SMC™ Plate Washer Evaluation Kit

Microparticle Assay

Catalog # 03-0165-00

Kit for the Quantitative Evaluation of Magnetic Plate Washer Performance

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

Manufactured & Distributed by:

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INTRODUCTION

The Single Molecule Counting (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 transfered to a microplate for analysis. The plate is loaded into the Erenna® or 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.

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SUPPLIES

The SMC™ Plate Washer Evaluation Reagent includes all reagents listed in *Table 1: Reagents Provided*. Additional reagents and supplies are required to run are listed in *Table 2: Additional Supplies Required (not provided)*. All reagents supplied are for Research Use Only.

Table 1: Reagents Provided

Item #	Description	Shipping Conditions	Storage Conditions	Component Part No.	Packaging Details
1	Plate Washer Evaluation Diluent	With cold pack	2 - 8°C	02-9991-00	5 x 21 mL
2	Plate Washer Evaluation Reagent	With cold pack	2 - 8°C	02-2165-00	5 x 1 mL
3	10X Wash Buffer	With cold pack	2 - 8°C	02-0111-03	1 x 1000 mL
4	Buffer D	With cold pack	2 - 8°C	02-0368-00	1 x 100 mL
5	Elution Buffer B	With cold pack	2 - 8°C	02-0297-00	1 x 100 mL

Storage Instructions

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

Supplied 10X Wash Buffer contains preservative. After dilution, the 1X Wash Buffer may be filter sterilized with Stericup® filter, EMD Millipore PN SCGPU11RE for storage of up to 1 month at 2 - 8°C.

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

Table 2: Additional Supplies Required (not provided)

Instrumentation

Item #	Product Description	Supplier	Product Number	Product Uses
1	12-Channel Manual Pipette 10 – 20 µL	--	--	Transferring 10 µL
2	12-Channel Manual Pipette 20 – 250 µL	--	--	Transferring 20 µL, 100 µL
3	Tube Rotator	--	--	Microparticle resuspension
4	Sphere Mag Plate	EMD Millipore	90-0003-02	Capturing/pelleting microparticles
5	Jitterbug™ Microplate Incubator/Shaker	EMD Millipore	70-0009-00	Incubating/Shaking at 25°C
6	VWR® Microplate Shaker	VWR International	12620-926	Plate shaking for overnight incubation, if recommended
7	Bio-Tek ELx™ 405 Microplate Washer	EMD Millipore	95-0004-05	Automated plate washing option
8	Tecan Hydroflex™ Microplate Washer	EMD Millipore	95-0005-02	Automated plate washing option
9	Centrifuge able to reach speed of 1,100 x g	--	--	Centrifuging samples, plates
10	ALPS™ 50V Microplate Heat Sealer	EMD Millipore	70-0018-00	Heat sealing 384-well plates before Erenna Reading

Additional Supplies Required (not provided) continued

Materials

Item #	Product Description	Supplier	Product Number	Product Uses
11	VistaLab™ 25 mL Reservoirs	Fisher Scientific	21-381-27C	Addition of Reagents
12	96-well V-bottom plate	Fisher Scientific	14-222-241	Assay plate
13	Nunc™ Clear Adhesive Plate Seal	Fisher Scientific	236366	Sealing assay plate
14	384-well round bottom plates	Fisher Scientific	12-565-384	Erenna® reading plate
15	Heat sealing foil	Fisher Scientific	NC0276513	Sealing plates for Erenna® reading
16	1L Stericup® Filter; 0.22 µm	EMD Millipore	SCGPU11RE	Filter sterilizing Erenna® system buffer
17	SMCxPRO™ 384-well plate, 1 plate with adhesive seal	EMD Millipore	02-1008-00	SMCxPRO™ reading plate, seal
18	SMCxPRO™ 384-well plate, case of 32	Edition Eight, LLC	ABB2-00160A	SMCxPRO™ reading plate
19	SMCxPRO™ aluminum adhesive plate seals	Fisher Scientific	276014	SMCxPRO™ reading plate seals
20	Universal plate cover	Fisher Scientific	253623	Covers assay plate
21	1**0**00 mL Container	--	--	Wash Buffer Dilution

Additional Supplies Required (not provided) continued

Reagents

Item #	Product Description	Supplier	Product Number	Product Uses
22	Elution Buffer (5 mL)	EMD Millipore	02-0002-04	Required for Erenna® maintenance
23	SMC™ 10X Wash Buffer (1 L)	EMD Millipore	02-0111-00	Automated plate washing
24	A**dditional** SMC™ 10X System/wash Buffer with Proclin (1 L)	EMD Millipore	02-0111-03	Use in Erenna® platform
25	De-ionized or Distilled water	--	--	Dilution of 10X Wash or System Buffer

Please contact your technical services representative for additional information or assistance selecting required but not provided supplies.

TECHNICAL HINTS

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. The following notes should be reviewed and understood before the assay is set up.

Assay Hints

1. Wipe down bench and pipettes with 70% isopropanol before use.
2. It is important to allow all reagents to warm to room temperature (20 - 25°C).
3. Use sterile filter pipette tips and reagent trays to avoid contamination.
4. Pre-wet tips (aspirate and dispense within well) twice before each transfer.
5. All washing must be performed with the wash buffer provided.
6. The recommended plate shaker settings are between #3 - #7 to provide maximal orbital mixing without splashing liquid or causing cross-contamination.
7. After the assay is complete, the plate should be read immediately.
    a. For Erenna® Immunoassay System, use heat sealing plate foil.
    b. For SMCxPRO™ Immunoassay System – use adhesive foil seal.
8. The plates may be stored at 2-8°C for up to 48 hours away from light if same day reading is not possible.
    a. Following transfer of the eluate, seal the plate before storing at 2 - 8°C
        i. For Erenna® Immunoassay System, use heat sealing plate foil
        ii. For SMCxPRO™ Immunoassay System – use aluminum adhesive plate seal
    b. Bring to RT then centrifuge the plate at 1,100 *x g* for 1 minute prior to reading.

Instrument Hints

9. For optimal Erenna® performance, execute the following prime of the instrument before reading:
    a. Cycle routine (10,000 µL at 1,000 µL/min)
    b. Bubble test (200 µL at 1,000 µL/min)
    c. Complete Erenna® calibration prior to reading the plate.

Note: If carry-over is experienced: perform a clean routine using a 384-well plate and 20 µL/well:

i. 3 wells of elution buffer
ii. 1 well of 10% bleach
iii. 5 wells of elution buffer

10. For optimal SMCxPRO™ performance, perform ASSIST testing on a daily basis (ideally at beginning of the day before assay is prepared).

PRECAUTIONS

- Use caution when handling biological samples. Wear protective clothing and gloves.
- Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Full Hazard Label:

Ingredient, Cat #		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 room temperature (RT) prior to use.
2. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
    a. Pour 100 mL of 10X Wash Buffer into a container capable of holding at least 1000 mL. Add 900 mL of deionized water.
    b. 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 a SMC™ Immunoassay

Post-Capture Wash

Wash plate once with an automated plate washer.

Plate Washer

a. BioTek; Post Capture Wash (POSTCAP) or
b. HydroFlex; Post Capture Wash (PCW)

If using automation please contact your technical service representative for the appropriate automation procedure.

Post-Detection Wash

Wash assay plate 4 times with a plate washer.

Plate Washer

a. BioTek; 4 cycle Pre-Transfer (4CYCPRE) or
b. HydroFlex; 4 cycle Pre-Transfer (*4cyPrTra)*

If using automation please contact your technical service representative for the appropriate automation procedure.

Final Aspiration

Plate Washer

a. BioTek; Final Aspirate (FINASP)
b. HydroFlex; Final Aspirate (FA_V1)

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 setting #5).

ASSAY READING

To read on the Erenna® Immunoassay System

1. Add 10 µL per well of Buffer D using reverse pipetting to Erenna® reading plate (Fisher Scientific PN 12-565-384) using a 12-channel manual P20.
2. Place assay plate onto sphere mag plate and allow beads to form a tight pellet for ≥ 2 minutes.
3. While keeping assay plate on the sphere mag plate, gently remove clear adhesive plate seal and transfer 10 µL of eluate from assay plate to reading plate by aspirating directly from the v-bottom of the plate, avoiding the pelleted beads, and changing tips with each dispensed row.
4. Seal reading plate with clear adhesive plate seal. Centrifuge plate for 1 minute at RT, approximately 1,100 *x g*.
5. Seal reading plate with heat sealing foil (Fisher Scientific PN NC0276513) according to manufacturer's instructions for the heat sealer.
6. Load completed reading plate onto the Erenna® Immunoassay System.
7. Set up SGXLink run table to read wells as unknowns in columns.
8. Analyze precision of Detected Events signal by columns, rows, and entire plate.

To read on the SMCxPRO™ Immunoassay System:

1. Secure the plate holder to the bottom of the SMCxPRO™ reading plate (EMD Millipore PN 02-1008-00).
2. Add 10 µL per well of Buffer D using reverse pipetting to the reading plate using a 12-channel manual P20.
3. Place assay plate onto sphere mag plate and allow beads to form a tight pellet for 2 minutes.
4. While keeping assay plate on the sphere mag plate, gently remove clear adhesive plate seal and transfer 10 µL of eluate from assay plate to reading plate by aspirating directly from the v-bottom of the plate, avoiding the pelleted beads, and changing tips with each dispensed row.
5. Place reading plate on plate holder and either cover with plate lid or seal with clear adhesive plate seal.
6. Place reading plate (on plate holder) into Jitterbug and shake for 1 minute at 25°C (Jitterbug setting #7), centrifuge plate for 1 minute at RT, approximately 1,100 *x g*.

   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 reading plate with SMCxPRO™ aluminum adhesive plate seal.
8. Remove the plate holder and load the sealed reading plate onto the SMCxPRO™ Immunoassay System.
9. Set up XPT file to read wells as unknowns in columns.
10. Start Read.
11. Analyze precision of Response Events signal by columns, rows, and entire plate.

Note: there is a smart warm up period of up to 30 minutes to wait for the read plate to be close to the internal instrument temperature. Once achieved the read will start automatically.

APPENDIX A: SMC™ Quick Assay Guide

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
8. Seal and incubate for 10 minutes at 25°C on microplate incubator/shaker.

10 minutes 25°C

9. Add 10 µL of **Buffer D** per well to the **reading plate.**
10. Place the **assay plate** on sphere mag plate for 2 minutes
11. Transfer 10 µL of eluted product from **assay plate** to **reading plate**.
12. Shake **reading plate** on Jitterbug at setting #7 for 1 minute; cover and centrifuge at 1,100 *x g* for 1 minute.
13. Seal **reading plate** with pierceable foil for Erenna® or aluminum adhesive plate seal for SMCxPRO™.

LOAD ON ERENNA® or SMCxPRO™ SYSTEM

Interpretation of Plate Washer Evaluation Reagent Data

1) Typical signal range is 50-250 RE for SMCxPro™ and 1,000 – 3,500 DE for Erenna®. (Signal is dependent on washer and reader).
2) Overall plate signal typically has less than 20 %CV
    a. Examine by row and column for %CV
    b. A row that deviates in signal from the rest of the rows can be caused by a clogged aspiration or dispense pin
3) For Hydroflex or Biotek
    a. For Hydroflex, a single column that deviates from other columns is typically an issue with a multichannel pipette not performing as expected.
    b. 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
    c. Excel conditional formatting by color gradient can be particularly useful in identifying wells that are contributing to %CV issues.
    d. Any high or low outlier wells can have their corresponding pins on the Biotek or Hydroflex washer cleaned.

TROUBLESHOOTING GUIDE

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 and system buffers) are not contaminated.
		Insufficient washes—washer may need to be cleaned or reprogrammed.
	Instrument needs cleaning	See Technical Hints for appropriate Erenna® cleaning protocol.
Signal 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 a vertical plate shaker at a speed where beads are in constant motion without causing splashing (~650 - 1000 RPM).
	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.
Beads are lost during the wash	Plate washer needs optimization/cleaning	Contact Tech Support or local BCS 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 mag plate (EMD Millipore PN 90-0003-02) 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.

ORDERING INFORMATION

To place an order or to obtain additional information about SMC™ products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds

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