User Guide

SMC® Plate Washer Evaluation Kit

Kit for the Quantitative Evaluation of Magnetic Plate Washer Performance

03-0165-00

Introduction

The SMC® Plate Washer Evaluation Reagent uses a fluorescent label to evaluate magnetic plate washer performance. A pre-formed immunocomplex is provided on magnetic microparticles (beads). The complex is diluted and transferred to a 96-well plate then washed in an automated plate washer. Elution buffer is then added and incubated. The elution buffer dissociates the labeled protein from the beads and is then transferred to an SMC® 384-well Read Plate for analysis. The plate is loaded into the SMCxPRO® System where the labeled molecules are detected and counted. The number of fluorescent molecules counted is used to evaluate performance and precision of a magnetic plate washer.

Supplies

The SMC® Plate Washer Evaluation Reagent includes all reagents listed below. Additional reagents and supplies are required to run this immunoassay, as listed in the next section, Additional Supplies Required (Not provided).

This kit and all reagents supplied are for research use only.

Reagents Included with the Kit

All items are shipped with a cold pack unless otherwise stated.

Description	Storage Conditions	Packaging Details	Component Number
Plate Washer Evaluation Diluent	2-8 °C	5 x 21 mL	02-9991-00
Plate Washer Evaluation Reagent	2-8 °C	5 x 1 mL	02-2165-00
10X Wash Buffer	2-8 °C	1 x 1000 mL	02-0111-03
Buffer D	2-8 °C	1 x 100 mL	02-0368-00
Elution Buffer B	2-8 °C	1 x 100 mL	02-0297-00
SMC® Commercial Plate	2-8 °C	5 plates	02-1PCP-00

Kit Storage

The SMC® Plate Washer Evaluation Kit should be stored at 2-8 °C.

Supplied 10X Wash Buffer contains a preservative. After dilution, the 1X Wash Buffer may be filter sterilized with Stericup® Filter, for storage of up to 1 month at 2-8 °C.

Proper kit performance can only be guaranteed if the materials are stored properly.

Additional Supplies Required (Not provided)

Catalogue numbers are provided to purchase products at SigmaAldrich.com or through sales quote, unless otherwise noted.

Instrumentation Equipment

- SMCxPRO® Ultrasensitive Immunoassay System for sample acquisition (95-0100-00)
- Orbital microplate shaker for assay plate incubation (for example, Boekel Scientific Jitterbug™ Shaker)
- Bio-Tek® 405 TSUVS Microplate Washer for assay plate washing (95-0004-05)
- Sphere Mag Plate for performing microparticle capture (90-0003-02)
- Rotisserie tube rotator for microparticle suspension
- Benchtop centrifuge with bucket rotors capable of reaching 1,100 x *g* for sample/plate centrifugation
- Single channel manual pipettes to accurately dispense 10-20 µL and 20-250 µL
- 12-channel manual pipettes to accurately dispense 10-20 µL and 20-250 µL
- Plate roller for complete plate sealing (Fisher Scientific, NC9185793)

Supplies

- 1 L Container with cap for Wash Buffer dilution
- Stericup® Quick Release Vacuum Filtration System, 0.22 µm, 1 L; for filter sterilizing 1X Wash Buffer (S2GPU11RE)
- 96-well V-bottom plate for assay setup (AXYP96450VCS)
- Universal plate cover to minimize plate well contamination (Fisher Scientific, 253623)
- VistaLab® 25 mL Reservoirs for addition of reagents (Fisher Scientific, 21-381-27C)
- Nunc™ Aluminum adhesive plate seals (Fisher Scientific, 276014)
- Container for Wash Buffer dilution

Reagents

- 10X Wash Buffer for automated assay plate washing, 1 L (02-0111-03)
- De-ionized or distilled water for dilution of 10X Wash Buffer

Assay Best Practices

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. In addition, proper training as well as instrument maintenance is critical for obtaining optimal results in performing SMC® assays. The following notes should be reviewed and understood before the assay is set up.

- Wipe down bench and pipettes with 70% isopropanol before use.
- It is important to allow all reagents to warm to room temperature (RT), 20-25 °C.
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.
- All washing must be performed with the Wash Buffer provided.
- An orbital microplate shaker for assay plate incubation (example, Boekel Scientific Jitterbug™ Shaker settings #5-7) provide maximal orbital mixing without splashing liquid or causing cross-contamination.
    - Jitterbug™ Shaker setting #5 ~ 1000 rpm
    - Jitterbug™ Shaker setting #7 ~ 1500 rpm

  Note: If using different orbital shaker, refer to recommended rpm ranges provided for each incubation step, and adjust speeds as necessary to ensure maximal orbital mixing without splashing liquid or causing cross-contamination.
- As the SMC® assay is extremely sensitive to dust particles, do not perform the assay or plate washing under direct airflow.
- Plate must also be protected from light after adding detection.
- After the assay is complete, seal the plate before reading immediately or storing temporarily at 2-8 °C. The SMCxPRO® Immunoassay System requires the use of aluminum adhesive plate seal.
- It is not recommended to store eluted products from SMC® assays overnight at 4 °C or frozen at –80 °C for later reading as performance cannot be guaranteed.
- If SMC® Read Plate has been stored at 4 °C, plate should be left at RT for 30 minutes to 1 hour on the benchtop before reading to avoid a rapid increase in temperature within SMC® Read Plate wells. Bring to RT then centrifuge the plate at 1,100 x *g* for 1 minute prior to reading.
- For optimal SMCxPRO® Immunoassay System performance, perform ASSIST testing daily (ideally at beginning of the day before assay is prepared).

Precautions

Use caution when handling biological samples. Wear protective clothing and gloves. Components of this reagent kit contain Sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing Sodium azide should be disposed of properly.

Ingredient	Cat. No.	Full Label	
10X Wash Buffer	02-0111-03		**Warning.** Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid release to the environment. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Assay Preparation

Reagent Preparation

1. Warm all reagents to RT prior to use.
2. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
   - Pour 100 mL of 10X Wash Buffer into a container capable of holding at least 1000 mL. Add 900 mL of deionized water.
   - Mix thoroughly by gentle inversion or with a clean, sterile stir bar.

   Note: 1X Wash Buffer may be filter sterilized (refer to Storage Instructions).
3. Mix Plate Washer Reagent Beads on a rotisserie spin rotator, or manually by repeat inversion, for ≥ 20 minutes until all beads are resuspended.

Assay Procedure

Plate Washer Reagent

1. Following initial mixing of the beads, add the entire vial, 1 mL of coated Beads to 21 mL of supplied Plate Washer Evaluation Diluent. Add the Coated Beads directly into the amber bottle of diluent. Mix diluted beads by gentle inversion for at least 30 more minutes to ensure adequate resuspension. There should be a total volume of 22 mL of diluted Coated Beads.
2. Pipette 200 µL per well of the Coated Beads into assay plate.
3. Seal assay plate with clear adhesive plate seal, apply pressure to seal to prevent leaking and cross-contamination.
4. No incubation is required in this protocol. Before initial wash step, centrifuge sealed plate at 1,100 x *g* for 1 minute and carefully remove clear adhesive plate seal to avoid splashing.
5. Perform all washes as would normally be required in an SMC® Immunoassay.

Post-Capture Wash

Wash plate once with a plate washer (Bio-Tek® 405 TSUVS; Post Capture Wash (POSTCAP)). If using automation, please contact your technical service representative for the appropriate automation procedure.

Post-Detection Wash

Wash the assay plate 4 times with wash buffer using the 4 cycle Pre-Transfer (4CYCPRE) program on the Bio-Tek® 405 TSUVS washer. If using automation, please contact your technical service representative for the appropriate automation procedure.

Final Aspiration

Perform Final Aspiration using Bio-Tek® 405 TSUVS; Final Aspirate (FINASP). If using automation, please contact your technical service representative for the appropriate automation procedure.

Elution

1. Dispense 10 µL Elution Buffer B per well using reverse pipetting without disturbing the bead pellet (It is recommended to change tips).
2. Seal assay plate with a clear adhesive plate seal.
3. Incubate plate for 10 minutes at 25 °C on microplate incubator/shaker (Jitterbug™ Shaker setting #5). Ensure plate is protected from light during this incubation.

Assay Reading

To read on the SMCxPRO® Immunoassay System

1. Secure the plate holder to the bottom of the SMC® Read Plate.
2. Add 10 µL per well Buffer D using reverse pipetting to the SMC® Read plate, using a 12-channel manual pipette (1-20 µL).
3. Place assay plate with Elution Buffer B onto sphere mag plate and allow beads to form a tight pellet for 2 minutes.
4. While keeping the assay plate containing eluate on sphere mag plate, gently remove clear adhesive seal and transfer 10 µL of eluate to the SMC® Read Plate containing Buffer D by aspirating directly from the V-bottom of the plate, avoiding the pelleted beads, and changing tips with each dispensed row.
5. Place SMC® Read Plate on plate holder and either cover with plate lid or seal with clear adhesive plate seal.
6. Place the plate into microplate incubator/shaker and shake for 1 minutes at 25 °C (Jitterbug™ Shaker setting #7), centrifuge plate for 1 minute at RT.

   Alternative to shaking option: If operator elects not to shake the plate at the neutralization step, the plate may be stored at room temperature, sealed and light protected, for a minimum of 30 minutes to allow the neutralization process to reach equilibrium by simple diffusion.
7. Seal SMC® Read Plate with aluminum adhesive plate seal.
8. Remove the plate holder from the sealed SMC® Read Plate and load sealed SMC® Read Plate it onto the SMCxPRO® Immunoassay System. Start read.
9. Set up XPT file to read wells as unknowns in columns.
10. Analyze precision of Response Events signal by columns, rows, and entire plate.

    Note: There is a warm-up period of up to 30 minutes to equilibrate plate temperature to internal instrument temperature. Once achieved the read will start automatically.

SMC® Assay Overview

1. Prepare all reagents as instructed.
2. Add 200 µL of diluted Plate Washer Evaluation Reagent Beads to assay plate.
3. Seal and centrifuge assay plate at 1,100 x *g* for 1 minute.
4. Perform Post-Capture Wash.
5. Perform Post-Detection Wash.
6. Perform Final Aspiration.
7. Remove from washer magnet and add 10 µL of Elution Buffer B to each well of assay plate.
8. Seal and incubate for 10 minutes at 25 °C on microplate incubator/shaker.

10 minutes at 25 °C

9. Add 10 µL of Buffer D per well to the SMC® Read Plate.
10. Place the assay plate on sphere mag plate for 2 minutes.
11. Transfer 10 µL of eluted product from assay plate to SMC® Read Plate.
12. Shake SMC® Read Plate on microplate incubator/shaker for 1 minute; cover and centrifuge at 1,100 x *g* for 1 minute.
13. Seal SMC® Read Plate aluminum adhesive plate seal for SMCxPRO® Immunoassay System.
14. Load on SMCxPRO® Immunoassay System.

Interpretation of Plate Washer Evaluation Reagent Data

Typical signal range is 50-250 RE for SMCxPRO® Immunoassay System.

Overall plate signal typically has less than 20 %CV.

- Examine by row and column for %CV
- A row that deviates in signal from the rest of the rows can be caused by a clogged aspiration or dispense pin

In rare cases, high inconsistency between columns can be caused by an x-y misalignment, but this would usually be visually identifiable with bead loss during the washing steps.

Excel conditional formatting by color gradient can be particularly useful in identifying wells that are contributing to %CV issues.

Any high or low outlier wells can have their corresponding pins on the Bio-Tek® washer cleaned.

Troubleshooting

Problem	Probable Cause	Solution
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using seal appropriately. Pipette with multichannel pipets without touching reagent in plate. Change tips when adding reagents if cross contamination is expected.
		Ensure reagents (including Wash Buffer) are not contaminated.
		Insufficient washes—washer may need to be cleaned or reprogrammed.
Sample variability is high	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm that there is no residual left in the wells following post-capture wash step and Final Aspirate. Ensure that you have < 2 µL or residual remaining in the well.
	Plate washer pins are clogged	Ream pins of washer head
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where beads are in constant motion without causing splashing (See Jitterbug™ Shaker settings in Assay Best Practices).
	Cross-well contamination	Ensure that the plate is sealed well at each incubation step. If splashing occurs on plate seal, centrifuge plate at 1,100 x *g* for 1 minute to remove material prior to removing the seal. A new plate seal should be used every time the plate is sealed.
		Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.

Problem	Probable Cause	Solution
Beads are lost during the wash.	Plate washer needs optimization/cleaning	Contact Tech Support or local Specialist to schedule washer programming. Refer to user guide for cleaning procedure.
	Insufficiently primed washer	Washer should be primed with wash buffer prior to running the post capture wash protocol.
	Beads came in contact with water	Washer should be primed with Wash Buffer sufficiently prior to plate wash. Viscosity of water changes the performance of the magnetic particles.
	Proper magnet was not used	Ensure that the SMC® magnetic plate shipped with the Bio-Tek® 405 TSUVS Plate Washer was present on plate wash stage prior to running wash protocol.
Microparticles do not resuspend into homogenous solution	Beads were not properly stored and may have been frozen	Labelled microparticles should be stored at 4 °C. If microparticles are frozen, they will not resuspend properly.

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