User Guide

SMC® Human IgE High Sensitivity Immunoassay Kit

Microparticle Assay

Human IgE High Sensitivity Immunoassay Kit for the Quantitative Determination of IgE in Human Plasma and Serum

03-0201-00

Introduction

The SMC® Human IgE High Sensitivity Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure IgE in human plasma and serum samples. The user coats a capture antibody specific for human IgE onto a 96-well microplate. After blocking the plate, the user pipettes standards and samples into the coated microplate wells. During incubation, the IgE present in the sample binds to the capture antibody on the coated plate. Unbound molecules are washed away during a wash step. The fluor-labeled detection antibody is added to each well and incubated. The detection antibody recognizes and binds to IgE that has been captured onto the plate, thus completing the immunosandwich. Following the final wash step, elution buffer is added and incubated. The elution buffer dissociates the bound protein sandwich from the plate surface releasing the labeled antibodies. The eluted antibodies are transferred to a SMC® 384-well Read Plate. The plate is loaded into the SMCxPRO® Immunoassay System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of IgE present in the sample when captured. The amount of IgE in unknown samples is interpolated from a standard curve.

Supplies

The SMC® Human IgE High Sensitivity Immunoassay Kit includes all reagents listed below; these components are lot matched and not intended to be used separately. 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 Part No.
IgE Standard	2-8 °C	1 lyophilized vial	02-8201-00
IgE Coating Antibody	-20 or -80 °C (Shipped with dry ice)	1 x 20 µL	02-2201-00
IgE Detection Antibody	2-8 °C	1 x 400 µL	02-1201-00
Standard Diluent	2-8 °C	1 x 50 mL	02-9959-00
Assay Buffer	2-8 °C	1 x 15 mL	02-9960-00
10X Wash Buffer **Note:** Contains Proclin™	2-8 °C	2 x 60 mL	02-0001-09
Coating Buffer	2-8 °C	1 x 20 mL	02-9958-00
Blocking Buffer	2-8 °C	1 x 30 mL	02-9957-00
Buffer 3	2-8 °C	1 x 5 mL	02-0554-00
Buffer C	2-8 °C	1 x 6 mL	02-9962-00
Elution Buffer B	2-8 °C	2 x 5 mL	02-0211-02
Assay Plate	2-8 °C	1 each	S30057-F
SMC® Commercial Plate	2-8 °C	1 plate	02-1PCP-00

Storage Instructions

The SMC® Human IgE High Sensitivity Immunoassay Kit should be stored at 2-8 °C.

Discard standards after one use. Proper kit performance can only be guaranteed if the materials are stored properly.

Additional Supplies Required (Not provided)

Catalogue numbers provided may be purchased from SigmaAldrich.com or through sales quote, unless otherwise noted.

Equipment

- SMCxPRO® Ultrasensitive Immunoassay System for sample acquisition (95-0100-00)
- Orbital microplate shaker for assay plate incubation (for example, Boekel Scientific Jitterbug™ Shaker)
- BioTek® 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
- Microcentrifuge capable of reaching 13,000 x *g* for reagent/sample 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

- Micro-centrifuge tubes for sample preparation and storage
- 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)
- MultiScreen®$_{HTS}$ 96-well Plate, hydrophilic PVDF membrane (MSBVN1210)
- 15 mL conical tube with cap for detection antibody dilution
- 96-well V-bottom plate for assay setup (AXYP96450VCS)
- Axygen™ Microplate Sealing Film and Tapes (Fisher Scientific, 14-222-344)
- Universal plate cover to minimize plate well contamination (Fisher Scientific, 253623)
- 12-Channel reagent reservoir (sterile) for standard serial dilution (Argos/Cole Parmer, 04395-33)
- VistaLab® 25 mL Reservoirs for addition of reagents (Fisher Scientific, 21-381-27C)
- Millex® Syringe Filter, 0.2 µm for detection antibody filtration (SLGPR33RS)
- Luer-Lok® Syringe, 5 mL; for Detection Antibody Filtration (Fisher Scientific, 14-829-45)
- Nunc™ Aluminum adhesive plate seals (Fisher Scientific, 276014)

Reagents

- 10X Wash Buffer for automated assay plate washing, 1 L (02-0111-00)
- 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.
- The standards prepared by serial dilution must be used within 10 minutes of preparation.

  Note: It is recommended that the standards are prepared as the last step prior to plate setup.
- All washing must be performed with the Wash Buffer provided.
- An orbital microplate shaker for assay plate incubation (Jitterbug™ Shaker settings #3-5) provide maximal orbital mixing without splashing liquid or causing cross-contamination.

  Jitterbug™ Shaker setting #3 ~ 750 rpm

  Jitterbug™ Shaker setting #4 ~ 875 rpm

  Jitterbug™ Shaker setting #5 ~1000 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	Catalogue Number	Full Label	
IgE Standard	02-8201-00	 	**Danger.** Harmful if swallowed or if inhaled. Toxic in contact with skin. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. May cause damage to organs Respiratory Tract through prolonged or repeated exposure if inhaled. Harmful to aquatic life with long lasting effects. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/ protective clothing. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. Get medical advice/ attention if you feel unwell. Take off contaminated clothing and wash before reuse. Store locked up. Dispose of contents/ container to an approved waste disposal plant**.**
Blocking Buffer	02-9957-00		**Warning**. May cause damage to organs Respiratory Tract through prolonged or repeated exposure if inhaled. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Get medical advice/ attention if you feel unwell. Dispose of contents/ container to an approved waste disposal plant.

Ingredient	Catalogue Number	Full Label	
Standard Diluent	02-9959-00		**Warning**. Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. If eye irritation persists: Get medical advice/ attention. Dispose of contents/ container to an approved waste disposal plant.
10X Wash Buffer	02-0001-09		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.
Assay Buffer	02-9960-00		**Warning.** Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. If eye irritation persists: Get medical advice/ attention. Dispose of contents/ container to an approved waste disposal plant.
Elution Buffer B	02-0211-02	No symbol required	Harmful to aquatic life. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant.

Assay Preparation

Plate Coating (Overnight)

1. Prepare a 1 mg/mL sub-stock of IgE Coating Antibody in 1X Buffer 3.
   - Allow Buffer 3 to equilibrate to room temperature (crystals may be present at refrigerated temperature).
   - Prepare 1X Buffer 3 by diluting 5 mL of provided Buffer 3 into 45 mL of deionized water and mix well.
   - Allow stock IgE Coating Antibody to equilibrate to room temperature and mix well by pulse vortexing briefly.
   - Refer to the Certificate of Analysis for the stock concentration of IgE Coating Antibody solution. For example, prepare 40 µL of a 1 mg/mL sub-stock solution of IgE Coating Antibody by adding 10 µL of a 4 mg/mL stock solution of IgE Coating Antibody to 30 µL of 1X Buffer 3 and mix well. Store remaining 1X Buffer 3 at 4 °C.
2. Dilute the 1 mg/mL sub-stock of IgE Coating Antibody 1:1,000 in Coating Buffer (For example, add 15 µL of 1 mg/mL IgE Coating Antibody to 14.985 mL of Coating Buffer.)
3. Pipette 100 µL of diluted Coating Antibody into each well of the assay plate. Ensure that the solution has evenly coated the bottom of each well.
4. Seal the plate and incubate without shaking overnight at 4 °C for a minimum of 12 hours.

   Note: For consistent results, use this plate and coating condition. Other configurations have not been tested.

Reagent Preparation

1. Warm all reagents to RT prior to use.
2. Store the Detection Antibody away from light until ready to use.
3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
   - Pour 50 mL of 10X Wash Buffer into a container capable of holding at least 500 mL. Add 450 mL of deionized water.
   - Mix thoroughly by gentle inversion or with a clean, sterile stir bar.

   Note: 1X Wash Buffer may be filter sterilized.
4. Following overnight plate coating, remove the Coating Antibody solution manually (careful, but firm ejection of liquid) or by aspirating with an automated plate washer and blot on paper towels. Ensure that wells are free of residual liquid.
5. Block the plate with 200 µL per well of Blocking Buffer. Incubate with shaking for 1 hour at 25 °C on microplate shaker/incubator (Jitterbug setting #5).

Sample Preparation

1. Prepare samples by one of the following methods:
   - 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.
   - If using a filter plate with prefilter: 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*.
2. Sample dilution:
   - Dilute the clarified samples 1:4,000 in two steps using 1X Buffer 3 for initial dilution and the Standard Diluent for the subsequent dilution.
   - (For example, add 10 µL of clarified sample to 990 µL of 1X Buffer 3 to make a 1:100 dilution then transfer 12.5 µL of 1:100 diluted sample to 487.5 µL of Standard Diluent to make a 1:4,000 dilution).
   - 50 µL per well of 1:4,000 diluted serum or plasma should be used.

   Note: If further sample dilution is required, samples can be diluted with the provided Standard Diluent.

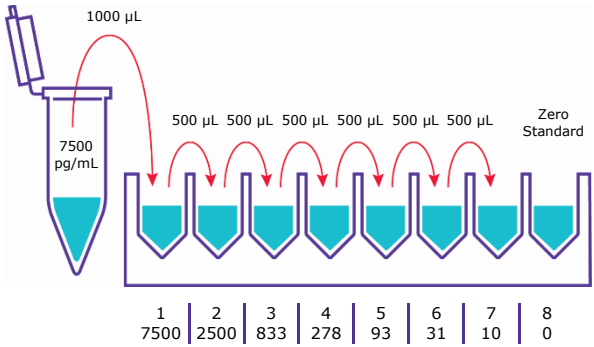
Initial Standard Stock Preparation

1. Reconstitute lyophilized standard in 250 µL of deionized water. Invert the vial several times to mix. Gently pulse vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes.
2. Refer to the standard value assignment on the Certificate of Analysis for the starting concentration of the IgE Standard in the vial.
3. Perform the necessary dilutions in Standard Diluent to achieve the final working concentration of 7500 pg/mL in a 1 mL final volume.

Standard Curve

Prepare the standard curve in a 12-channel reagent reservoir. Perform 1:3 serial dilutions of the 7500 pg/mL Standard 1 for Standards 2 through 7 to achieve a curve from 7,500 to 10 pg/mL. Standard 8 is the Blank (Standard Diluent only).

Run the standards in triplicate.



1. Add 1000 µL Standard Diluent to wells 2 through 8 of a 12-channel reservoir.
2. Transfer 1000 µL of 7,500 pg/mL working stock (Standard 1) into well 1.
3. Transfer 500 µL from well 1 into well 2, mixing thoroughly. Continue serial dilutions from well 2 stopping at well 7, mixing thoroughly each time. Use a fresh tip with each transfer.

Assay Procedure

Target Capture

1. Remove Blocking Buffer from wells manually or by aspirating with an automated plate washer and blot on paper towels. Ensure that wells are free of residual liquid.
2. Pipette 50 µL per well of Standards and diluted Samples to blocked assay plate.
3. Seal assay plate with clear adhesive plate seal, apply pressure to seal to prevent leaking and cross-contamination.
4. Incubate for 2 hours at 25 °C on microplate incubator/shaker (Jitterbug™ Shaker setting #4).
5. Approximately 10 minutes prior to the end of target capture incubation, prepare the Detection Antibody using one of the following methods:
   - Centrifuge 20X Detection Antibody at 14,000 x *g* for 5 minutes. Prepare 1X Detection Antibody by adding 350 µL of the centrifuged supernatant into 6,650 µL of Assay Buffer.
   - Prepare 1X Detection Antibody by adding 350 µL of 20X Detection Antibody into 6,650 µL of Assay Buffer and filter the diluted Detection Antibody using the syringe with a 0.2 µm filter into a clean tube.
6. When incubation is complete, carefully remove clear adhesive plate seal to avoid splashing.

Post-Capture Wash

Wash the assay plate six times (plate washer) or three times (hand wash) with 200 µL of 1X Wash Buffer.

Plate Washer

- BioTek® 405 TSUVS (95-0004-05) washer is recommended.
- For instructions regarding BioTek® plate washer programming, please contact Customer Support.

Hand Wash

1. Dump liquid with firm motion and gently blot on paper towels.
2. Pipette 200 µL/well 1X Wash Buffer.
3. Dump liquid into waste with firm motion.
4. Repeat steps 2 and 3 two times.
5. Blot assay plate thoroughly on paper towels ensuring all wells are free of residual liquid.

Detection

1. After removal from plate washer, dispense 50 µL per well of Detection Antibody (It is recommended to change tips).
2. Seal assay plate with clear adhesive plate seal.
3. Incubate for 1 hour at 25 °C on microplate incubator/shaker (Jitterbug™ Shaker setting #5). Ensure plate is protected from light during this incubation.
4. After incubation, carefully remove clear adhesive plate seal to avoid splashing.

Post-Detection Wash

Wash the assay plate six times (plate washer) or three times (hand wash) with 200 µL of 1X Wash Buffer.

Plate Washer

- BioTek® 405 TSUVS (95-0004-05) washer is recommended.
- For instructions regarding BioTek® plate washer programming, please contact Customer Support.

Hand Wash

1. Dump liquid with firm motion and gently blot on paper towels.
2. Pipette 200 µL/well 1X Wash Buffer.
3. Dump liquid into waste with firm motion.
4. Repeat steps 2 and 3 two times.
5. Blot assay plate thoroughly on paper towels ensuring all wells are free of residual liquid.

Elution

1. After washing the plate, dispense 50 µL Elution Buffer B per well of assay plate. 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).

Assay Reading

To read on the SMCxPRO® Immunoassay System

1. Add 10 µL per well of Buffer C using reverse pipetting to a fresh 96-well assay plate, using a 12-channel manual pipette (1-20 µL).
2. After incubation, gently remove clear adhesive seal and transfer 40 µL of eluate to the new assay plate containing Buffer C changing tips with each dispensed row.
3. Seal this plate with a clear adhesive plate seal.
4. Place the plate (containing eluted, neutralized antibody solution) into microplate incubator/shaker and shake for 2 minutes at 25 °C (Jitterbug™ Shaker setting #5), centrifuge plate for 1 minute at RT, approximately 1,100 x *g*.
5. Gently remove clear adhesive plate seal and transfer 40 µL of neutralized eluate solution per well to corresponding wells of the SMC® Read Plate (02-1PCP-00), placed over the included plate holder.
6. Seal SMC® Read Plate with clear adhesive plate seal. Centrifuge plate for 1 minute at RT, approximately 1,100 x *g*. Remove plate sealer, inspect SMC® Read Plate wells and remove bubbles if they are present.
7. Firmly seal SMC® Read Plate with aluminum adhesive plate seal using the recommended plate roller.
8. Remove the plate holder from the sealed SMC® Read Plate and load it onto the SMCxPRO® Immunoassay System. Start read.

   Note: There is a warmup period of up to 30 minutes to wait for the SMC® Read Plate to be close to the internal instrument temperature. Once achieved the read will start automatically.

SMC® Assay Overview

1. Add 100 µL of Diluted Coating Antibody to each well.
2. Seal coated plate and incubate overnight at 4 °C. Do Not Shake.

Overnight 4 °C

3. Remove Coating Antibody from Coated Assay Plate.
4. Block plate with 200 µL/well of Blocking Buffer for 1 hour at 25 °C on microplate incubator/shaker with shaking.
5. Prepare all reagents, standard curve and samples as directed.

1 hour 25 °C

6. Remove Blocking Buffer from Blocked Assay Plate.
7. Add 50 µL of Standard/1:4,000 Diluted Samples to Assay Plate.
8. Seal and incubate for 2 hours at 25 °C on microplate incubator/shaker.

2 hours at 25 °C

9. Wash Assay Plate 6 times (plate washer) or 3 times (hand wash) with 200 µL 1X Wash Buffer.
10. Add 50 µL of Detection Antibody per well.
11. Seal and incubate for 1 hour at 25 °C on microplate incubator/shaker.

1 hour 25 °C

12. Wash Assay Plate 6 times (plate washer) or 3 times (hand wash) with 200 µL 1X Wash Buffer.
13. Add 50 µL of Elution Buffer B per well.
14. Seal and incubate for 10 minutes at 25 °C on microplate incubator/shaker.

10 minutes 25 °C

15. Neutralize 40 µL of eluted antibody with 10 µL Buffer C per well.
16. Transfer 40 µL of neutralized eluate to SMC® Read Plate.
17. Seal SMC® Read Plate with aluminum adhesive plate seal for SMCxPRO® Immunoassay System.
18. Load on SMCxPRO® Immunoassay System.

Assay Characteristics

Sensitivity

Assay sensitivity measures the true limit of quantitation of an analyte and is often defined by the Lower Limit of Quantification (LLOQ). LLOQ is calculated as the lowest concentration that can achieve CVs of < 20% and the percent recovery of the standard point is still between 80%-120%. The LLOQ of IgE is 31 pg/mL. The reported value is the average of multiple assays (n = 11 assays). Please note that the published LLOQ is data generated during kit verification and can have minor variation between kit lots. For lot specific LLOQ data, please see the certificate of analysis.

Precision

The assay variations of SMC® Human IgE High Sensitivity Immunoassay Kit were studied using five normal plasma samples run in triplicate by 3 different operators on 3 different days.

- Mean intra-assay variation was 7%
- Mean inter-assay variation was 12%

Specificity/Cross-Reactivity

- Cross-reactivity to the following analytes were tested with the following results:
- IgA – Not cross-reactive
- IgD – Not cross-reactive
- IgM – Not cross-reactive
- Total IgG (G1,G2,G3,G4) – Not cross-reactive

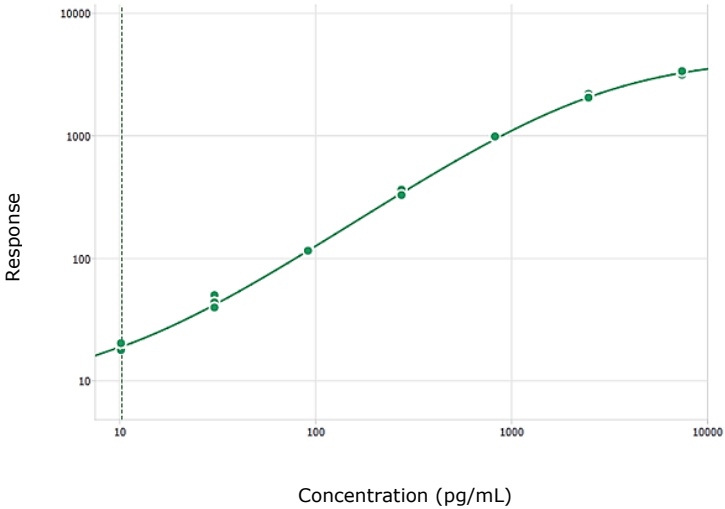
Spike Recovery

The data represent mean percent recovery of three different concentrations of standard spiked into samples (n=4 plasma samples, 5 serum samples).

Sample ID	Serum Recovery %	Plasma Recovery %
Sample 1	95	96
Sample 2	95	86
Sample 3	73	87
Sample 4	96	89
Sample 5	71	N/A
Average	**86**	**89**

Graph of Typical Reference Curve

Typical SMCxPRO® Human IgE Immunoassay Standard Curve, not to be used to calculate data.



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.
	Plate was over-incubated	Confirm plate incubation times are as recommended, particularly for the Detection incubation.
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 filtered according to the Assay Preparation section. Unprocessed samples could lead to higher imprecision.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where liquid is in constant motion without causing splashing (See Jitterbug™ Shaker setting in Assay Best Practices section).
	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
Published LLoQ was not achieved	Improper dilution/reconstitution of the standard reference material	Confirm appropriate kit protocol was followed when preparing standard curve.
		Ensure standards are prepared before starting capture incubation.
Microparticles do not resuspend into homogenous solution	Samples may be causing interference due to excess particulate matter	Samples should be properly processed prior to testing to remove particulate matter or lipids.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8				
B	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8				
C	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8				
D	Sample 1	Sample 2	Etc.									
E	Sample 1	Sample 2	Etc.									
F												
G												
H												

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