



Guava[®] easyCheck[™] Kit

For Research Use Only
4600-3265, Rev D
Catalog No. 4500-0025
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Application

The Guava® easyCheck™ Kit is used to verify the performance of the Guava® easyCyte™ and easyCyte HT systems by assessing counting accuracy and fluorescence detection using a standardized fluorescent bead reagent. The kit contains the Guava easyCheck Bead Reagent and Guava Check Diluent. To assure that the easyCyte System provides reliable, accurate results, run the easyCheck procedure every day that the instrument is used before performing any assay.

For research use only. Not for use in diagnostic procedures.

Test Principle

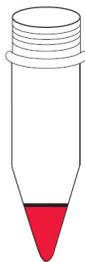
The Guava® easyCheck™ Kit is used to verify the performance of your Guava® easyCyte™ System by assessing counting accuracy and fluorescence detection using a standardized fluorescent bead reagent. Running the Guava easyCheck procedure before performing any assays is critical to assure that your Guava easyCyte systems provide reliable, accurate results. Guava easyCheck Bead Reagent is provided as a concentrated fluorescent bead suspension of known concentration. To run the Guava easyCheck procedure, mix the bead reagent with Guava Check Diluent at the recommended dilution to yield a bead suspension at a specific target concentration. The Guava easyCheck software module analyzes the results from three sequential data acquisitions of the bead suspension. The count results are displayed on the screen for comparison with the known target values. Particle counts that are outside the expected range are displayed in red. Count results, forward scatter and side scatter signal intensity, and mean fluorescence intensities are displayed. The averages and %CV values for these results are calculated and also displayed. The results and the instrument settings are saved to a spreadsheet file. The results from all Guava easyCheck procedures are appended to a file, providing a history of data that allows you to monitor the performance of your Guava System over time.

Summary of Protocol

Add 380 μL of Guava®
Check Diluent to a tube



Add 20 μL of easyCheck™
Bead Reagent



Mix thoroughly and run
on easyCyte™ system



Materials Provided

Guava® easyCheck™ Kit (Catalog No. 4500-0025) includes the following reagents:

- Guava® easyCheck™ Bead Reagent (Part No. 4200-0075)
- Guava® Check Diluent (Part No. 4200-0050)

Materials Required but Not Supplied

- Guava® Instrument with GuavaSoft™ Software
- Micropipettors and disposable micropipettor tips
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalog No. 16466-030, or equivalent)
- Vortex mixer
- Disposable gloves
- 20% bleach solution
- Deionized water
- Guava Instrument Cleaning Fluid (ICF) [Catalog No. 4200-0140], optional

For 96-Well Plate Procedure

- 96-well microplates, round bottom (BD Falcon™ Catalog No. 353910 or 353918) or flat bottom (BD Falcon Catalog No. 353075 or 353915), or equivalent. Refer to the appropriate Guava® System user's guide for other compatible microplates.
- 1.5-mL microcentrifuge tubes (VWR Catalog No. 16466-030 or equivalent) for cleaning
- Reagent reservoirs, 50 mL (VWR Catalog No. 82026-354 or equivalent)

For Microcentrifuge Tube Procedure

- Sample acquisition tubes, 1.5-mL microcentrifuge tubes (VWR Catalog No. 16466-030 or equivalent), or 1.2-mL titer tubes (E&K Scientific, Catalog No. 604508-RC or equivalent), for use on the SL Guava® Systems
- 0.5-mL microcentrifuge tubes (VWR Catalog No. 16466-036 or equivalent) for sample acquisition on the HT Guava Systems

Precautions

- The Guava® easyCheck™ Kit is for research use only.
- Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling or using this reagent.
- All materials should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.
- The Guava Check Diluent contains sodium azide, which is toxic if ingested. Reagents containing sodium azide should be considered a poison. If products containing sodium azide are swallowed, seek medical advice immediately and show product container or label. (Refer to NIOSH, National Institute for Occupational Safety and Health; CAS#: 2628-22-8; and also to GHS, The Globally Harmonized System of Classifi-

cation and Labeling of Chemicals.) Aqueous solutions of sodium azide, when mixed with acids, may liberate toxic gas. Any reagents containing sodium azide should be evaluated for proper disposal. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent build-up in plumbing. Check with regulatory agencies to determine at what concentration sodium azide may cause a product to be regulated as hazardous.

- Avoid microbial contamination of the solution, which may cause erroneous results.
- Safety Data Sheets (SDS) for kit reagents are available from our website <https://www.luminexcorp.com>.

Storage

- Store Guava® easyCheck™ Kit refrigerated at 2 to 8°C. Do not freeze. Refer to the expiration date on the package label. Do not use the kit after the expiration date.
- Shield the Guava easyCheck Bead Reagent from excessive exposure to light.

Before You Begin

Allow the Guava easyCheck Bead Reagent and Guava Check Diluent to warm to room temperature before use.

Thoroughly mix the Guava easyCheck Bead Reagent for at least 5 seconds using a vortex mixer set at moderate speed. Ensure that the beads are well mixed before removing an aliquot to prepare the diluted bead sample.

Time considerations: Once diluted the beads are ready to acquire. No incubation is necessary. Acquiring three replicates of the diluted beads on the easyCyte™ takes approximately 5 minutes.

Preparing the easyCheck™ Bead Sample

1. Transfer 380 µL of the Guava® Check Diluent to a microcentrifuge tube or sample acquisition titer tube using a calibrated pipettor.
2. Thoroughly mix the easyCheck™ bead vial. Using a calibrated pipettor, pipette 20 µL of the easyCheck Bead Reagent. Carefully wipe off excess solution adhering to the exterior of the pipette tip.
3. Transfer the 20 µL of the bead reagent into the 380 µL of diluent. Mix the bead solution thoroughly.
4. If using an SL Guava System, you may vortex the sample, however, be sure to cap the tube first.
5. If using 0.5-mL microcentrifuge tube on an HT system, do not vortex. The instrument will automatically mix the sample.
6. The diluted beads are ready to acquire on the appropriate Guava easyCyte™ system. To ensure proper sampling and reliable results, thoroughly mix the bead suspension immediately before loading the tube on the easyCyte instrument for data acquisition.

Running the easyCheck™ Procedure

Run the Guava® easyCheck™ procedure on your Guava System at the start of the day before the system is used for sample acquisitions. This ensures that your system is functioning properly.

Starting the System

1. Start the easyCyte™ system.
2. Start GuavaSoft™ Software.
3. Click **easyCheck** from the main menu.

Entering Guava® easyCheck™ Bead Information

1. Enter or confirm that the bead lot number shown on the Guava® easyCheck™ screen is the same as the lot number on the Guava easyCheck Bead Reagent vial (alternately, you may use the kit lot number). If not, enter the correct number.
2. Enter or check the Expected Particles/mL value that corresponds to the concentration of beads in your diluted sample. This value should be approximately 50,000 if you prepared the Guava easyCheck Bead Reagent at the recommended 20-fold dilution.

NOTE: Check the information card that comes with the Guava easyCheck Kit for the exact concentration of the diluted beads for each lot.

3. Enter or confirm the expiration date of the Guava easyCheck Bead Reagent (or of the kit).
4. Proceed to the following instructions appropriate for your instrument—either single-loader (SL) or high-throughput (HT).

Running the Beads on the Single-Loader (SL) Guava® Systems

1. Click **Adjust Settings**.
The system automatically sets a threshold to exclude background noise.
2. Mix the Guava® easyCheck™ Bead sample thoroughly and load it on the Guava System. Click **Run 1st Replicate**.
The system acquires 1000 events and displays the results in the easyCheck Results table for Replicate 1.
3. Mix the Guava easyCheck Bead sample thoroughly and load it on the Guava System. Click **Run 2nd Replicate**.
The system acquires 1000 events and displays the results in the row for Replicate 2.
4. Mix the Guava easyCheck Bead sample thoroughly and load it on the Guava System. Click **Run 3rd Replicate**.
The system acquires 1000 events and displays the results in the row for Replicate 3. The mean values and %CV for each parameter are automatically calculated and displayed.
Particle count results that fall outside the expected range (more than 10% deviation from the target value) appear in red, while counts that are within the expected range appear in dark green.

If the results are within the expected range, you may proceed with your assays. If the system does not meet established target values, proceed to “Guava® easyCheck™ Results”.

Running the Beads on the High-Throughput (HT) Guava® Systems

1. Click **Start**. The tray automatically ejects.
2. Load the following on the instrument:
 - 0.5-mL tube or microplate containing the diluted Guava® easyCheck™ Beads
 - 1.5-mL microcentrifuge tubes containing approximately 1.5 mL of DI water in locations w2, w4, and w5
 - 1.5-mL microcentrifuge tube containing approximately 100 µL of bleach in location w1
 - empty 1.5-mL microcentrifuge tubes in locations w3 and w6
3. On the tray map displayed in the software dialog box, click the location of the diluted Guava easyCheck Beads (tube or well), and click **OK**. The tray automatically loads and the Guava® easyCheck procedure begins. The following steps are performed:
 - The Guava System automatically sets a threshold to exclude background noise.
 - The Guava System then automatically mixes the Guava easyCheck Bead sample thoroughly and automatically runs all the replicates.
 - The system acquires 1000 events for each replicate and displays the results in the easyCheck Results table for each replicate as it is acquired. The mean and %CV values for each parameter are automatically calculated and displayed.
 - Particle count results that fall outside the expected range (more than 10% deviation from the target value) appear in red, while counts that are within the expected range appear in dark green.
4. If the results are within the expected range, proceed with your assays. If the system does not meet established target values, proceed to “Guava® easyCheck™ Results” in the following section.

Guava® easyCheck™ Results

The software displays the %CVs and the averages for the particles/mL (bead count), FSC and SSC intensities, and the mean fluorescence intensities (MFIs) for each fluorescence parameter for the three replicates.

- If any result for Particles/mL falls outside $\pm 10\%$ of the expected value, the result is outside the acceptable range and appears in red.
- If the %CV for Particles/mL is $>10\%$, it appears in red.
- Compare the MFI results to the ranges on the card that comes with easyCheck™ Kit. If any result falls outside $\pm 15\%$ of the expected value, the result is outside the acceptable range.
- The %CV for FSC and SSC Intensities, and MFIs should be $<5\%$.

If the system does not meet established target values, clean the instrument.

1. Click **Quick Clean** from the easyCheck screen. Follow the prompts to load the tubes and run the cleaning cycle.
2. Rerun the easyCheck procedure with your prepared bead sample.
3. If the results from the second run are within the expected range, proceed with your assays. If the results are still not within the expected range, prepare and run a new dilution of Guava® easyCheck Beads.

4. If the procedure fails again, see the section, Troubleshooting Tips or refer to the appropriate Guava System user guide for more information.

Troubleshooting Tips

1. Thoroughly mix all suspensions of the Guava® easyCheck™ Beads before pipetting the reagent or loading the diluted beads onto the instrument. Inadequate mixing results in erroneous bead counts. Avoid high vortexing speeds, which can cause foaming of the solution.
2. The Guava easyCheck Reagents should equilibrate to room temperature before preparing a dilution of the bead reagent. Diluted beads should be at room temperature before acquired on the Guava System.
3. Cover or cap all tubes when vortexing to avoid volume loss while mixing. Sample loss may affect the concentration of particles in the diluted bead suspension.
4. To ensure accurate results, use pipettors that have been properly maintained and calibrated.
5. If the data acquisition rate slows or does not reach 1000 events, click **Abort** to discontinue the run. Check the volume left in the tube or well. If it is less than or around 50 μL , prepare a freshly diluted suspension for use. If the volume is still sufficient, most likely a clog has occurred. Do the following:
 - On the single-sample Guava easyCyte™ Systems, replace the sample with a tube containing approximately 100 μL of bleach, and then click **Backflush**. When the backflush is complete, exit the Guava easyCheck module and perform a Guava Clean procedure.
 - On the HT Guava Systems, click **Backflush**, select the location for the tube of bleach, and click **OK**. When the backflush is complete, exit the Guava easyCheck module and perform a Guava Clean procedure.
 - On all instruments, return to the Guava easyCheck module. If necessary, allow the SL Guava System to warm up at least 5 minutes before proceeding. Refer to the appropriate easyCyte System user's guide for information.
6. If the 1st Replicate or 2nd Replicate result is out of range, you can discontinue the run immediately by clicking **Abort**. Perform a Quick Clean procedure and rerun the Guava easyCheck procedure. If the results continue to fall outside the expected range, prepare a new dilution of Guava easyCyte Beads. Rerun the Guava easyCheck procedure with the fresh beads.
7. The diluted Guava easyCheck Bead suspension can be stored in a tightly capped tube protected from light at room temperature for use throughout the same day (within 6 to 8 hours). If the volume of diluted Guava easyCheck Beads prepared was 500 μL , then this suspension will be sufficient for three Guava easyCheck procedures. If the volume of diluted Guava easyCheck Beads prepared was 200 μL , then this suspension will be sufficient for only one Guava easyCheck procedure. Old samples should be discarded and a fresh dilution should be made daily.
8. The signal intensities measured by all installed detectors are included with the count results in the spreadsheet file and can be viewed on screen by checking the Show History box. We recommend reviewing this file periodically to monitor instrument performance over time. If the scatter or fluorescence values from a single lot of Guava easyCheck Beads fall outside the recommended ranges found on the card that comes in the kit, call Luminex Technical Support for advice.
9. For more troubleshooting information, refer to the appropriate Guava System user's guide or contact Luminex Technical Support.

Limitations

The results are dependent upon proper use of reagents and instruments.

Related Kits

For a complete list of reagent kits, go to www.luminexcorp.com.

| Reagent Kit | Catalog No. |
|------------------------------------------|-------------|
| Guava® ViaCount™ Reagent (40 mL) | 4000-0040 |
| Guava® ViaCount™ Reagent (240 mL) | 4000-0041 |
| Guava® ViaCount™ Flex Reagent Kit | 4500-0110 |
| Guava® Annexin Red Kit | FCCH100108 |
| Guava® Nexin® Reagent (100 tests) | 4500-0450 |
| Guava® MitoPotential Red Kit | FCCH100105 |
| Guava® MitoDamage Kit | FCCH100106 |
| Guava® MitoLive Kit | FCCH100107 |
| Guava® MitoStress Kit | FCCH100109 |
| Guava® Cytochrome c Kit | FCCH100110 |
| Guava® Oxidative Stress Characterization | FCCH025111 |
| Guava® MitoCaspase 3/7 Kit | FCCH100178 |
| Guava® TUNEL | 4500-0121 |

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