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QuantiGene™ Plex Automation Setup Kit

USER GUIDE

for 384-well Assays

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ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0025573

Revision	Date	Description
A.0	12 July 2021	Baseline for this revision history.

Contents

QuantiGene™ Plex Automation Setup Kit

Product description

The QuantiGene™ Plex Automation Setup Kit is used to adjust an automated plate washer, as needed, for a QuantiGene™ Plex 384-well assay wash without any performance or bead loss issues. The QuantiGene™ Plex Automation Setup Kit can also be used for setting up and validating other automation equipment for QuantiGene™ Plex 384-well assays, such as liquid handlers.

For the most current version of user documentation, visit our website **http://www.thermofisher.com**.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C. When stored as indicated, all reagents are stable until the expiration date.

Contents	Amount
Automation Setup Beads	1 x 7.5 mL
SAPE Wash Buffer Concentrate (10X)[1]	2 x 225 mL
QuantiGene™ Plex 384-well Flat Bottom Magnetic Separation Plate	6 plates
Plate Seal	6 seals

[1] Contains sodium azide. See CAUTION.

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Required materials not supplied

- FLEXMAP 3D™ or INTELLIFLEX™ instrument
- Microtiter™ plate shaker (must have 3 mm orbit at 600–800 rpm)
- Vortex mixer
- Adjustable single and multichannel pipettes with disposable tips
- Reagent reservoirs
- Nuclease-free water
- Tubes, beakers, flasks, and cylinders for preparation of reagents
- Automated plate washer suited for washing 384-well plates

Note: The QuantiGene™ Plex Assays (384-well) were assessed using the BioTek™ ELx405TS plate washer with a flat magnet (BioTek™ Part No. 7103017). See "Magnetic plate washer settings" on page 11 for the correct settings for the BioTek™ ELx405TS plate washer.

Settings for other automated magnetic plate washers need to be adjusted to the QuantiGene™ Plex 384-well Flat Bottom Magnetic Separation Plates.

Workflow

Assay protocol

Prepare 1X SAPE Wash Buffer

Mix 225 mL of SAPE Wash Buffer Concentrate (10x) with 2,025 mL nuclease-free water.

Prepare serial dilution of the Automation Setup Beads

1. Vortex the Automation Setup Beads, then add 1,250 µL to tube 1.
2. Add 250 µL of 1X SAPE Wash Buffer to tubes 2–5.
3. Vortex, then transfer 1,000 µL from tube 1 to tube 2. Vortex again.
   Repeat this step for the remaining tubes in the series.

Define the plate map

Mark the bead dilution, wash buffer, and residual volume wells using the recommended plate layout.

Add bead dilutions and 1X SAPE Wash Buffer to the plate

1. Add 50 µL of each bead dilution to the designated wells of the plate.
2. Add 50 µL of 1X SAPE Wash Buffer to the remaining wells of the plate.

Wash the plate, then determine residual volume

1. Wash the plate 15 times using the washer settings recommended for your magnetic plate washer.
2. Determine the residual volume in 10–15 of the designated plate wells.

Add 1X SAPE Wash Buffer, then run the plate

1. Add 50 µL of 1X SAPE Wash Buffer to each well containing beads.
2. Seal the plate, then shake at room temperature for 5 minutes.
3. Run the plate on a FLEXMAP 3D™ or INTELLIFLEX™ instrument.

Analyze the results

Export the CSV file containing the bead counts from each well.

2 Methods

Procedural guidelines

- Thoroughly read this user guide.
- For FLEXMAP 3D™ and INTELLIFLEX™ instruments, initiate the startup protocol to warm up the lasers for at least 30 minutes.
  - Ensure that the Luminex™ instrument is calibrated to the manufacturer's instructions.
- Do not invert the QuantiGene™ Plex 384-well Flat Bottom Magnetic Separation Plate during the assay or allow contents from one well to mix with another well.
- Use a multichannel pipette and reagent reservoirs whenever possible to achieve optimal assay precision.

Prepare 1X SAPE Wash Buffer

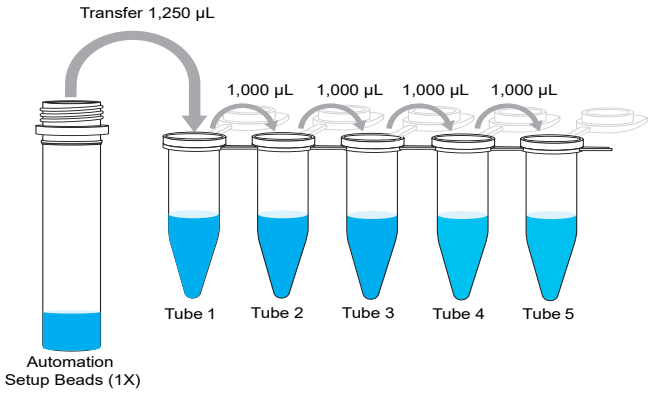
1. Bring the SAPE Wash Buffer Concentrate (10X) to room temperature, then vortex for 15 seconds.
2. Mix 225 mL of the SAPE Wash Buffer Concentrate (10X) with 2,025 mL nuclease-free water. Mix gently to avoid foaming.

1X SAPE Wash Buffer can be stored at 2°C–8°C for up to 6 months.

Prepare serial dilution of the Automation Setup Beads

1. Label 5 tubes: 1, 2, 3, 4, and 5.
2. Vortex the Automation Setup Beads for 30 seconds, then add 1,250 µL to tube 1.
3. Add 250 µL of 1X SAPE Wash Buffer to tubes 2–5.
4. Vortex, then transfer 1,000 µL from tube 1 to tube 2. Vortex again for 30 seconds.
5. Transfer 1,000 µL of the mixed beads from tube 2 to tube 3, then vortex for 30 seconds.

6. Repeat step 5 for tubes 4 and 5.



Define the plate map

Mark the bead dilution, wash buffer, and residual volume wells using the recommended plate layout on page 13 of this manual.

Perform the assay

Add bead dilutions and 1X SAPE Wash Buffer to the plate

1. Add 50 µL of each bead dilution to the plate in triplicate (see "Recommended plate layout" on page 13).
2. Add 50 µL of 1X SAPE Wash Buffer to the remaining wells of the plate.

Wash the plate, then determine residual volume

1. Wash the plate 15 times using the washer settings recommended for your specific magnetic plate washer.

   Note: See "Magnetic plate washer settings" on page 11 for the wash settings of the BioTek™ ELx405TS plate washer with flat magnet. Test and optimize the recommended settings for your magnetic plate washer using the instructions in this protocol. The most critical parameters to optimize for are:
   - Bead loss
   - Residual volume

2. Remove the plate from the automated plate washer and determine the residual volume in 10–15 wells of the plate using a single channel pipette (see "Recommended plate layout" on page 13 for the wells to test for residual volume).
   - Only use wells that contain wash buffer, not the wells that contain beads.
   - Residual volume in the wells should be less than 10 µL.

Add 1X SAPE Wash Buffer, then run the plate

1. Add 50 µL of 1X SAPE Wash Buffer to each well containing beads.
2. Seal the plate with a new plate seal, then incubate at room temperature for 5 minutes on a plate shaker set at 800 rpm.
3. Remove the plate seal and run the plate on a FLEXMAP 3D™ or INTELLIFLEX™ instrument (see "Set up the instrument" on page 9).

Note: Prior to running the instrument, initiate the startup protocol to warm up the lasers for at least 30 minutes.

Set up the instrument

Select bead regions 20, 35, and 55 for the measurement of the QuantiGene™ Plex Automation Setup Kit.

If given the option between calibrating with Low or High RP1 target values, we recommend RP1 Low target value settings for the QuantiGene™ Plex Assay. Use the following parameters to complete protocol definition:

Sample size	DD gate	Timeout	Bead event/bead region
50 µL	5,000 - 25,000	45 seconds	50

Note: Prior to running the assay, ensure that the probe height has been calibrated with the QuantiGene™ Plex 384-well Flat Bottom Magnetic Separation Plate supplied with the kit. For details on how to adjust the probe height, see the FLEXMAP 3D™ or INTELLIFLEX™ user manual.

Analyze the results

In xPONENT, export the CSV file which contains the bead count for every well. The bead counts for the first three bead dilutions should be ≥50 for every well and for every bead region/ID.

Note: The bead dilutions from tubes 4 and 5 may show bead counts below 50.

Troubleshooting

Troubleshooting

Observation	Probable cause	Recommended solution
Residual volume in wells is >10 µL	Incorrect comb height	Lower the comb height of the washer.
All bead wells show bead counts <50	Beads diluted incorrectly	Dilute the beads. The supplied stock serves as the first dilution.
	Comb positioned incorrectly	Adjust the comb height or position.
A few wells of the first three bead dilutions show bead counts <50	Beads not vortexed enough	Vortex beads thoroughly when repeating the experiment.
	Washer not set up correctly	Change the plate washer settings.

B

Magnetic plate washer settings

Magnetic plate washer settings

The following settings are for reference only as a starting point. For plate washers from other manufacturers, adjust accordingly. Always validate your own unit before beginning the experiment.

Example: BioTek™ ELx405TS with flat magnet (Part No. 7103017)

Setting	Description
Wash	• 1. "Move" carrier home, soak 2 minutes • 2. "Wash" 80 µL of buffer for 1 cycle • Travel rate = 1 CW • Volume = 80 µL/well • Buffer A
Dispense	• Z-position: 105 (13.34 mm above carrier) • X-position: -15 (0.69 mm left of carrier) • Y-position: 0 (center of the well) • Delay start of vacuum until 80 µL/wells is dispersed.
Aspiration	• Travel rate = 1 CW • Z-position: 30 (3.81 mm above carrier) • X-position: -9 (0.41 mm left of center) • Y-position: 4 (0.30 mm front of center) • 3. “Move” carrier home, soak 15 seconds • 4. "Wash" 80 µL of buffer for 1 cycle • 5. “Move” carrier home, soak 15 seconds • 6. "Wash" 80 µL of buffer for 1 cycle • 7. “Move” carrier home, soak 15 seconds • 8. “Aspirate” (same conditions as the wash) • Travel Rate: 1 CW • Delay 0 msec

Recommended plate layout

Recommended plate layout

x = determine residual volume

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	Tube 1 beads				x																			
B	Tube 2 beads					x																		
C	Tube 3 beads					x																		
D	Tube 4 beads						x			x														
E	Tube 5 beads										x													
F																								
G												x												
H																	x							
I																		x						
J																								
K																			x					
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