

User Guide

# SMC® Amyloid Beta 1-40 High Sensitivity Immunoassay Kit

Microparticle Assay

Amyloid Beta 1-40 Immunoassay Kit for the Quantitative Determination of Amyloid Beta 1-40 in Human, Mouse, and Rat Plasma and Cerebrospinal Fluid

**03-0145-00**

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## Introduction

The SMC<sup>®</sup> Amyloid Beta 1-40 High Sensitivity Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure Amyloid Beta 1-40 in human, mouse, and rat plasma and cerebrospinal fluid (CSF) samples. A capture antibody specific for Amyloid Beta 1-40 has been pre-coated onto paramagnetic microparticles (beads). The user pipettes beads, standards, and samples into uncoated microplate wells. During incubation, the Amyloid Beta 1-40 present in the sample binds to the capture antibody on the coated beads. Unbound molecules are washed away during the subsequent wash steps. Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to Amyloid Beta 1-40 that has been captured onto the beads, thus completing the sandwich. Elution buffer is added to dissociate the protein sandwich, releasing the fluor-labeled antibodies. The eluted antibodies are transferred to a SMCxPRO<sup>®</sup> 384-well plate. The plate is loaded into the SMCxPRO<sup>®</sup> 384-well System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of Amyloid Beta 1-40 present in the sample. The amount of Amyloid Beta 1-40 in unknown samples is interpolated from a standard curve.

## Supplies

The SMC<sup>®</sup> Amyloid Beta 1-40 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. All items are shipped with a cold pack unless otherwise stated.

<b>Description</b>	<b>Storage Conditions</b>	<b>Packaging Details</b>	<b>Component Number</b>
Assay Buffer	2-8 °C	2 x 20 mL	02-9998-00
SMC <sup>®</sup> Amyloid Beta 1-40 Coated Beads	2-8 °C	1 x 550 µL	02-0953-00
SMC Abeta Standard Diluent	2-8 °C	2 x 20 mL	02-0954-00
SMC <sup>®</sup> Amyloid Beta Detection	2-8 °C	1 x 270 µL	02-1145-40
SMC <sup>®</sup> Amyloid Beta 1-40 Standard	2-8 °C	1 lyophilized vial	02-0950-00
10X Wash Buffer	2-8 °C	1x 50 mL	02-0001-03
Buffer D	2-8 °C	1 x 6 mL	02-0446-00
Elution Buffer B	2-8 °C	1 x 5 mL	02-0211-02

For research use only. Not for use in diagnostic procedures.

## Kit Storage

The SMC<sup>®</sup> Amyloid Beta 1-40 High Sensitivity Immunoassay Kit should be stored at 2-8 °C. Discard standards after one use.

Supplied 10X Wash Buffer does not contain preservative. After dilution, the 1X Wash Buffer may be filter sterilized with Stericup<sup>®</sup> filter, for storage of up to 1 month at 2-8 °C. If not filter sterilized, all remaining 1X wash buffer should be discarded upon experiment completion.

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](https://www.sigmaaldrich.com) unless otherwise noted.

### Equipment

- SMCxPRO<sup>®</sup> Ultrasensitive Immunoassay System for sample acquisition (95-0100-00)
- Orbital microplate shaker for assay plate incubation (e.g., Boekel Scientific Jitterbug<sup>™</sup>) Temperature control for assay by equipment may be necessary. Please confirm temperatures required for this assay as referenced in the protocol can be accommodated.
- Bio-Tek<sup>®</sup> 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)

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## Supplies

- Micro-centrifuge tubes for sample preparation and storage
- 1 L Container with cap for wash buffer dilution
- Stericup® Filter, 0.22 µm, 1 L; for filter sterilizing 1X Wash Buffer (S2GPU11RE)
- MultiScreen®<sub>HTS</sub> BV 96-Well Filter Plate for sample filtration (MSBVN1210)
- 15 mL conical tube with cap for capture bead and detection antibody dilution
- 96-well V-bottom plate for assay setup (Fisher Scientific, 14-222-241)
- 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)
- SMCxPRO® 384-well plate with adhesive seal (02-1008-00)
- SMCxPRO® 384-well plate, bulk case of 32 (ABB2-00160A)
- 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



## 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.

## Hazard Labels

Ingredient	Catalogue No.	Label	
SMC® Ab40 Coated Beads	02-0953-00	No Symbol Required.	Harmful to aquatic life with long lasting effects. Avoid release to the environment.
Amyloid Beta Detection Antibody	02-1145-40	No Symbol Required.	Harmful to aquatic life with long lasting effects. Avoid release to the environment.
Amyloid Beta 40 Standard	02-0950-00		<b>Warning.</b> Harmful if swallowed. May cause damage to organs Respiratory Tract through prolonged or repeated exposure if inhaled. Do not breathe dust/fume/gas/mist/vapours/spray. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Get medical advice/attention if you feel unwell. Rinse mouth. Dispose of contents/ container to an approved waste disposal plant.
Assay Buffer	02-9998-00		<b>Warning.</b> 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 No.	Label	
SMC Abeta Standard Diluent	02-0954-00		<p><b>Warning.</b> 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.</p>
10X Wash Buffer	02-0001-03		<p><b>Warning.</b> 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.</p>

## 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. 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.
- 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.
- Boekel Scientific Jitterbug™ Shaker settings #3-5 provide maximal orbital mixing without splashing liquid or causing cross-contamination.

**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.

- Plate must also be protected from light after adding detection.
- After the assay is complete, the plate should be read immediately.
- The plates may be stored at 2-8 °C for up to 48 hours away from light if same day reading is not possible.
- After the assay is complete, seal the plate before storing at 2-8 °C.

**Note:** For SMCxPRO® Immunoassay System, use aluminum adhesive plate seal.

- Bring to RT then centrifuge the plate at 1,100 x g for 1 minute prior to reading.

For optimal SMCxPRO® performance, perform ASSIST testing daily (ideally at beginning of the day before assay is prepared).



## SMC<sup>®</sup> Assay Overview

1. Prepare all reagents, standard curve, and samples as instructed.
2. Add 100  $\mu\text{L}$  of Standard/1:10 Plasma/1:50 diluted CSF samples and 100  $\mu\text{L}$  of Coated Beads to assay plate.
3. Seal and incubate for 2 hours at 25 °C on appropriate microplate incubator/shaker.



2 hours 25 °C

4. After capture incubation, centrifuge assay plate at 1,100  $\times g$  for 1 minute.
5. Perform Post-Capture Wash.
6. Remove from washer magnet and add 20  $\mu\text{L}$  of Detection Antibody per well.
7. Seal assay plate and incubate for 1 hour at 25 °C on microplate incubator/shaker.



1 hour at 25 °C

8. Perform Post-Detection Wash.
9. Seal the assay plate and perform the post-detection shake for 2 minutes on microplate incubator/shaker.
10. Perform the Final Aspiration.
11. Remove from washer magnet and add 10  $\mu\text{L}$  of Elution Buffer B to each well.
12. Seal assay plate and incubate for 10 minutes at 25 °C on microplate incubator/shaker.



10 minutes at 25 °C

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13. Add 10  $\mu$ L of Buffer D to neutralize the eluted antibody.
  14. Transfer 18  $\mu$ L of neutralized eluate to the **reading plate**.
  15. Seal **reading plate** aluminum adhesive plate seal for SMCxPRO<sup>®</sup> System.
  16. Load on SMCxPRO<sup>®</sup> System.

# Assay Preparation

## Sample Preparation

1. Sample handling:
  - The stability of Amyloid Beta is critical because the peptides tend to aggregate in samples. For this reason, the pre-analytic sample preparation is major influencing parameter within the analysis of amyloid peptides. Samples should be collected according to clinical approved standard procedures and immediately stored at  $\leq -20$  °C. Avoid repeated thawing and freezing of samples and standards.
  - Keep samples on ice throughout assay and mix gently before adding to wells.
2. Prepare plasma samples by one of the following methods:
  - Preferred Method: Stack the filter plate on top of a 96-well plate. Place 250  $\mu\text{L}$  of sample into a filter plate well and spin for  $\geq 10$  minutes at 1,100  $\times g$ .
  - Optional Method: Centrifuge samples at  $> 13,000 \times g$  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.
3. Prepare cerebrospinal fluid samples by one of the following methods:

Immediately after collection, centrifuge the CSF at 1,100  $\times g$  for 10 minutes at 4 °C to remove any particulates. Do not filter CSF samples. Either use immediately for analysis or aliquot and store frozen in small volumes at  $\leq -70$  °C. Repeated freeze/thaw cycles can result in incorrect concentration values. The CSF samples must be free of blood contamination which may compromise results.
4. Sample Dilution – Plasma

Dilute the clarified samples 1:10 using the Standard Diluent (e.g., for triplicates, transfer 40  $\mu\text{L}$  of clarified sample to the sample preparation plate and add 360  $\mu\text{L}$  Standard Diluent).
5. Sample Dilution - Cerebrospinal Fluid

Dilute the clarified samples 1:50 using the Standard Diluent (e.g., for triplicates, transfer 8  $\mu\text{L}$  of clarified sample to the sample preparation plate and add 392  $\mu\text{L}$  Standard Diluent.

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## Reagent Preparation

1. Warm all reagents to room temperature (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 (refer to Storage Instructions)
  - 4. Mix Amyloid Beta 1-40 Antibody Coated Beads on a rotisserie spin rotator, or manually by repeat inversion, for  $\geq 20$  minutes until all beads are resuspended.

## Initial Standard Stock Preparation

Reconstitute lyophilized standard in 250  $\mu$ L 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.

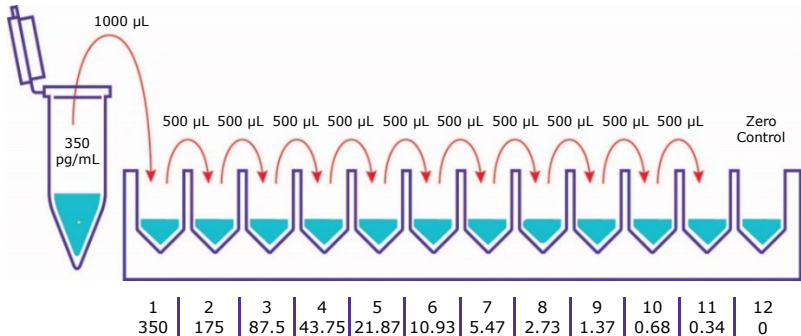
Refer to the standard value assignment on the Certificate of Analysis for the starting concentration of the Amyloid Beta 1-40 Standard in the vial.

Perform the necessary dilutions in Standard Diluent to achieve the final working concentration of 350 pg/mL in a 1.0 mL final volume.

## Standard Curve

Prepare the standard curve in a 12-channel reagent reservoir. Perform dilution using 1:2 serial dilutions of the 350 pg/mL Standard 1 for Standards 2 through 11 to achieve a curve from 350 pg/mL to 0.34 pg/mL. Standard 12 is the Blank (Standard Diluent only).

Run the standards in triplicate.



**NOTE:** Pipette gently into reservoir wells to avoid creating bubbles.

1. Add 500 µL Standard Diluent to wells 2 through 12 of a 12-channel reservoir dilution plate.
2. Transfer 1000 µL 350 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 11, mixing thoroughly each time. Use a fresh tip with each transfer.

# Assay Procedure

## Target Capture

1. Pipette 100  $\mu\text{L}$  of Standards or 1:10 diluted plasma samples or 1:50 diluted CSF Samples per well of 96 well assay plate.
2. Retrieve the Amyloid Beta 1-40 Coated Bead vial from the rotator and transfer its full contents to 11.0 mL of supplied Assay Buffer. Rinse the bead vial with 0.55 mL of fresh Assay Buffer and ensure that all beads have been transferred from the original vial. Mix by gentle inversion. There should be a total volume of 12.1 mL of diluted Amyloid Beta 1-40 Coated Beads.
3. Using a multichannel pipette, add 100  $\mu\text{L}$  per well of diluted Amyloid Beta 1-40  $\mu\text{L}$  Coated Beads into the assay plate.
4. Seal the assay plate with clear adhesive plate seal, applying pressure to the seal to prevent leaking and cross-contamination.
5. Incubate for 2 hours at 25 °C on microplate incubator/shaker set at approximately 650-875 rpm or Jitterbug™ setting #4.
6. A minimum of 10 minutes prior to the end of target capture incubation, prepare the Detection Antibody working stock:
  - Prepare 1X detection antibody by adding 250  $\mu\text{L}$  of 20X detection antibody into 4,750  $\mu\text{L}$  of Assay Buffer and filter the diluted detection antibody using the syringe with a 0.2  $\mu\text{m}$  filter into a clean tube.
7. When incubation is complete, centrifuge the assay plate at 1,100 x *g* for 1 minute, place the plate on the washer magnet, and carefully remove clear adhesive plate seal to avoid splashing.

## Post-Capture Wash

Wash plate once with a plate washer using the Post Capture Wash (POSTCAP) program on the Bio-Tek® 405 TSUVS washer.

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

## Detection Antibody Incubation

1. After removal from the plate washer, place the assay plate onto the sphere mag plate and allow beads to form a tight pellet at the well corners for 2 minutes.
2. Using a multichannel pipette, dispense 20  $\mu\text{L}$  per well of Detection Antibody using reverse pipetting without disturbing the bead pellets.
3. Seal the assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.

5. Incubate for 1 hour at 25 °C on microplate incubator/shaker set at approximately 1000 rpm or Jitterbug™ setting #5. Ensure plate is protected from light during this incubation.
6. When incubation is complete, centrifuge at 1,100 x g for 1 minute then carefully remove the clear adhesive plate seal to avoid splashing.

### Post-Detection Wash

Wash the assay plate 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.

### Post-Detection Shake

1. After 4 cycle Pre-Transfer wash, visually verify that each well contains ~200 µL of wash buffer.
2. Seal the assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.
3. Place the plate on the microplate/incubator shaker set at approximately 500-750 rpm or Jitterbug™ setting #3 for 2 minutes. **Ensure plate is protected from light during this incubation.**
4. Remove the plate from the shaker, and centrifuge at 1,100 x g for 1 minute. Carefully remove clear adhesive plate seal to avoid splashing and place it on the plate washer to perform Final Aspiration.

### Final Aspiration

Perform the Final Aspirate program (FINASP) program on the Bio-Tek® 405 TSUVS washer.

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

### Elution

1. After removal from the plate washer, place the assay plate onto the sphere mag plate and allow beads to form a tight pellet at the well corners.
2. Dispense 10 µL Elution Buffer B per well using reverse pipetting without disturbing the bead pellet.
3. Seal assay plate with a new clear adhesive plate seal. Apply pressure to the seal to prevent leaking and cross-contamination.
4. Incubate the plate for 10 minutes at 25 °C on microplate incubator/shaker set at 1000-1500 rpm or Jitterbug™ setting #5. **Ensure plate is protected from light during this incubation.**
5. When incubation is complete, centrifuge at 1,100 x g for 1 minute.

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## Assay Reading on the SMCxPRO® Immunoassay System

1. Place the assay plate with Elution Buffer B onto the sphere mag plate and allow beads to form a tight pellet for 2 minutes.
2. Keeping the assay plate on the magnet, carefully remove the adhesive plate seal. Using a multichannel pipette, add 10  $\mu\text{L}$  of Buffer D to center of wells containing Elution Buffer B. Use a fresh tip with each dispense.
3. Set a manual 12-channel pipette to 18  $\mu\text{L}$  and put 12 tips onto the pipettor. Transfer 18  $\mu\text{L}$  of neutralized eluate solution per well to corresponding wells of the SMCxPRO® reading plate, placed over the included plate holder by aspirating directly from the v-bottom of the plate, avoiding the pelleted beads, and changing tips with each dispensed row.
4. Seal the 384-well reading plate with new clear adhesive plate seal. Centrifuge plate for 1 minute at RT, approximately 1,100 x *g*. Remove the seal, inspect reading plate wells and remove bubbles if they are present.
5. Firmly seal the reading plate with aluminum plate seal using the recommend plate roller.
6. Remove the plate holder from the sealed reading plate and load it onto the SMCxPRO® Immunoassay System. Start read.

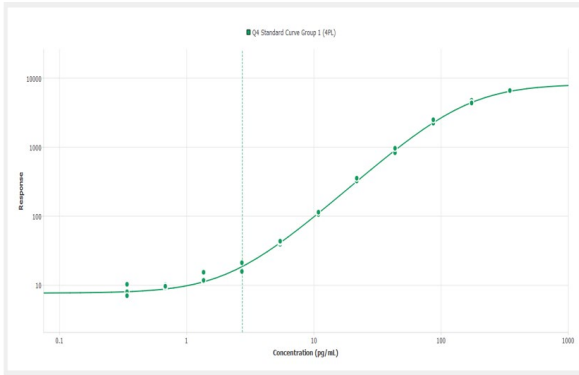
**Note:** There is a smart warm up period of up to 30 minutes to equilibrate plate temperature to internal instrument temperature. Once achieved the read will start automatically.



# Assay Characteristics

## Typical Reference Curve

Typical SMCxPRO® Immunoassay System Standard Curve is shown on below (not to be used to calculate data).



## 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 Amyloid Beta 1-40 is 2.73 pg/mL.

## Precision

The assay variations of SMC<sup>®</sup> Amyloid Beta 1-40 Immunoassay kits were studied using five normal plasma samples run in triplicate by 3 different operators on 3 different days.

- Mean intra-assay variation was <5%
- Mean inter-assay variation was <10%

## Cross-Reactivity

- Mouse and Rat plasma samples (n = 8) 100% detectability

## Spike Recovery

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

<b>Sample ID</b>	<b>Plasma Recovery (%)</b>	<b>CSF Recovery (%)</b>
Sample 1	117	101
Sample 2	114	98
Sample 3	103	94
Sample 4	109	105
Sample 5	109	100
Average	110	100

## Troubleshooting

<b>Problem</b>	<b>Probable Cause</b>	<b>Solution</b>
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.
	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 $\mu$ L or residual remaining in the well.
	Samples may have high particulate matter or other interfering substances	Samples should be processed according to the Assay Preparation section. Unprocessed samples could lead to higher imprecision.
Sample variability is high	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 (~650-1500 rpm).
	Cross-well contamination	<p>Ensure that the plate is sealed well at each incubation step. If splashing occurs on plate seal, centrifuge plate at 1,100 x <i>g</i> for 1 minute to remove material prior to removing the seal. A new plate seal should be used every time the plate is sealed.</p> <p>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.</p>

<b>Problem</b>	<b>Probable Cause</b>	<b>Solution</b>
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 mag plate Catalogue No. 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 $\mu$ L following the post-capture and final aspiration protocols.
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 or lipids.

# Well Map

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

For research use only. Not for use in diagnostic procedures.

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## Terms of Sale

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"PRODUCT" means SMCxPRO® Immunoassay Instrument, Cat. No. 95-0100-00, 70-0100-00, 95-0100-00-JPN.

"Commercial Product" means any product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

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- Using the PRODUCT as a component of a Commercial Product
- Reselling or licensing the PRODUCT
- Using the PRODUCT in clinical or therapeutic applications including producing materials for clinical trials
- Using the PRODUCT to provide a service to any third party
- Using the PRODUCT in collaboration or to enable a commercial entity
- Commercial Use of the PRODUCT to make a "home brew" assays, which includes a LDT (Lab Developed Test) assay(s) or any related commercial testing
- Transfer of PRODUCT to a site that is not the site where the Product was originally installed.

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