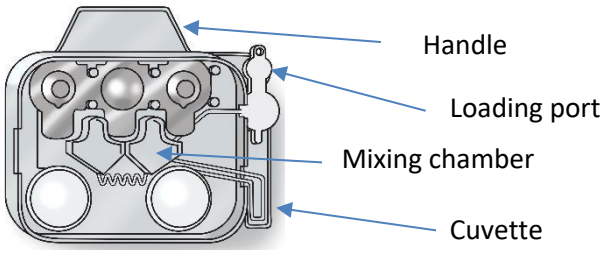
1. Calibrated pipettes and tips (20-200µL)
2. Pulse vortex mixer (Scientific Industries, Vortex-Genie Pulse, SKU: SI-P236)
3. Accellix Vortex Reagent Tube Adaptor
4. Phosphate Buffer Saline (1X PBS)- Mg^{2+}/Ca^{2+} free

Instrument Requirements

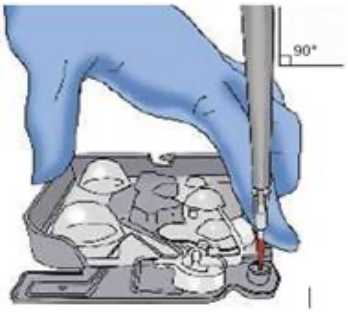
Accellix Instrument with software version 3.8.2 and above

Assay Protocol

1. Open the assay kit pouch by holding it by the top edge and tearing it open from the tear notch.
2. Carefully remove the cartridge and reagent tube from the pouch, holding it by the handle.
   Note: Be sure to hold the cartridge by the handle on the top of the cartridge and avoid touching the area near the cuvette.

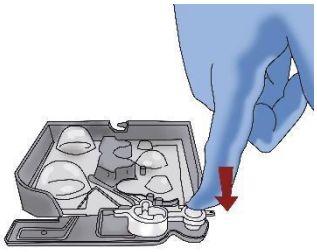


3. Place the cartridge, label-side down, on a clean surface.
4. Remove the reagent tube from its pouch by tearing open from the tear notch.
5. Uncap the reagent tube, accurately pipette, and dispense 40µL of PBS to the bottom of the tube.
6. Recap the tube, place tube in the Reagent Tube Adaptor on the preprogrammed pulse vortex mixer and press Start.
   Note: The mixer is programmed to mix the reagent/sample tube for 2 minutes at 1500rpm (vortex cycle = 2 seconds on, 1 second off). For more information on how to use the vortex, see the Operating Instructions for the Vortex Genie Pulse Mixer.
   Note: The reagent is dried on the bottom of the tube and must be completely reconstituted for the assay to perform accurately.
7. Immediately after the 2-minute pulse vortex step, uncap the tube and transfer 20µl of the reconstituted reagent while flipping open the sample plug of the sample loading port on the microfluidic cartridge.
   - Firmly insert the end of the pipette tip into the hole at the bottom of the loading port, holding it at a 90-degree angle and make contact with the base.



- Depress pipette plunger to the second stop (this ensures that the sample moves into the microfluidic channel connected to the loading port).

8. With the cartridge still lying flat, firmly press the sample plug into place over the sample loading port.



- Verify that the sample fluid moves down into the channel leading to the front mixing chamber on the cartridge.

9. Initiate the assay run by pressing 'Start' on the Accellix touchscreen. Follow the on-screen instructions to proceed.
10. Insert the cartridge into the instrument cartridge slot with the label facing away from the operator. A "click" can be heard and felt when pressing on the right-hand corner of the cartridge handle which confirms the cartridge has reached the proper position. Once the cartridge has been inserted properly, close the Accellix Instrument Door.
11. When prompted, enter the Sample ID using the on-screen keyboard or a barcode scanner.
12. Verify the entry and press next to initiate the automated data acquisition and analysis.
13. Total running time is approximately 16 minutes.
14. At the conclusion of the run, follow instructions on the screen and remove the cartridge from the Accellix Instrument.
15. The resulting data files are saved on the Accellix internal hard drive and will be copied from the Accellix instrument to a configured network drive or other storage destination. The storage destination can be set up at the time of instrument installation.

    Note: the PDF report and results CSV filenames will contain a date and time stamp, cartridge serial number (Cartridge ID), instrument serial number (Device ID), and Sample ID.

Expected results:

At the end of the QC Assay run, PDF, CSV and FCS files will be generated in the results folder. The PDF and CSV files include an Accellix Results Summary as well as an Accellix QC Assay Detailed Results reporting on optical alignment, sensitivity, linearity, and calibration parameters of the Accellix Instrument.

For each parameter, the per-bead group or per-fluorescent channel results and acceptance criteria are shown in the PDF report and in the results CSV file. If a parameter's measured value falls within acceptance criteria, "PASS" is displayed in green. If a parameter's measured value falls outside of acceptance criteria, "FAIL" is displayed in red. The PDF report also contains relevant graphs illustrating within-run results and cumulative results over time.

Note: For any parameter failure please refer to the troubleshooting section below.

Note: For a detailed Parameter definition guide, please refer to the Accellix QC Assay Metrics below.

Files Generated and Usage

File Type and Name	Purpose / Usage
PDF file ending with _QC-Results	Detailed QC Assay Results Report
PDF file named Assay Results	Assay Results Summary Report
FCS ending with _Events.fcs	FCS for Accellix Troubleshooting
CSV file ending with _Events	CSV for Accellix Troubleshooting
CSV file ending with _QC-Results	CSV for plotting trends over time

Accellix QC Assay Metrics

Bead Count: The bead count for each group is reported to ensure that there are sufficient events detected for statistically robust measurements of Forward Scatter (FSC) and Median Fluorescence Intensity (MFI).

Linearity: Linear regression analysis characterizes the correlation between six bead fluorescence intensity levels (groups 1-6) in each optical channel and the expected molecules of equivalent fluorochrome (MEFL). The correlation coefficient (R2 value) is reported as an indicator of detector linearity.

Sensitivity:

Stain Index: The stain index (SI), or the separation between the dimmest bead group and the blank bead group in each fluorescent channel, is an indicator of instrument sensitivity. Stain index is defined as:

(MFI of Dimmest Positive Group – MFI of Blank Group)/ (2 x rSD of Blank Group in same channel) = Stain Index

% CV: The coefficient of variation (CV) of MFI and FSC signals in their respective channels is another indicator of instrument sensitivity and is defined as the robust standard deviation of the signal divided by the median of that signal.

Calibration: The QC Assay contains the same beads (group 7) that are used to calibrate the voltages for each detector channel. The percent difference between the bead MFI in each channel relative to the target MFI used during calibration is reported as a way to verify that the instrument remains calibrated.

Results Summary: A table showing summarized results for bead count, optical linearity, sensitivity, and calibration is displayed on-screen at the completion of the run and in the PDF report. It indicates overall pass/fail for each of the four parameters and provides instructions in the case that the assay fails.

Detailed Results: For each parameter, the per-bead group or per-fluorescent channel results and acceptance criteria are shown in the PDF report and in the results CSV file. If a parameter's measured value falls within acceptance criteria, "PASS" is displayed. If a parameter falls outside of acceptance criteria, "FAIL" is displayed. The PDF report also contains relevant graphs illustrating within-run results and cumulative results over time.

Troubleshooting

1. If any single parameter fails during a run, the assay fails. The operator will need to restart the instrument and re-run the assay. If the assay fails three times in a row, operator should contact Accellix Support: support@accellix.com
2. If an error is displayed on the screen of the Accellix Instrument at any point during a run, follow the instructions displayed.
3. If a failure occurs, or if the screen instructs to contact Accellix support, please take a picture of the front and back sides of the cartridge, email Accellix Support (support@accellix.com) with the instrument and cartridge details (Device ID, Cartridge ID), description of the error, attach the images as well as the associated results file if available.

Storage, Stability and Disposal

1. Store Accellix assay kits at room temperature (15-25°C) in their original packaging until used.
2. Reagents are photo sensitive. When working with them, assure minimal exposure to light.
3. Accellix assay kits are valid for use until the expiration date printed on the cartridge next to the hourglass icon and on the box (i.e., for the date: 2 of Jan 2030, it will expire on 1 of Jan 2030, at 23:59).
4. Used cartridges, and reagent tubes should be disposed of with proper biohazard precautions in accordance with local regulations.

Warnings and Precautions

1. Accellix RTF Assay kits are not intended for diagnosis of human or animal disease.
2. For use by a trained operator only.
3. The Accellix RTF Assay kits should be handled observing standard safety precautions (do not ingest; do not inhale).
4. Do not use a damaged cartridge, tube or one with damaged packaging.
5. Do not use an expired cartridge or tube.
6. Do not re-use a cartridge.
7. Do not open the instrument door when an assay is in progress, as this will abort the assay and the cartridge will not be reusable.

***Hazard Pictograms:**

Signal Word:	Danger
Hazard Statement:	H332 - Harmful if inhaled. H317 - May cause an allergic skin reaction. H350 - May cause cancer. H341 - Suspected of causing genetic defects.

Precautionary Statements

Prevention:	P202 - Do not handle until all safety precautions have been read and understood. P280 - Wear protective gloves. Wear eye or face protection. Wear protective clothing. P261 - Avoid breathing vapor. P272 (OSHA) - Contaminated work clothing must not be allowed out of the workplace.
Response:	P308 + P313 – IF EXPOSED: Get medical attention. P304 + P340 + P312 - IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or physician if you feel unwell. P302 + P352 + P363 - IF ON SKIN: Wash with plenty of soap and water. Wash contaminated clothing before reuse. P333 + P313 - If skin irritation or rash occurs: Get medical attention.

This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest; do not inhale).