SMC™ Human IgE High Sensitivity Immunoassay Kit

Plate Assay

Catalog # 03-0201-00

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

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

Manufactured & Distributed by:

EMD Millipore Corporation
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St. Louis, Missouri 63103
United States of America
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INTRODUCTION

The Single Molecule Counting (SMC™) Human IgE High Sensitivity Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure IgE in human serum and plasma 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. These antibodies are separated during transfer to a final microplate. The reading plate is loaded onto 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 IgE present in the sample when captured. The amount of IgE in unknown samples is interpolated from a standard curve.

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SUPPLIES

The SMC™ Human IgE Immunoassay Kit includes all reagents listed in *Table 1: Reagents Provided*. Additional reagents and supplies are required to run this immunoassay and are listed in *Table 2: Additional Supplies Required (not provided)*. All reagents supplied are for Research Use Only.

Table 1: Reagents Provided

Item #	Description	Shipping Conditions	Storage Conditions	Component Part No.	Packaging Details
1	IgE Standard	With cold pack	2 - 8°C	02-8201-00	1 lyophilized vial
2	IgE Coating Antibody	With dry ice	-20 or -80°C	02-2201-00	1 x 20 µL
3	IgE Detection Antibody	With cold pack	2 - 8°C	02-1201-00	1 x 400 µL
4	Standard Diluent	With cold pack	2 - 8°C	02-9959-00	1 x 50 mL
5	Assay Buffer	With cold pack	2 - 8°C	02-9960-00	1 x 15 mL
6	10X Wash Buffer Note: Contains 0.05% Proclin	With cold pack	2 - 8°C	02-0001-09	2 x 60 mL
7	Coating Buffer	With cold pack	2 - 8°C	02-9958-00	1 x 20 mL
8	Blocking Buffer	With cold pack	2 - 8°C	02-9957-00	1 x 30 mL
9	Buffer 3	With cold pack	2 - 8°C	02-0554-00	1 x 5 mL
10	Buffer C	With cold pack	2 - 8°C	02-9962-00	1 x 6 mL
11	Elution Buffer B	With cold pack	2 - 8°C	02-0211-02	2 x 5 mL
12	Assay Plate	With cold pack	2 - 8°C	S30057-F	1 each

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.

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	Jitterbug™ Microplate Incubator/Shaker	EMD Millipore	70-0009-00	Incubating/Shaking at 25°C
4	VWR® Microplate Shaker	VWR International	12620-926	Plate shaking for overnight incubation, if recommended
5	Bio-Tek ELx™ 405 Microplate Washer	EMD Millipore	95-0004-05	Automated plate washing option
6	Centrifuge able to reach speed of 1,100 x g	--	--	Centrifuging samples, plates
7	Micro-Centrifuge	--	--	Centrifuge samples, provided Detection Antibody
8	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
9	12-Channel Reagent Reservoir (sterile)	Argos/Cole Parmer	04395-33	Standard curve dilution
10	VistaLab™ 25 mL Reservoirs	Fisher Scientific	21-381-27C	Addition of Reagents
11	MultiScreenHTS BV 96-Well Filter Plate	EMD Millipore	MSBVN1210	Sample filtration
12	96-well V-bottom plate	Fisher Scientific	14-222-241	Mixing eluate and neutralization buffer C
13	5 mL Luer-Lok™ Syringe	Fisher Scientific	14-829-45	Detection Antibody filtration
14	0.2 µm Syringe Filter	EMD Millipore	SLGPR33RS	Detection Antibody filtration
15	Nunc™ Clear Adhesive Plate Seal	Fisher Scientific	236366	Sealing assay plate
16	384-well round bottom plates	Fisher Scientific	12-565-384	Erenna® reading plate
17	Heat sealing foil	Fisher Scientific	NC0276513	Sealing plates for Erenna® reading
18	1L Stericup® Filter; 0.22 µm	EMD Millipore	S2GPU11RE	Filter sterilizing Erenna® system buffer
19	SMCxPRO™ 384-well plate, 1 plate with adhesive seal	EMD Millipore	02-1008-00	SMCxPRO™ reading plate, seal
20	SMCxPRO™ 384-well plate, case of 32	EMD Millipore	ABB2-00160A	SMCxPRO™ reading plate
21	SMCxPRO™ aluminum adhesive plate seals	Fisher Scientific	276014	SMCxPRO™ reading plate seals
22	Plate Roller	Fisher Scientific	NC9185793	Creates secure/even seal for each well of SMCxPRO™ reading plate
23	Universal plate cover	Fisher Scientific	253623	Covers assay plate

Additional Supplies Required (not provided) continued

Materials continued

Item #	Product Description	Supplier	Product Number	Product Uses
24	500 mL Container	--	--	Wash Buffer Dilution
25	Micro-centrifuge tubes	--	--	Sample storage, standard preparation

Reagents

Item #	Product Description	Supplier	Product Number	Product Uses
26	Elution Buffer (5 mL)	EMD Millipore	02-0002-04	Required for Erenna® maintenance
27	SMC™ 10X System/wash Buffer with Proclin (1 L)	EMD Millipore	02-0111-03	Use in Erenna® platform
28	De-ionized or Distilled water	--	--	Dilution of 10X System Buffer

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

TECHNICAL HINTS

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

Assay Hints

1. Wipe down bench and pipettes with 70% isopropanol before use.
2. It is important to allow all reagents to warm to room temperature (20 - 25°C).
3. Use sterile filter pipette tips and reagent trays to avoid contamination.
4. Pre-wet tips (aspirate and dispense within well) twice before each transfer.
5. The standards prepared by serial dilution must be used within 10 minutes of preparation.
    a. It is recommended that the standards are prepared as the last step prior to plate setup.
6. All washing must be performed with the wash buffer provided.
7. The recommended plate shaker setting is #5 to provide maximal orbital mixing without splashing liquid or causing cross-contamination.
8. After the assay is complete, the plate should be read immediately.
    a. For Erenna® Immunoassay System, use heat sealing plate foil.
    b. For SMCxPRO™ Immunoassay System – use adhesive seal.
9. The plates may be stored at 2-8°C for up to 48 hours away from light if same day reading is not possible.
    a. After the assay is complete, seal the plate before storing at 2 - 8°C
        i. For Erenna® Immunoassay System, use heat sealing plate foil
        ii. For SMCxPRO™ Immunoassay System – use aluminum adhesive plate seal
    b. Bring to RT then centrifuge the plate at 1,100 *x g* for 1 minute prior to reading.

Instrument Hints

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

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

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

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

PRECAUTIONS

- Use caution when handling biological samples. Wear protective clothing and gloves.
- Components of this reagent kit contain approximately 0.08% 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 Hazard Label

Ingredient, Cat #		Full Label	
02-8201-00	IgE Standard		**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.
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.

Full Hazard Label Continued

Ingredient, Cat #		Full Label	
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.
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.
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.
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.
    a. Allow Buffer 3 to equilibrate to room temperature *(crystals may be present at refrigerated temperature)*.
    b. Prepare 1X Buffer 3 by diluting 5 mL of provided Buffer 3 into 45 mL of deionized water and mix well.
    c. Allow stock IgE Coating Antibody to equilibrate to room temperature and mix well by pulse vortexing briefly.
    d. 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 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:
    a. Pour 50 mL of 10X Wash Buffer into a container capable of holding at least 500 mL. Add 450 mL of deionized water.
    b. Mix thoroughly by gentle inversion or with a clean, sterile stir bar.

    NOTE: 1X Wash Buffer may be filter sterilized *(refer to Storage Instructions)*
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).

ASSAY PREPARATION (continued)

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.

ASSAY PREPARATION (continued)

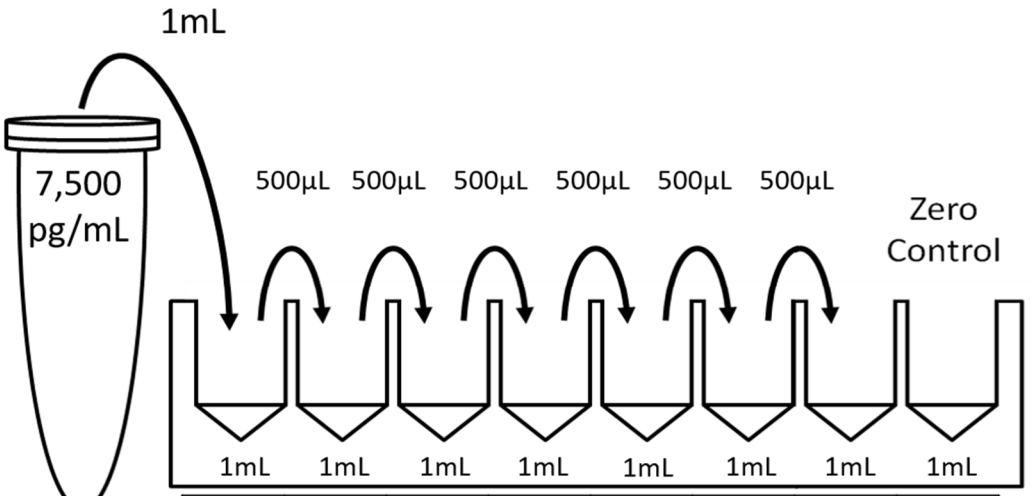
Initial Standard Stock Preparation

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 7,500 pg/mL in a 1.0 mL final volume.

Standard Curve

Prepare the standard curve in a 12-channel reagent reservoir. Perform 1:3 serial dilutions of the 7,500 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	2	3	4	5	6	7	8
7500	2500	833	278	93	31	10	0

1. Add 1 mL Standard Diluent to wells 2 through 8 of a 12-channel reservoir.
2. Transfer 1 mL 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 setting #5).

   For convenience, optional overnight incubation (16-18 hours) at 4°C can be performed on VWR® microplate shaker (setting #5)*. The next day, following overnight incubation, the plate should be equilibrated to room temperature by shaking for 20 minutes on microplate incubator/shaker* (Jitterbug setting #5)
5. Approximately 10 minutes prior to the end of target capture incubation, prepare the detection antibody using one of the following methods:
   a. 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.
   b. 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

a. BioTek ELx™ 405 (95-0004-05) or TS 405 washers are recommended.
b. For instructions regarding BioTek plate washer programming, please contact Customer Support.

Hand Wash

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

ASSAY PROCEDURE (continued)

Detection

1. After washing the plate, dispense 50 µL per well of Detection Antibody.
2. Seal assay plate with clear adhesive plate seal.
3. Incubate for 1 hour at 25°C on microplate incubator/shaker (Jitterbug setting #5).
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

a. BioTek ELx™ 405 (95-0004-05) or TS 405 washers are recommended.
b. For instructions regarding BioTek plate washer programming, please contact Customer Support.

Hand Wash

a. Dump liquid with firm motion and gently blot on paper towels.
b. Pipette 200 µL/well 1X Wash Buffer.
c. Dump liquid into waste with firm motion.
d. Repeat steps b. and c. two times.
e. 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.
2. Seal assay plate with a clear adhesive plate seal.
3. Incubate plate for 10 minutes at 25°C on microplate incubator/shaker (Jitterbug setting #5).

ASSAY READING

To read on the Erenna® Immunoassay System

1. Add 10 µL per well of Buffer C using reverse pipetting to Erenna® reading plate (Fisher Scientific PN 12-565-384) using a 12-channel manual P20.
2. Transfer 40 µL of eluate from assay plate to reading plate changing tips with each dispensed row.
3. Seal reading plate with clear adhesive plate seal. Centrifuge plate for 1 minute at RT, approximately 1,100 *x g*. Remove plate sealer, inspect reading plate wells and remove bubbles if they are present.
4. Seal reading plate with heat sealing foil (Fisher Scientific PN NC0276513) according to manufacturer's instructions for the heat sealer.
5. Load completed reading plate onto the Erenna® Immunoassay System.

To read on the SMCxPRO™ Immunoassay System

1. Add 10 µL per well of Buffer C using reverse pipetting to a new 96 well assay plate, using a 12-channel manual P20.
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 new assay plate (containing neutralized eluate) into Jitterbug and shake for 2 minutes at 25ºC (Jitterbug setting #5), then 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 SMCxPRO™ reading plate (EMD Millipore PN 02-1008-00). (*Ensure the reading plate is sitting on the included plate holder*)
6. Seal reading plate with clear adhesive plate seal. Centrifuge plate for 1 minute at RT, approximately 1,100 *x g*. Remove plate sealer, inspect reading plate wells and remove bubbles if they are present.
7. Firmly seal reading plate with aluminum adhesive plate seal using the recommended plate roller.
8. 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.

APPENDIX A: SMC™ Quick Assay Guide

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 or overnight at 4°C on microplate incubator/shaker.

2 hours 25°C/ Overnight 4°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.

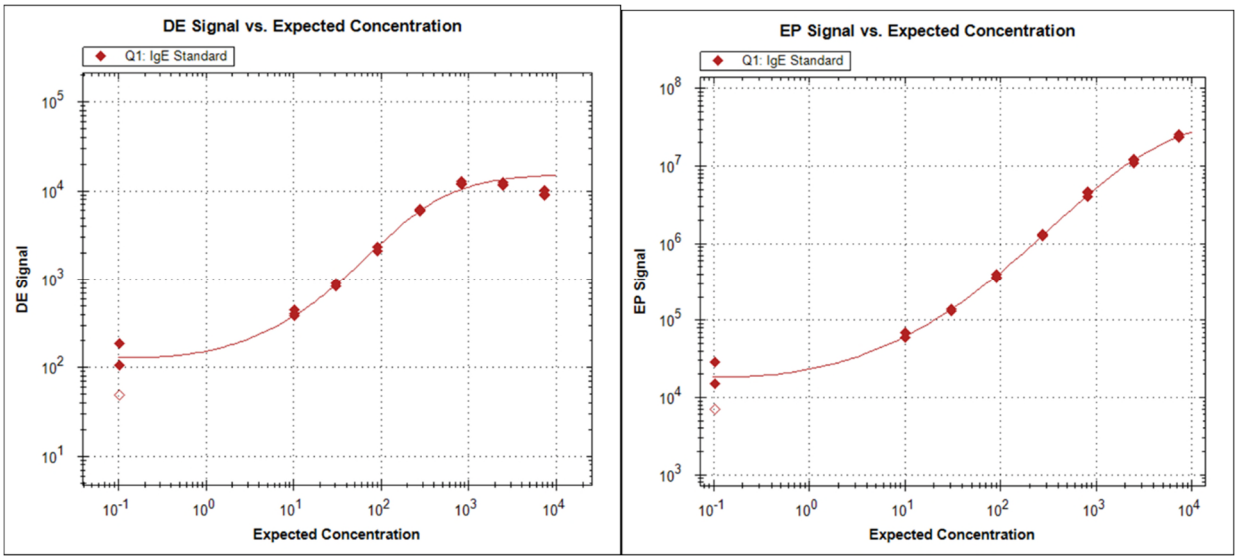
10 minutes 25°C

15. Neutralize 40 µL of eluted antibody with 10 µL Buffer C per well.
16. Transfer 40 µL of neutralized eluate to Reading Plate.
17. Seal the reading plate with pierceable foil for Erenna® or aluminum adhesive plate seal for SMCxPRO™

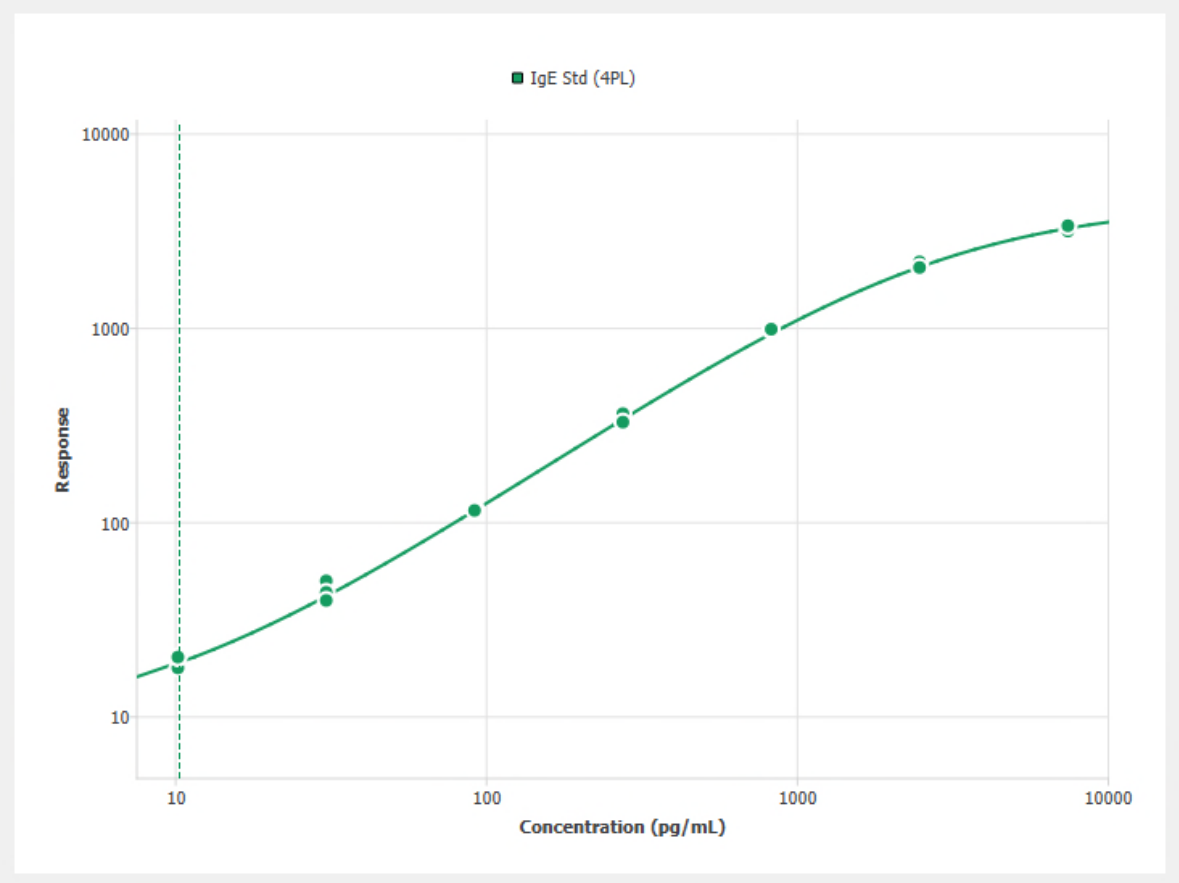
LOAD ON ERENNA® or SMCxPRO™ SYSTEM

GRAPH OF TYPICAL REFERENCE CURVE

Typical Erenna® Immunoassay System Standard Curve in DE and EP signal, not to be used to calculate data.



Typical SMCxPRO™ Immunoassay System Standard Curve, not to be used to calculate data.



ASSAY CHARACTERISTICS

A. 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).

B. Precision

The assay variations of SMC™ Human IgE 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%.

C. 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

D. Spike Recovery

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

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using seal appropriately. Pipette with multichannel pipets without touching reagent in plate. Change tips when adding reagents if cross contamination is expected.
		Ensure reagents (including wash and system buffers) are not contaminated.
	Instrument needs cleaning	See Technical Hints for appropriate Erenna® cleaning protocol.
	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. 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 Assay Preparation section. 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 liquid is in constant motion without causing splashing (~650 - 1000 RPM).
	Cross-well contamination	Ensure that the plate is sealed well at each incubation step. If splashing occurs on plate seal, centrifuge plate at 1,100 x g for 1 minute to remove material prior to removing the seal. A new plate seal should be used every time the plate is sealed.
		Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.
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.

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

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

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