SMC™ Human IL-6 High Sensitivity Immunoassay Kit

Microparticle Assay

Catalog # 03-0089-01

Immunoassay kit for the quantitative determination of **Interleukin 6 (IL-6)** in human EDTA plasma

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

Manufactured & Distributed by:

EMD Millipore Corporation
3050 Spruce Street
St. Louis, Missouri 63103
United States of America
emdmillipore.com

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INTRODUCTION

The SMC™ Human IL-6 High Sensitivity Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure IL-6 in human EDTA plasma samples. A capture antibody specific for human IL-6 has been pre-coated onto paramagnetic microparticles (beads). The user pipettes beads, standards, and samples into uncoated microplate wells. During incubation, the IL-6 present in the sample binds to the capture antibody on the coated beads. Unbound molecules are washed away during the wash steps. Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to IL-6 that has been captured onto the beads. Following a stringent wash step to remove unbound detection, the beads are transferred to a clean plate. After a final aspirate, elution buffer is then added and incubated. The elution buffer dissociates the bound protein sandwich from the bead surface releasing the labeled antibodies. These antibodies are separated during transfer to a final microplate. The plate is loaded into the Erenna® or SMCxPRO™ System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of IL-6 present in the sample when captured. The amount of IL-6 in unknown samples is interpolated from a standard curve.

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SUPPLIES

The SMC™ Human IL-6 High Sensitivity Immunoassay Kit includes all reagents listed in *Table 1: Reagents Provided*. Additional reagents and supplies may be required to run this immunoassay, as 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	Assay Buffer	With cold pack	2-8°C	02-0306-00	1 x 15 mL
2	IL-6 Coated Beads	With cold pack	2-8°C	02-0600-00	1 x 500 µL
3	Standard Diluent	With cold pack	2-8°C	02-0015-02	1 x 15 mL
4	IL-6 Detection Antibody	With cold pack	2-8°C	02-0599-00	1 x 20 µL
5	Human IL-6 Standard	On dry ice	≤ -70°C	02-0007-00	1 x 150 µL
6	10X Wash Buffer Note: Contains 0.5% Proclin	With cold pack	2 - 8°C	02-0001-06	1 x 50 mL
7	Buffer D	With cold pack	2 - 8°C	02-0359-00	1 x 3 mL
8	Elution Buffer B	With cold pack	2 - 8°C	02-0211-02	1 x 5 mL

Storage Instructions

- The SMC™ Human IL-6 High Sensitivity Immunoassay Kit should be stored at 2 - 8°C. The Standard analyte should be stored at ≤ -70°C.
- Discard standards after one use.
- 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	Micro-Centrifuge	--	--	Centrifuge samples, provided Detection Antibody
11	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
12	12-Channel Reagent Reservoir (sterile)	Argos/Cole Parmer	04395-33	Standard curve dilution
13	VistaLab™ 25 mL Reservoirs	Fisher Scientific	21-381-27C	Addition of Reagents
14	MultiScreenHTS BV 96-Well Filter Plate	EMD Millipore	MSBVN1210	Sample filtration
15	96-well V-bottom plate	Fisher Scientific	14-222-241	Assay plate
16	5 mL Luer-Lok™ Syringe	Fisher Scientific	14-829-45	Detection Antibody filtration
17	0.2 µm Syringe Filter	EMD Millipore	SLGP033RS	Detection Antibody filtration
18	Nunc™ Clear Adhesive Plate Seal	Fisher Scientific	236366	Sealing assay plate
19	384-well round bottom plates	Fisher Scientific	12-565-384	Erenna® reading plate
20	Heat sealing foil	Fisher Scientific	NC0276513	Sealing plates for Erenna® reading
21	1L Stericup® Filter; 0.22 µm	EMD Millipore	S2GPU11RE	Filter sterilizing Erenna® system buffer
22	SMCxPRO™ 384-well plate, 1 plate with adhesive seal	EMD Millipore	02-1008-00	SMCxPRO™ reading plate, seal
23	SMCxPRO™ 384-well plate, case of 32	Edition Eight, LLC	ABB2-00160A	SMCxPRO™ reading plate
24	SMCxPRO™ aluminum adhesive plate seals	Fisher Scientific	276014	SMCxPRO™ reading plate seals
25	Plate Roller	Fisher Scientific	NC9185793	Creates secure/even seal for each well of SMCxPRO™ reading plate
26	Universal plate cover	Fisher Scientific	253623	Covers assay plate

Additional Supplies Required (not provided) continued

Reagents

Item #	Product Description	Supplier	Product Number	Product Uses
27	500 mL Container	--	--	Wash Buffer Dilution
28	Micro-centrifuge tubes	--	--	Sample storage, standard preparation
29	Elution Buffer (5 mL)	EMD Millipore	02-0002-04	Required for Erenna® maintenance
30	SMC™ 10X Wash Buffer (1 L)	EMD Millipore	02-0111-00	Automated plate washing
31	SMC™ 10X System/wash Buffer with Proclin (1 L)	EMD Millipore	02-0111-03	Use in Erenna® platform
32	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 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 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 heat sealing plate foil (for reading on Erenna® Immunoassay System) or adhesive sealer (for reading on SMCxPRO™ Immunoassay System) 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.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 Erenna® 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.)
- For optimal SMCxPRO™ performance, perform ASSIST testing on a daily basis (ideally at beginning of the day before assay is prepared).

SAMPLE INFORMATION

- The SMC™ Human IL-6 High Sensitivity Immunoassay validation data have been compiled using EDTA plasma.
- Ensure sample is clear of precipitants and other visible particulate matter before testing with the SMC™ Human IL-6 Immunoassay.

PRECAUTIONS

- Use caution when handling biological samples. Wear protective clothing and gloves.
- Proclin-containing solutions and their containers must be disposed of in a safe way and in accordance with local, regional and national regulations.
- The chemical, physical and toxicological properties Proclin 950 at 5% have not been thoroughly investigated. At this concentration, this biocidal preservative is irritating to eyes and skin, and may be detrimental if enough is ingested (quantities above those found in the kit). ProClin 950 is a potential sensitizer by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals. The potential for these adverse health effects is unknown for the highly diluted, small volume of ProClin in this kit, but unlikely if handled appropriately with the requisite good laboratory practices and universal precautions. For full concentration information, please refer to the SDS.
- 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.

Full Hazardous Label:

Ingredient, Cat #		Full Label	
02-0001-06	10X Wash Buffer		**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 the following reagents to room temperature prior to use: Standard Diluent, Assay Buffer, Coated Beads, Elution Buffer B, Buffer D, Detection Antibody and 10X Wash Buffer.
2. Store the Detection Antibody away from light until ready to use.
3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
    a. Pour 50 mL of the 10X Wash Buffer into a container capable of holding at least 500 mL.
    b. Add 450 mL of deionized water.
    c. Mix thoroughly by gentle inversion or with a clean, sterile stir bar.
4. Mix IL-6 Coated Beads (coated microparticles) on a rotisserie spin rotator, or manually by repeat inversion, for 10 - 20 minutes until all beads are completely resuspended.

Sample Preparation

1. Prepare samples by one of the following methods:
    a. If using a filter plate with prefilter (EMD Millipore PN MSBVN1210 or equivalent): Stack the filter plate on top of a 96-well receptacle plate. Place ≤ 250 µL of sample into a filter plate well and spin for ≥ 10 minutes at 1,100 x g.
    b. If using a microcentrifuge: Centrifuge samples at >13,000 x g for 10 minutes immediately prior to use. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer.

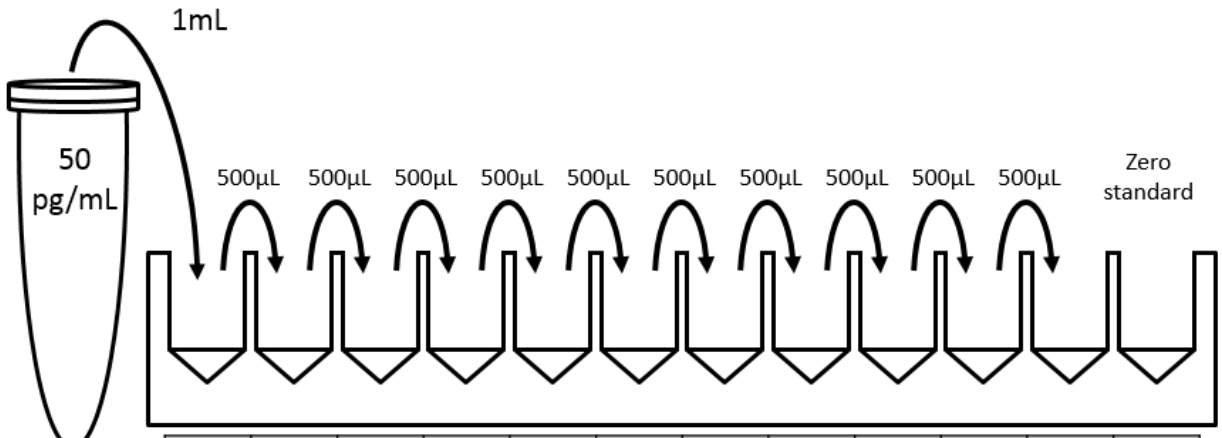
Initial Standard Stock Preparation

1. Quick spin the Human IL-6 Standard vial in a microcentrifuge and pipette mix prior to preparing standards. Use care when opening the stock standard vial to prevent loss of materials and contamination of specimens or plates with aerosols.
2. Refer to the standard value assignment on the Certificate of Analysis for the starting concentration of the Human IL-6 Standard in the vial.
3. To make your Analyte Working Stock, perform the necessary serial dilutions, in Standard Diluent, to achieve the final working concentration of 50 pg/mL in a 1 mL final volume. Ensure that all pipetting steps transfer ≥10 µL of liquid to achieve the best precision.
4. Discard standard after one use.

HUMAN IL-6 ASSAY PROCEDURE

Standard Curve

Prepare the standard curve dilutions in a 12-channel reservoir. Perform 1:3 serial dilutions of the Analyte Working Stock for dilutions 2,3,4,and 5 then 1:2 dilutions for 6 to 11, to achive a curve from 50 pg/mL to 0,01 pg/mL. Run the standards in triplicate. \



1	2	3	4	5	6	7	8	9	10	11	12
50	16.67	5.56	1.85	0.62	0.31	0.15	0.08	0.04	0.02	0.01	0

1. Add 1,000 µL Standard Diluent to wells 2, 3, 4, and 5 of a 12-channel reservoir.
2. Add 500 µL Standard Diluent to wells 6 through 12.
3. Add 1,000 µL of the 50 pg/mL Analyte Working Stock from standard preparation into well 1.
4. Transfer 500 µL from well 1 into well 2, mixing thoroughly. Continue serial dilutions from well 2, stopping at well 11, mixing thoroughly each time. Use a fresh tip with each transfer.

Target Capture

1. Pipette 75 µL per well of Standards or Samples to Plate 1 (96-well polypropylene).
2. Mix microparticles (coated beads) by gentle inversion until all beads are completely resuspended.
3. Immediately before adding to the assay plate, add the vial of Coated Beads to 10.5 mL of the supplied Assay Buffer. Mix by gentle inversion. Ensure that all beads have been transferred.
4. Pipette 100 µL per well of the Coated Beads into Plate 1.
5. Cover Plate 1 with a plate sealing film.
6. Incubate for 2 hours at 25°C on microplate incubator / shaker (if using EMD Millipore PN 70-0009-00, use setting # 5).

HUMAN IL-6 ASSAY PROCEDURE (continued)

7. Approximately 10 minutes prior to the end of Target Capture incubation, prepare the Detection Antibody. Add 10 µL of Detection Antibody to 90 µL of Assay Buffer to make a 1:10 dilution. Then add 75 µL of the diluted Detection Antibody to 2925 µL of Assay Buffer. Filter the diluted detection antibody using the syringe with a 0.2 µm filter into a clean tube.
8. When target capture incubation is complete, carefully remove temporary plate cover to avoid splashing.

Post-Capture Wash

The plate can be washed with a plate washer or a manual washer.

1. **Plate Washer**
    a. BioTek; Post Capture Wash (POSTCAP)
    b. HydroFlex; Post Capture Wash (PCW)
2. **Manual Post-Capture Wash Protocol**
    a. Place Plate 1 onto magnet (EMD Millipore PN 90-0003-02).
    b. Wait 2 minutes for beads to settle (ensure all beads are amassed as a pellet near magnet).
    c. Aspirate the supernatant (beads remain visible).
    d. Add 200 µL of Wash Buffer.
    e. Wait ≥ 2 minutes. To ensure that the beads remain amassed, do not suspend or remove plate from the magnet during this time.
    f. Aspirate buffer.

Detection

1. Immediately remove Plate 1 from the magnet and add 20 µL per well of Detection Antibody.
2. Cover Plate 1 with plate sealing film.
3. Incubate for 1 hour at 25°C on microplate incubator / shaker (if using EMD Millipore PN 70-0009-00, use setting # 5).
4. Carefully remove plate sealing film to avoid splashing.

HUMAN IL-6 ASSAY PROCEDURE (continued)

Pre-Transfer Wash

The plate can be washed with a plate washer or a manual washer.

1. **Plate Washer**
    a. BioTek; 4 cycle Pre-Transfer (4CYCPRE)
    b. HydroFlex; 4 cycle Pre-Transfer (*4cyPrTra)*
2. **Manual Pre-Transfer Wash Protocol**
    a. Place Plate 1 onto magnet (EMD Millipore PN 90-0003-02).
    b. Add 100 µL of Wash Buffer to each well of Plate 1.
    c. Wait 2 minutes.
    d. Aspirate the supernatant and discard into waste, change tips.
    e. Add 200 µL of Wash Buffer to each well.
    f. Wait ≥ 2 minutes. To ensure that the beads remain amassed, do not suspend or remove beads from the magnet during this time.
    g. Aspirate buffer from each well, discard into waste and change tips.
    h. Repeat steps e - g three more times for a total of four washes.
    i. Add 200 µL of Wash Buffer to each well of Plate 1.
    j. Remove Plate 1 from magnet.

Manual Plate Transfer

1. **If using Sphere Mag Plate with SBS Footprint (EMD Millipore PN 90-0003-02 or DynaMag™-96 Side Skirted Magnet (Thermo Fisher PN 12027))**
    a. Prepare manual transfer station:
        i. Set manual 12-channel pipette to 100 µL.
        ii. Fill a reservoir with Wash Buffer.
        iii. Place Plate 2 on a magnet.
    b. In the first row of Plate 1, pipette up and down seven to ten times gently to minimize bubbles & to resuspend beads.
    c. Transfer 200 µL (100 µL x 2) of suspended beads from Row A of Plate 1 to Row A of Plate 2.
    d. Change tips.
    e. Aspirate 100 µL of Wash Buffer from reservoir and dispense into Row A of Plate 1.
    f. Pipette up and down seven to ten times to resuspend any remaining beads, and then transfer 100 µL of suspended beads to Row A of Plate 2.
    g. Change tips.
    h. Repeat steps b - h for remaining 7 rows.

i. Inspect Plate 1 for any remaining beads.
    i. Yes, beads are present in Plate 1:
        - Add 100 µL of Wash Buffer to wells containing beads.
        - Gently mix by pipetting to resuspend the bead pellet.
        - Transfer the contents of each well containing beads to Plate 2 on magnet.
    ii. No, beads are not present in Plate 1.
j. Discard Plate 1.
k. Magnetized bead pellet should be visible in Plate 2.

Final Aspiration

1. Plate Washer
    a. BioTek; Final Aspirate (FINASP)
    b. HydroFlex; Final Aspirate (FA_V1)
2. Manual Final Wash Aspirate
    a. While Plate 2 is on the magnet, wait 2 minutes.
    b. Aspirate the supernatant and discard into waste.

Elution

1. Immediately remove Plate 2 from the magnet.
2. Add 10 µL Elution Buffer B per well.
3. Cover Plate 2 with a plate sealing film.
4. Incubate plate for 10 minutes at 25°C on microplate incubator / shaker (if using EMD Millipore PN 70-0009-00, use setting # 5).
5. Add 10 µL per well of Buffer D to Plate 3 (384-well polypropylene plate (Nunc PN 264573 or equivalent)) using a 12-channel manual P20.
6. Place Plate 2 on sphere magnet bed (EMD Millipore PN 90-0003-02), remove plate sealing film, and allow beads to form a tight pellet for 2 minutes.
7. Set manual 8-channel pipette to 15 µL and transfer eluate to Plate 3 by rows, avoiding the pelleted beads.
8. Cover Plate 3 with a universal plate cover and spin plate for 5 minutes at RT, approximately 1,100 x g.
9. Cover Plate 3 with heat sealing foil (EMD Millipore PN 02-01-0216-00 or equivalent), according to manufacturer's instructions for the heat sealer.

Run on Erenna® Immunoassay System

1. Load completed assay Plate 3 onto the Erenna® Immunoassay System.

To run on the SMCxPRO™ Immunoassay System:

1. Add 10 µL per well of Buffer D to the SMCxPRO™ reading plate.

NOTE: This plate comes individually wrapped with a lid; handle by touching only the sides of the plate. Leave the plate on the plate holder at all times except when loading into SMCxPRO™ for analysis.

2. Place Plate 2 on sphere magnet bed (EMD Millipore PN 90-0003-02), remove plate sealing film, and allow beads to form a tight pellet for 2 minutes..
3. Set manual 8-channel pipette to 15 µL and transfer eluate to Plate 3 by rows, avoiding the pelleted beads.
4. Cover reading plate (plate 3) with a universal plate cover and spin plate for 2 minutes at RT, approximately 1,100 *x g*.
5. Seal reading plate with adhesive plate sealer (Thermo PN 276014). If need to read on Erenna® after SMCxPRO™ then seal with piercable heat sealer (EMD Millipore PN 02-01-0216-00 or equivalent).
6. Remove the plate holder and load sealed reading plate onto the SMCxPRO™ Immunoassay System.
7. There is a warm up period of 30 minutes to bring the plate to the appropriate temperature prior to the start of a new plate read.
8. After 30 minutes, hit 'Start' to start the plate read.

For both platforms, if the assay plate cannot be read on the same day, the sealed reading plate can be stored at 4°C overnight (plate can be stored for up to 48 hours at 4°C). Note: For Aurora plates, ensure plate remains on the plate holder.

The following day remove the reading plate from 4 °C and bring to RT for 15 min, spin for 2 minutes at RT, approximately 1,100 x g; for the SMCxPRO™ platform, place in instrument, wait for 30 minutes and click 'start' to start plate reading.

APPENDIX A: SMC™ Quick Assay Guide

1. Prepare all reagents, standard curve, and samples as instructed.
2. Add 75 µL of Standard/Samples and 100 µL of **Coated Beads** to **Plate 1**.
3. Cover and incubate for 2 hours at 25°C on microplate incubator/shaker.

2 hours 25°C

4. Perform Post-Capture Wash (**Plate 1**).
5. Remove from magnet and add 20 µL of **Detection Antibody** per well.
6. Cover and incubate for 1 hour at 25°C on microplate incubator/shaker.

1 hour 25°C

7. Perform Pre-Transfer Post-Detection Wash (**Plate 1**).
8. Perform Manual Plate Transfer (**Plate 2**).
9. Perform Final Aspiration (**Plate 2**).
10. Remove from magnet and add 10 µL of **Elution Buffer B** to each well.
11. Cover and incubate for 10 minutes at 25°C on microplate incubator/shaker.

10 minutes 25°C

12. Add 10 uL of **Buffer D** per well to **Plate 3**.
13. Transfer contents of **Plate 2** to **Plate 3**.
14. Cover and centrifuge for 5 minutes Erenna® Plate or 2 minutes SMCxPRO™ plate at 1,100 x g.
15. Cover **Plate 3** with pierceable plate seal cover for Erenna® plate or adhesive plate sealer for SMCxPRO™ plate .

LOAD ON ERENNA® OR SMCxPRO™ SYSTEM

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and pipeting 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. Ensure beads were not lost during wash.
	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.

TROUBLESHOOTING GUIDE (continued)

Problem	Probable Cause	Solution
Sample variability is high (continued)	Cross-well contamination (continued)	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.
Published LLoQ was not achieved	Improper dilution/reconsititution 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

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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EMD Millipore Corporation
3050 Spruce Street
St. Louis, Missouri 63103
United States of America

Toll-Free US: (800) 645-5476
Fax: (800) 645-5439
emdmillipore.com