SMC™ Assay Buffer Optimization Kit Instructions

Assay Development Kit

Catalog # 03-0122-00

SMC™ Kit for the optimization of assay buffers for

SMC™ Bead-Based Immunoassays

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

Manufactured & Distributed by:

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INTRODUCTION

The Erenna® Bead-Based SMC™ Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure analyte in a matrix. The Erenna® Assay Buffer Optimization Kit allows the operator to optimize their immunoassays with appropriate salt and detergent concentrations. The optimization of salt and detergent concentrations will minimize background, reduce non-specific binding, and improve immunoassay stringency. Immunoassay buffer optimization can also improve results for assay validation.

The buffers in this kit consist of a stock concentration of a base assay buffer (Optimization Assay Buffer A), a high salt assay buffer (Optimization Assay Buffer B) and a high detergent assay buffer (Optimization Assay Buffer C). Optimization Assay Buffers A, B, and C contain a proprietary mixture of blockers to decrease non-specific antibody binding. By following the instructions in this package insert, the operator can create enough optimization screening buffers to conduct approximately 60 optimization experiments. Extra buffer can be utilized to run immunoassays with the chosen assay buffer, or that buffer mixture can be purchased separately from EMD Millipore Corporation

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REAGENTS

The SMC™ Assay Buffer Optimization Kit includes all reagents listed in Reagents Provided. Additional reagents and supplies may be required to run this immunoassay, as listed in the section titled General Supplies Required But Not Provided. All reagents supplied are for Research Use Only.

Reagents Provided

Item #	Description	Shipping Conditions	Storage Conditions	Component Part No.	Packaging Details
1	Optimization Assay Buffer A	With cold pack	2-8°C	02-0826-00	1 x 500 mL
2	Optimization Assay Buffer B	With cold pack	2-8°C	02-0828-00	1 x 500 mL
3	Optimization Assay Buffer C	With cold pack	2-8°C	02-0830-00	1 x 100 mL

Storage Instructions

The SMC™ Assay Buffer Optimization Kit should be stored at 2–8°C.

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

General Supplies Required But Not Provided

Reagents

1. Erenna® 10X System/Wash Buffer w/ Proclin (1 L bottle) (EMD Millipore PN 02-0111-03)
2. Erenna® 10X Wash Buffer (1 L bottle) (EMD Millipore PN 02-0111-00) if using an automated plate washer
3. Elution Buffer (EMD Millipore PN 02-0002-04) for maintenance
4. De-ionized or distilled water

Washing Options

Automated

a. Bio-Tek ELx405™ Microplate Washer (EMD Millipore PN 95-0004-05) *or*
b. Tecan HydroFlex™ microplate washer (EMD Millipore PN 95-0005-02)

Manual

a. Sphere Mag Plate SBS Footprint (EMD Millipore PN 90-0003-02) *or*
b. DynaMag™-96 Side Skirted Magnet (Thermo Fisher PN 12027)

Instrumentation / Materials

1. Jitterbug™ Microplate incubator / shaker (EMD Millipore PN 70-0009-00 or equivalent)
2. ALPS™ 50V microplate heat sealer (Thermo Fisher PN AB1443A or equivalent)
3. Centrifuge with plate rotor capable of reaching a speed of 1,100 xg
4. 12-channel pipettes capable of transferring 20 µL - 250 µL
5. 8- or 12-channel pipette capable of transferring 15 µL
6. Rotisserie rotator
7. Microcentrifuge
8. MultiScreen$_{HTS}$ BV 96-Well Filter Plate (EMD Millipore PN MSBVN1210 or equivalent)
9. 96-well V-bottom polypropylene plate, 500 µL (Axygen PN P-96-450V-C)
10. 384-well round bottom polypropylene plate, 120 µL (Thermo Fisher PN 264573)
11. 0.2 µm syringe filter (EMD Millipore PN SLGPR33RS or equivalent)
12. Universal plate cover (Thermo Fisher PN 253623 or equivalent)
13. Sealing tape (Thermo Fisher PN 236366 or equivalent)
14. Heat sealing plate foil (EMD Millipore PN 02-01-0216-00 or equivalent)
15. 12-channel reagent reservoirs for preparing standards
16. 5 mL syringe
17. Microcentrifuge tubes
18. Container capable of holding 300 mL
19. 500 mL graduated cylinder

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

WHEN TO USE THIS KIT

This kit should be used in conjunction with other SMC™ Assay Development and Labeling Kits:

Catalog #	Kit Name
03-0077-02	Erenna® SMC™ Capture Reagent Labeling Kit
03-0076-02	Erenna® SMC™ Detection Reagent Labeling Kit
03-0078-00	Erenna® SMC™ Bead Based Immunoassay Development Kit

Complementary Assay Development Kits

It is recommended to optimize the assay buffer **after** the best conditions for capture and detection antibody concentrations have been determined in the initial immunoassay development experiment. The instructions for initial assay development are included in the Erenna® SMC™ Bead Based Immunoassay Development Kit (03-0078-02).

Note: this protocol requires the use of Standard Diluent, 10X Wash Buffer, Elution Buffer B and Buffer D.

TECHNICAL HINTS DUE TO HIGH SENSITIVITY

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.

- Wipe down bench and pipettes with 70% isopropanol before use. It is important to allow all reagents to warm to room temperature (20 - 25°C).
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Use filter tips while transferring standard.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.
- The standards prepared by serial dilution must be used within 10 minutes of preparation. It is recommended that the standards are prepared as the last step prior to plate setup.
- The detection antibody is light sensitive and must be protected from light at all times.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the wash buffer provided.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- The plates should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate with the pierceable foil seal and store the plate at 2-8°C for up to 48 hrs. Bring to room temperature then centrifuge the plate at 1,100 x g for 5 minutes prior to reading on the Erenna.
- The plate shaker should be set at a speed to provide maximum orbital mixing without splashing liquid on the sealer or outside the wells. For the recommended plate shaker, this would be a setting of 3 - 5.
- For optimal instrument performance, complete a cycle routine (10,000 µL at 1,000 µL/min) followed by a bubble test, and an instrument calibration prior to reading the plate.
- If a clean routine is required, run using three wells of elution buffer (EMD Millipore PN 02-0002-04), one well of 10% bleach and five wells of elution buffer (EMD Millipore PN 02-0002-04). (Note: This elution buffer is not provided and should be ordered separately.)

PRECAUTIONS

- Use caution when handling biological samples. Wear protective clothing and gloves.
- Components of this reagent kit contain approximately 0.1% 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.

ASSAY BUFFER OPTIMIZATION SCREENING

Preparation of Assay Buffer Optimization Screen

1. Warm all reagents to room temperature prior to use.
2. Label 16 centrifuge tubes 1-16.
3. Create a matrix of 16 buffers by following the below table. The final volume in each tube will be 10 mL.

Buffer #	Assay Buffer A (mL)	Assay Buffer B (mL)	Assay Buffer C (mL)
1	8.3	1.5	0.2
2	8.0	1.5	0.5
3	7.5	1.5	1.0
4	6.5	1.5	2.0
5	5.3	4.5	0.2
6	5.0	4.5	0.5
7	4.5	4.5	1.0
8	3.5	4.5	2.0

Buffer #	Assay Buffer A (mL)	Assay Buffer B (mL)	Assay Buffer C (mL)
9	3.8	6.0	0.2
10	3.5	6.0	0.5
11	3.0	6.0	1.0
12	2.0	6.0	2.0
13	2.8	7.0	0.2
14	2.5	7.0	0.5
15	2.0	7.0	1.0
16	1.0	7.0	2.0

Note: Buffer #1 is equivalent to Assay/Discovery Buffer (02-0474-00).

4. If larger volumes are desired, scale up appropriately.
5. Store the buffers tightly capped at 2-4°C, or continue to screen.

ASSAY BUFFER OPTIMIZATION SCREENING (continued)

Optimization Screen (Bead Based Assay)

1. Transfer the 16 buffers to a 96 deep well block as per the suggested plate map. **The 1 mL aliquot will be used for bead dilution; the 500 µL aliquot will be used for detection antibody dilution.**

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Buffer 1:**	1 mL		**Buffer 5:**	1 mL		**Buffer 9:**	1 mL		**Buffer 13:**	1 mL	
B		500 µL			500 µL			500 µL			500 µL	
C	**Buffer 2:**	1 mL		**Buffer 6:**	1 mL		**Buffer 10:**	1 mL		**Buffer 14:**	1 mL	
D		500 µL			500 µL			500 µL			500 µL	
E	**Buffer 3:**	1 mL		**Buffer 7:**	1 mL		**Buffer 11:**	1 mL		**Buffer 15:**	1 mL	
F		500 µL			500 µL			500 µL			500µL	
G	**Buffer 4:**	1 mL		**Buffer 8:**	1 mL		**Buffer 12:**	1 mL		**Buffer 16:**	1 mL	
H		500 µL			500 µL			500 µL			500 µL	

2. Prepare 6 mL of the standard analyte to desired concentration in Standard Diluent (provided in the Immunoassay Development kit). Based on the initial immunoassay development experiment, choose a standard analyte concentration that will yield approximately 3000 DE in prototyping standard curves.
3. Add bulk coated beads to each 1 mL aliquot of optimization buffer, based on the best condition(s) chosen from the initial immunoassay development. E.g., for a 5 µg/well assay, add 5 µL of beads to 995 µL optimization buffer. For a 10 µg/well assay, add 10 µL of beads to 990 µL optimization buffer. Mix each 1 mL mixture by pipette. (A 250 µL multichannel pipette can be used for this step).
4. Prepare a 96-well polypropylene plate **(Plate 1).** Add 100 µL of coated beads prepared in each optimization buffer to six wells, as per the following plate map.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Buffer 1 100 µL coated beads/well**			**Buffer 5 100 µL coated beads/well**			**Buffer 9 100 µL coated beads/well**			**Buffer 13 100 µL coated beads/well**		
B												
C	**Buffer 2 100 µL coated beads/well**			**Buffer 6 100 µL coated beads/well**			**Buffer 10 100 µL coated beads/well**			**Buffer 14 100 µL coated beads/well**		
D												
E	**Buffer 3 100 µL coated beads/well**			**Buffer 7 100 µL coated beads/well**			**Buffer 11 100 µL coated beads/well**			**Buffer 15 100 µL coated beads/well**		
F												
G	**Buffer 4 100 µL coated beads/well**			**Buffer 8 100 µL coated beads/well**			**Buffer 12 100 µL coated beads/well**			**Buffer 16 100 µL coated beads/well**		
H												

ASSAY BUFFER OPTIMIZATION SCREENING (continued)

5. Add 100 µL of analyte to Rows **A, C, E, and G** as per the following plate map.
6. Add 100 µL of Standard Diluent to **Rows B, D, F,** and **H** as per the following plate map.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Buffer 1 + Analyte			Buffer 5 + Analyte			Buffer 9 + Analyte			Buffer 13 + Analyte		
B	Buffer 1 + Standard Dil.			Buffer 5 + Standard Dil.			Buffer 9 + Standard Dil.			Buffer 13 + Standard Dil.		
C	Buffer 2 + Analyte			Buffer 6 + Analyte			Buffer 10 + Analyte			Buffer 14 + Analyte		
D	Buffer 2 + Standard Dil.			Buffer 6 + Standard Dil.			Buffer 10 + Standard Dil.			Buffer 14 + Standard Dil.		
E	Buffer 3 + Analyte			Buffer 7 + Analyte			Buffer 11 + Analyte			Buffer 15 + Analyte		
F	Buffer 3 + Standard Dil.			Buffer 7 + Standard Dil.			Buffer 11 + Standard Dil.			Buffer 15 + Standard Dil.		
G	Buffer 4 + Analyte			Buffer 8 + Analyte			Buffer 12 + Analyte			Buffer 16 + Analyte		
H	Buffer 4 + Standard Dil.			Buffer 8 + Standard Dil.			Buffer 12 + Standard Dil.			Buffer 16 + Standard Dil.		

7. Cover the plate with an adhesive seal and continue with the immunoassay protocol.
    a. Incubate analyte for 2 hours with shaking (Jitterbug, setting #5).
    b. Perform Post-Capture Wash.
8. Approximately 10 minutes prior to the end of the analyte incubation, prepare the Detection Antibody. Based on the initial immunoassay development conditions, prepare 1 mL of a 20X intermediate dilution (if feasible based on stock concentration) in **Buffer 1.** Filter the intermediate diluted detection antibody using the syringe with a 0.2 µm filter into a clean tube.
9. Dilute the intermediate detection antibody stock 20-fold by adding 25 µL of the intermediate stock into the 475 µL aliquot of each assay buffer (refer to Table 4 for Detection Antibody preparation layout).

ASSAY BUFFER OPTIMIZATION SCREENING (continued)

10. Add 20 µL of diluted detection antibody per well, as per the following plate map.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Buffer 1 20 µL detection antibody**			**Buffer 5 20 µL detection antibody**			**Buffer 9 20 µL detection antibody**			**Buffer 13 20 µL detection antibody**		
B												
C	**Buffer 2 20 µL detection antibody**			**Buffer 6 20 µL detection antibody**			**Buffer 10 20 µL detection antibody**			**Buffer 14 20 µL detection antibody**		
D												
E	**Buffer 3 20 µL detection antibody**			**Buffer 7 20 µL detection antibody**			**Buffer 11 20 µL detection antibody**			**Buffer 15 20 µL detection antibody**		
F												
G	**Buffer 4 20 µL detection antibody**			**Buffer 8 20 µL detection antibody**			**Buffer 12 20 µL detection antibody**			**Buffer 16 20 µL detection antibody**		
H												

11. Cover the plate with an adhesive seal and continue with the immunoassay protocol.
    a. Incubate detection antibody for 1 hour with shaking (Jitterbug, setting #5).
    b. Perform Pre-Transfer Post-Detection Wash.
    c. Transfer Beads to a clean 96-well plate **(Plate 2).**
    d. Perform Final Aspiration.
    e. Add 10 µL of Elution Buffer B to each well.
    f. Cover and Incubate for 10 minutes with shaking (Jitterbug, setting #5).
    g. Add 10 µL Buffer D to a 384-well plate **(Plate 3).**
    h. Transfer 10 µL of solution from Plate 2 to Plate 3.
    i. Cover and centrifuge for 5 minutes at 1,100 *x g*.
    j. Cover Plate 3 with pierceable plate seal cover.
12. Load completed Plate 3 onto the Erenna® Immunoassay System.

INTERPRETATION OF RESULTS

Export the plate data to a spreadsheet program. The above protocol will yield DE results consistent with Table 8 below.

Calculate the slope and Signal: Background ratio (Table 9). The analyte concentration used in this experiment was 15 pg/mL. The chosen condition balanced low background, high slope, and good signal: background for the tested conditions.

Increased Detergent (↓) / Increased Salt (→)

Condition	DE mean	Condition	DE mean	Condition	DE mean	Condition	DE mean
1	2540	**5**	1452	**9**	1187	**13**	971
	166		68		49		45
2	2463	**6**	1584	**10**	1232	**14**	1019
	65		56		42		48
3	2600	**7**	1608	**11**	1337	**15**	1089
	77		54		37		40
4	2777	**8**	1649	**12**	1410	**16**	1214
	94		69		91		53

Example Optimization Screening Data (Analyte Conc. 15 pg/mL)

#	slope [pg/mL]/DE	S: B	#	slope [pg/mL]/DE	S: B	#	slope [pg/mL]/DE	S: B	#	slope [pg/mL]/DE	S: B
1	158	15	**5**	92	21	**9**	76	24	**13**	62	21
2	160	38	**6**	102	28	**10**	79	29	**14**	65	21
3	168	34	**7**	104	30	**11**	87	37	**15**	70	28
4	179	30	**8**	105	24	**12**	88	16	**16**	77	23

Example Slope & Signal: Background Data (Analyte Conc. 15 pg/mL)

INTERPRETATION OF RESULTS (continued)

Assay Buffer Optimization Considerations

- **Very low DE counts** (<50 counts) may be less reproducible. Consider precision (%CV) of the 0 pg/mL replicates before choosing an optimized buffer condition.
- **High DE counts** at 0 pg/mL analyte (>300 counts) suggest opportunity for further optimization. Achieving a very low background will also be limited by the choice/ availability of antibodies and the target. Consider fit-for-purpose (i.e., is sensitivity appropriate for the target and sample matrix) before further optimization.
- **Salt and detergent concentrations** may impact biological integrity of critical components when measuring analyte in biological samples. Consider the assay target if choosing very high or very low salt/detergent conditions.

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and pipetting 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.
		Change tips for each dilution of the standard curve.
		Insufficient washes—washer may need to be cleaned or reprogrammed.
	Instrument needs cleaning	See Technical Guidelines for appropriate Erenna® cleaning protocol.
	Plate was over-incubated	Confirm correct incubation times were followed.
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.
	Samples may have high particulate matter or other interfering substances	Samples should be centrifuged or filtered according to the PI and lab SOPs. Unprocessed samples could lead to higher imprecision.
	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. Should splashing occur on the plate sealer pulse spin plate to remove excess 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.

TROUBLESHOOTING GUIDE (continued)

Problem	Probable Cause	Solution
Beads are lost during the wash (continued)	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.
Published LLoQ was not achieved	Improper dilution/reconstitution of the standard reference material	Confirm appropriate kit protocol was followed when preparing standard curve.
		Check plate washer to confirm no beads were lost during washes and that plate contains <2 uL following the post-capture and final aspiration protocols.
		Ensure time from thawing the standard to starting the capture incubation is ≤10 minutes.
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.
	Samples may be causing interference due to excess particulate matter	Samples should be properly processed prior to testing to remove particulate matter/lipids.

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

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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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