

**Guava® Check Kit**

Cat. No. 4500-0020

**For Verifying the Performance of the Guava Systems****For Research Use Only; Not for Use in Diagnostic Procedures****PRODUCT DESCRIPTION AND INTENDED USE**

The Guava® Check Kit is used to verify the performance of your Guava PCA, Guava EasyCyte™ Plus, or Guava EasyCyte Mini System by assessing counting accuracy and fluorescence detection using a standardized fluorescent bead reagent. The kit contains Guava Check Beads and Diluent. Running the Guava Check Procedure daily, before performing any assays, is important to assure that your Guava Systems provide reliable, accurate results. Guava Check Beads are provided as a concentrated fluorescent bead suspension of known concentration. To run the Guava Check 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 Check Software Module analyzes the results from three sequential data acquisitions of this bead suspension. The count results are displayed on the screen for comparison with the known target value. Particle counts that are outside the expected range are displayed in red. Count results, forward scatter and side scatter (optional on some systems) signal intensity, and mean fluorescence intensities are displayed. The averages and %CV values for these results are calculated and also displayed. These results and the instrument settings are saved to a spreadsheet file. The results from all Guava Check Procedures are appended to a file, providing a history of data that allows you to monitor the performance of your Guava System.

**MATERIALS PROVIDED**

Guava® Check Kit (Cat. No. 4500-0020) includes the following reagents:

- Guava® Check Bead Reagent (Part No. 4200-0070)
- Guava® Check Diluent (Part No. 4200-0050)

**HANDLING AND STORAGE**

1. Store the Guava® Check Kit refrigerated (2 to 8°C). Do not freeze. Refer to the expiration date on the package label. Do not use the reagent after the expiration date.
2. Shield the Guava Check Bead Reagent from excessive exposure to light.

**WARNINGS AND PRECAUTIONS**

1. The Guava® Check Kit reagents are intended for research use only.
2. Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling or using this reagent.

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3. 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.
4. The Guava Check Diluent contains sodium azide, which is toxic. Contact with acids liberates toxic gas. Flush plumbing with copious amounts of water when disposing of azide compounds to avoid potentially explosive conditions arising from azide deposits in pipes.
5. Avoid microbial contamination of the solution, which may cause erroneous results.
6. Safety Data Sheets (SDS) for kit reagents are available from our website [www.luminexcorp.com](http://www.luminexcorp.com).

**EQUIPMENT AND MATERIALS REQUIRED**

- Guava® Instrument with Guava CytoSoft™ Software
- Guava Check Kit
- Micropipettors and disposable micropipettor tips
- For the Guava PCA and Guava EasyCyte™ Mini Systems:
  - Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Cat. No. 16466-030 or equivalent) *or*
  - Sample acquisition tubes, 1.2-mL titer tubes (E&K Scientific, Cat. No. 604508-RC, or equivalent)
- For the Guava EasyCyte Plus System:
  - Microplates, 96-well round-bottom, polystyrene (Falcon Cat. No. 353910 or 353918, or equivalent) *or*
  - Sample acquisition tubes, 0.5-mL microcentrifuge tubes (VWR, Cat. No. 16466-036, or equivalent) *and*
  - Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Cat. No. 16466-030, or equivalent) for cleaning
- Vortex mixer
- Disposable gloves
- 20% bleach solution
- Guava Instrument Cleaning Fluid (ICF) [Cat. No. 4200-0140], optional
- Deionized water

**PREPARING THE GUAVA® CHECK SAMPLE**

1. Allow the Guava® Check Beads and Diluent to warm to room temperature before use.
2. Thoroughly mix the Guava Check Beads using a vortex mixer set at moderate speed. Take care to ensure that the Guava Check Bead Reagent is well mixed before removing an aliquot for dilution to prepare diluted Guava Check Beads.

**Guava® Check Beads Prepared for the PCA and EasyCyte™ Mini Systems Using Microcentrifuge Tubes**

1. Transfer 950 µL of the Guava® Check Diluent to a microcentrifuge tube or sample acquisition titer tube using a calibrated pipettor.
2. Using a calibrated pipettor, pipette 50 µL of the Guava Check Bead Reagent.
3. Carefully wipe off excess solution adhering to the exterior of the pipette tip.
4. Transfer the 50 µL of the Guava Check Bead Reagent into the 950 µL of diluent. Mix the solution by repeatedly withdrawing and expelling a

portion of the suspension with a 1000-µL micropipettor. This should sufficiently mix the beads. However, if vortexing is desired, be sure to cap the tube before vortexing.

5. The diluted Guava Check Beads are ready to use on the Guava System. To ensure proper sampling and reliable results from the Guava Check Procedure, thoroughly mix the bead suspension immediately before loading the tube on the Guava Instrument for data acquisition.
6. Prepare a second tube containing 100 µL of Guava Check Diluent to be used for setting the threshold.

**Guava® Check Beads Prepared for the EasyCyte™ Plus System Using Microcentrifuge Tubes**

1. Transfer 475 µL of the Guava® Check Diluent to a 0.5-mL microcentrifuge tube.
2. Using a calibrated pipettor, pipette 25 µL of the Guava Check Bead Reagent.
3. Carefully wipe off excess solution adhering to the exterior of the pipette tip.
4. Transfer the 25 µL of the Guava Check Bead Reagent into the 475 µL of diluent. Mix the solution by repeatedly withdrawing and expelling a portion of the suspension with a 1000-µL micropipettor. Do not vortex as inadequate mixing and spillage often occur when vortexing a solution in a 0.5-mL microcentrifuge tube.

The diluted Guava Check Beads are ready for use.

**Guava® Check Beads Prepared for the EasyCyte™ Plus System Using Microplates**

1. Transfer 190 µL of the Guava® Check Diluent to one well of a microplate.
2. Using a calibrated micropipettor, pipette 10 µL of the Guava Check Bead Reagent.
3. Carefully wipe off any excess solution that may be adhering to the exterior of the pipette tip.
4. Transfer the 10 µL of the Guava Check Bead Reagent into the 190 µL of diluent. Mix the solution by repeatedly withdrawing and expelling a portion of the suspension with a 200-µL micropipettor.

The diluted Guava Check Beads are ready to use.

**RUNNING THE GUAVA® CHECK PROCEDURE**

Run the Guava® Check Procedure on your Guava System at the start of the work day before the system is used for sample acquisitions. This ensures that your system is functioning properly for CytoSoft™ Software.

**NOTE:** Turn on your instrument at least 15 minutes before running the Guava Check Procedure to ensure that the system is warmed up.

**Running the Procedure on the PCA and EasyCyte™ Mini Systems**

1. Enter or confirm that the bead lot number shown on the Guava® Check screen is the same as the lot number on the Guava Check Bead Reagent vial. 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.

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

3. Enter or confirm the expiration date of the Guava Check Bead Reagent.

4. Load the tube of Guava Check Diluent on the instrument.
5. Click **Adjust Settings**. The system automatically sets a threshold to exclude background noise.
6. Mix the Guava Check 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 Check Results table for Replicate 1.
7. Click **Run 2nd Replicate**.  
The system acquires 1000 events and displays the results in the row for Replicate 2.
8. 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.
9. 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.
10. 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 step 11.
11. Exit the Guava Check Software Module and select **Clean and Shut Down** on the menu bar to enter Guava Clean.
12. Perform a thorough cleaning using Guava Clean with Guava ICF and water, then return to the Guava Check Software Module. Allow the system to warm up at least 5 minutes. Rerun the procedure with your prepared buffer and bead samples.
13. If results from the second run are within the expected range, proceed with your assays. If the results from the second run are still not within the expected range, prepare and run a new dilution of Guava Check Beads and a new aliquot of Guava Check Diluent.
14. If the procedure fails again, see the section, *Troubleshooting Tips* or refer to the appropriate Guava System user's guide for more information.

### Running the Procedure on the EasyCyte™ Plus System

1. Enter or confirm that the bead lot number shown on the Guava® Check screen is the same as the lot number on the Guava Check Bead Reagent vial. 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.  
**NOTE:** Check the information card that comes with the Guava Check Beads for the exact concentration of the diluted beads for each lot.
3. Enter or confirm the expiration date of the Guava Check Bead Reagent.
4. Click **Start**. The tray automatically ejects.
5. Load the tube or microplate containing the diluted Guava Check Beads on the instrument.
6. Load 1.5-mL microcentrifuge tubes containing approximately 1.5 mL deionized water in locations W2, W4, and W5.
7. Load a 1.5-mL microcentrifuge tube containing approximately 100 µL 20% bleach in location W1.
8. Load empty 1.5-mL microcentrifuge tubes in locations W3 and W6.
9. On the tray map displayed in the software dialog box, indicate the location of the diluted Guava Check Beads (tube or well). Click **OK**. The tray automatically loads and the Guava Check 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 Check Bead sample thoroughly and automatically runs all the replicates.
  - The system acquires 1000 events and displays the results in the Check 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.
10. If the results are within the expected range, proceed with your assays. If the system does not meet established target values, proceed to step 11.
  11. Exit the Guava Check Software Module and select **Clean and Shut Down** on the menu bar to enter Guava Clean.
  12. Perform a thorough cleaning using Guava Clean with Guava ICF and water, and then return to the Guava Check Software Module. Rerun the procedure with your prepared bead sample.
  13. If the results from the second run are within the expected range, proceed with your assays. If the results from the second run are still not within the expected range, prepare and run a new dilution of Guava Check Beads.
  14. If the procedure fails again, see the section, *Troubleshooting Tips* or refer to the appropriate Guava System user's guide for more information.

### TROUBLESHOOTING TIPS

1. It is important to thoroughly mix all suspensions of the Guava® Check Beads before pipetting the reagent or loading the diluted samples onto the instrument for data acquisition. Inadequate mixing results in erroneous bead counts. Mix at medium speed to avoid creating bubbles in the suspension.
2. Guava Check Reagents should be equilibrated to room temperature before preparing a dilution of the bead reagent. Test sample should be at room temperature before acquiring data 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 Guava Check 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 and do the following:
  - On the PCA and EasyCyte™ Mini Systems, replace the sample with a tube of deionized water or 20% bleach, and then click **Backflush** to backflush the fluidics system to remove a blockage. Exit the Guava Check Software Module and perform a thorough cleaning using Guava Clean.
  - On the EasyCyte Plus System, click **Quick Clean** or **Backflush**, then select the correct location for the tube of deionized water or 20% bleach, and then click **OK** to clean or backflush the fluidics system to remove a blockage. Exit the Guava Check Software Module and perform a thorough cleaning using Guava Clean.
  - On all instruments, return to the Guava Check Software Module. Allow the Guava PCA System to warm up at least 5 minutes before proceeding.
6. If the 1st Replicate or 2nd Replicate result is out of range, you can discontinue the run immediately by clicking **Abort**. Perform a thorough cleaning as described in Tip 5 and rerun the Guava Check Procedure. If the results continue to fall outside the expected range, prepare a fresh sample

of Guava Check Diluent for setting the threshold (Guava PCA and EasyCyte™ EasyCyte Mini Systems only) and a new dilution of Guava Check Beads. Rerun the Guava Check Procedure with the fresh samples.

7. During acquisition, if the progress bar stops moving to the right, or if the counts shown in the sample information box on the left side panel suddenly do not continue to go up, then it is most likely that all the diluted Guava Check Bead suspension has been used up. Check the volume left in the tube or well. If it is less than or around 50 µL, then prepare a freshly diluted suspension for use. If the volume is still sufficiently above the dead volume, then most likely a clog has occurred. See Tip 5 for instructions on removing the clog.
8. The diluted Guava Check 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 Check Beads prepared was 500 µL, then this suspension will be sufficient for three Guava Check Procedures. If the volume of diluted Guava Check Beads prepared was 200 µL, then this suspension will be sufficient for only one Guava Check Procedure. Old samples should be discarded and a fresh dilution should be made daily.
9. The signal intensities measured by all installed detectors are included with the count results in the spreadsheet file. We recommend reviewing this file periodically to monitor instrument performance over time. If the scatter or fluorescence values from a single lot of Guava Check Beads fall outside the recommended ranges found on the lot-specific card, call Technical Support for advice.

For more troubleshooting tips, refer to the appropriate Guava System user's guide.

### LIMITATIONS

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

### TRADEMARKS

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