SMC™ Glucagon High Sensitivity Immunoassay Kit

Microparticle Assay

Catalog # 03-0153-00

SMC™ Glucagon High Sensitvity Immunoassay Kit for the Quantitative Determination of Glucagon in Human, Mouse, and Rat 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 Single Molecule Counting (SMC™) Glucagon High Sensitvity Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure glucagon in human, mouse, and rat plasma samples. A capture antibody specific for glucagon has been pre-coated onto paramagnetic microparticles (beads). The user pipettes beads, standards, and samples into uncoated microplate wells. During incubation, the glucagon 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 glucagon that has been captured onto the beads, thus completing the immunosandwich. Elution buffer is then added and incubated. The elution buffer dissociates the bound protein sandwich from the beads 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 glucagon present in the sample when captured. The amount of glucagon in unknown samples is interpolated from a standard curve.

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SUPPLIES

The SMC™ Glucagon High Sensitvity 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	Assay Buffer	With cold pack	2 - 8°C	02-0212-00	2 x 20 mL
2	Glucagon Coated Beads	With cold pack	2 - 8°C	02-2153-00	1 x 550 µL
3	Standard Diluent	With cold pack	2 - 8°C	02-0216-00	2 x 20 mL
4	Glucagon Detection Antibody	With cold pack	2 - 8°C	02-1153-00	1 x 270 µL
5	Glucagon Standard	With cold pack	2 - 8°C	02-8153-00	1 lyophilized vial
6	10X Wash Buffer	With cold pack	2 - 8°C	02-0001-03	1 x 50 mL
7	Buffer D	With cold pack	2 - 8°C	02-0446-00	1 x 6 mL
8	Elution Buffer B	With cold pack	2 - 8°C	02-0211-02	1 x 5 mL

Storage Instructions

The SMC™ Glucagon 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® filter, EMD Millipore PN SCGPU11RE 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.

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	EMD Millipore	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
27	500 mL Container	--	--	Wash Buffer Dilution

Additional Supplies Required (not provided) continued

Reagents

Item #	Product Description	Supplier	Product Number	Product Uses
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

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 settings are between #3 - #7 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.

Component	Catalogue#	Full Label	
Standard Diluent	02-0216-00		**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.
Glucagon Standard	02-8153-00		**Warning.** 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.
10X Wash Buffer	02-0001-03		**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 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. Mix glucagon Antibody Coated Beads on a rotisserie spin rotator, or manually by repeat inversion, for ≥ 20 minutes until all beads are resuspended.

Sample Preparation

1. Sample Stability:

    - The recommended sample type for this assay is plasma collected in P800 tubes (or equivalent containing protease inhibitors for stabilization of metabolic markers) to adequately protect Glucagon. Carefully follow blood collection and handling directions provided by tube manufacturer.

    - Collected samples should be stored on ice temporarily.

        i. Aliquot samples while fresh and store samples at minus (-) 20^0C or minus (-) 80^0C.

        ii. Avoid multiple freeze thaw cycles.

2. 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*.
3. Sample dilution:
    - Dilute the clarified samples 1:10 using the Standard Diluent
      (e.g. for triplicates, transfer 40 µL of clarified sample to the sample preparation plate and add 360 µL Standard Diluent).
    - 100 µL per well of 1:10 diluted plasma should be used.

ASSAY PREPARATION (continued)

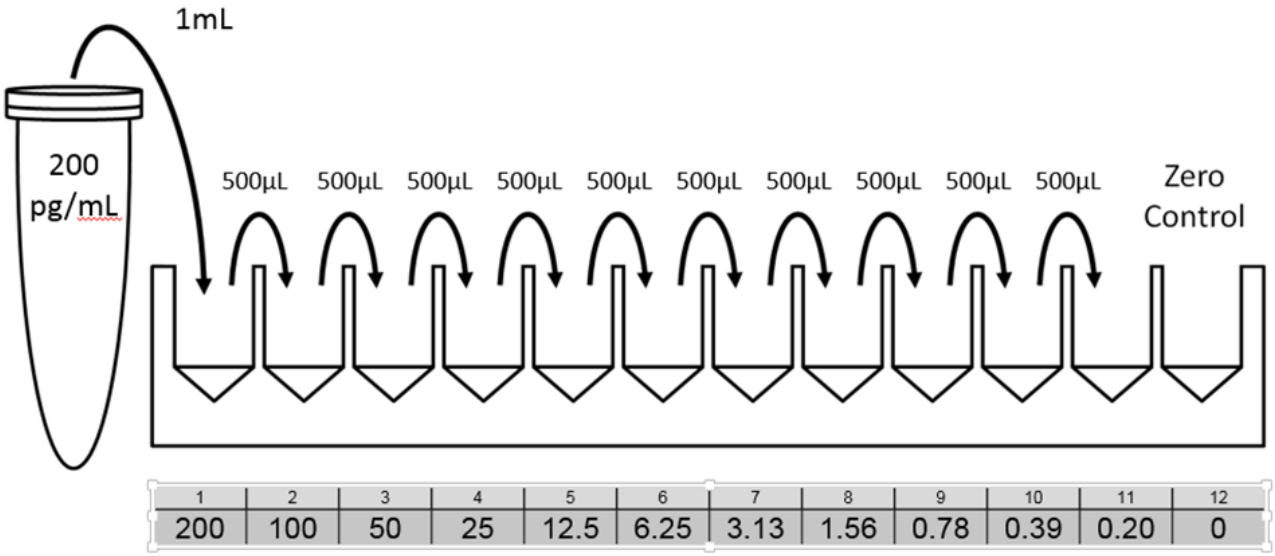
Initial Standard Stock Preparation

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 glucagon Standard in the vial.
3. Perform the necessary dilutions in Standard Diluent to achieve the final working concentration of 200 pg/mL in a 1.0 mL final volume.

Standard Curve

Prepare the standard curve in a 12-channel reagent reservoir. Perform 1:2 serial dilutions of the 200 pg/mL Standard 1 for Standards 2 through 11 to achieve a curve from 200 pg/mL to 0.20 pg/mL. Standard 12 is the Blank *(Standard Diluent only).*

Run the standards in triplicate.



1	2	3	4	5	6	7	8	9	10	11	12
200	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0

1. Add 500 µL Standard Diluent to wells 2 through 12 of a 12-channel reservoir dilution plate.
2. Transfer 1,000 µL 200 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 µL per well of Standards or 1:10 diluted plasma samples to assay plate.
2. Following mixing of the coated beads, immediately before adding to the assay plate, add the entire vial of coated Beads to 11.0 mL of supplied Assay Buffer. Rinse bead vial with 0.55 mL of Assay Buffer and ensure that all beads have been transferred. Mix by gentle inversion. There should be a total volume of 12.1 mL of diluted Coated Beads.
3. Pipette 100 µL per well of the Coated Beads into assay plate.
4. Seal assay plate with clear adhesive plate seal, apply pressure to seal to prevent leaking and cross-contamination.
5. Incubate for 2 hours at 25°C on microplate incubator/shaker (Jitterbug setting #4).

   Optional

   For convenience, 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 30 minutes on microplate incubator/shaker* (Jitterbug setting #4)
6. 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 250 µL of the centrifuged supernatant into 4,750 µL of Assay Buffer.
   b. Prepare 1X detection antibody by adding 250 µL of 20X detection antibody into 4,750 µL of Assay Buffer and filter the diluted detection antibody using the syringe with a 0.2 µm filter into a clean tube.
7. When incubation is complete, centrifuge at 1,100 *x g* for 1 minute and carefully remove clear adhesive plate seal to avoid splashing.

Post-Capture Wash

Wash plate once with a plate washer.

Plate Washer

a. BioTek; Post Capture Wash (POSTCAP) or
b. HydroFlex; Post Capture Wash (PCW)

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

ASSAY PROCEDURE (continued)

Detection

1. After removal from plate washer, dispense 20 µL per well of Detection Antibody without disturbing the bead pellet. *(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 setting #5).
4. After incubation, carefully remove clear adhesive plate seal to avoid splashing.

Post-Detection Wash

Wash assay plate 4 times with a plate washer.

Plate Washer

a. BioTek; 4 cycle Pre-Transfer (4CYCPRE) or
b. HydroFlex; 4 cycle Pre-Transfer (*4cyPrTra)*

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 assay plate with clear adhesive plate seal and apply pressure to the seal to prevent leaking and cross-contamination.
3. Place plate on microplate/incubator shaker for 90 seconds (Jitterbug setting #3).
4. Remove the plate from the Jitterbug, carefully remove clear adhesive plate seal to avoid splashing and place it on the plate washer to perform Final Aspiration.

Final Aspiration

Plate Washer

a. BioTek; Final Aspirate (FINASP)
b. HydroFlex; Final Aspirate (FA_V1)

Elution

1. Dispense 10 µL Elution Buffer B per well using reverse pipetting without disturbing the bead pellet. *(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 setting #5).

ASSAY READING

To read on the Erenna® Immunoassay System

1. Add 10 µL per well of Buffer D using reverse pipetting to Erenna® reading plate (Fisher Scientific PN 12-565-384) using a 12-channel manual P20.
2. Place assay plate onto sphere mag plate and allow beads to form a tight pellet for ≥ 2 minutes.
3. While keeping assay plate on the sphere mag plate, gently remove clear adhesive plate seal and transfer 10 µL of eluate from assay plate to reading plate by aspirating directly from the v-bottom of the plate, avoiding the pelleted beads, and changing tips with each dispensed row.
4. Seal reading plate with clear adhesive plate seal. Centrifuge plate for 1 minute at RT, approximately 1,100 *x g*.
5. Seal reading plate with heat sealing foil (Fisher Scientific PN NC0276513) according to manufacturer's instructions for the heat sealer.
6. Load completed reading plate onto the Erenna® Immunoassay System.

To read on the SMCxPRO™ Immunoassay System:

1. Add 10 µL per well of Buffer D using reverse pipetting to a fresh 96 well assay plate, using a 12-channel manual P20.
2. Place assay plate with Elution Buffer B onto sphere mag plate and allow beads to form a tight pellet for 2 minutes.
3. While keeping the assay plate containing eluate on sphere mag plate, gently remove clear adhesive seal and transfer 10 µL of eluate to the assay plate containing Buffer D by aspirating directly from the V-bottom of the plate, avoiding the pelleted beads, and changing tips with each dispensed row.
4. Seal this plate with a clear adhesive plate seal.
5. Place the plate (containing eluted, neutralized antibody solution) into Jitterbug and shake for 2 minutes at 25ºC (Jitterbug setting #5), centrifuge plate for 1 minute at RT, approximately 1,100 X g.
6. Gently remove clear adhesive plate seal and transfer 20 µL of neutralized eluate solution per well to corresponding wells of the SMCxPRO™ reading plate (EMD Millipore PN 02-1008-00), placed over the included plate holder.
7. 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.
8. Firmly seal reading plate with aluminum adhesive plate seal using the recommended plate roller.
9. 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 wait for the read plate to be close to the internal instrument temperature. Once achieved the read will start automatically.

APPENDIX A: SMC™ Quick Assay Guide

1. Prepare all reagents, standard curve, and samples as instructed.
2. Add 100 µL of Standard/ 1:10 diluted plasma samples and 100 µL of **Coated Beads** to **assay plate**.
3. Seal and incubate for 2 hours at 25°C or overnight (16-18 hours) at 4°C on appropriate microplate incubator/shaker.

2 hours 25°C / Overnight 4°C

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

1 hour 25°C

8. Perform Post-Detection Wash**.**
9. Perform Post-Detection Shake for 90 seconds on Jitterbug setting #3**.**
10. Perform Final Aspiration.
11. Remove from washer magnet and add 10 µL of **Elution Buffer B** to each well
12. Seal and incubate for 10 minutes at 25°C on microplate incubator/shaker.

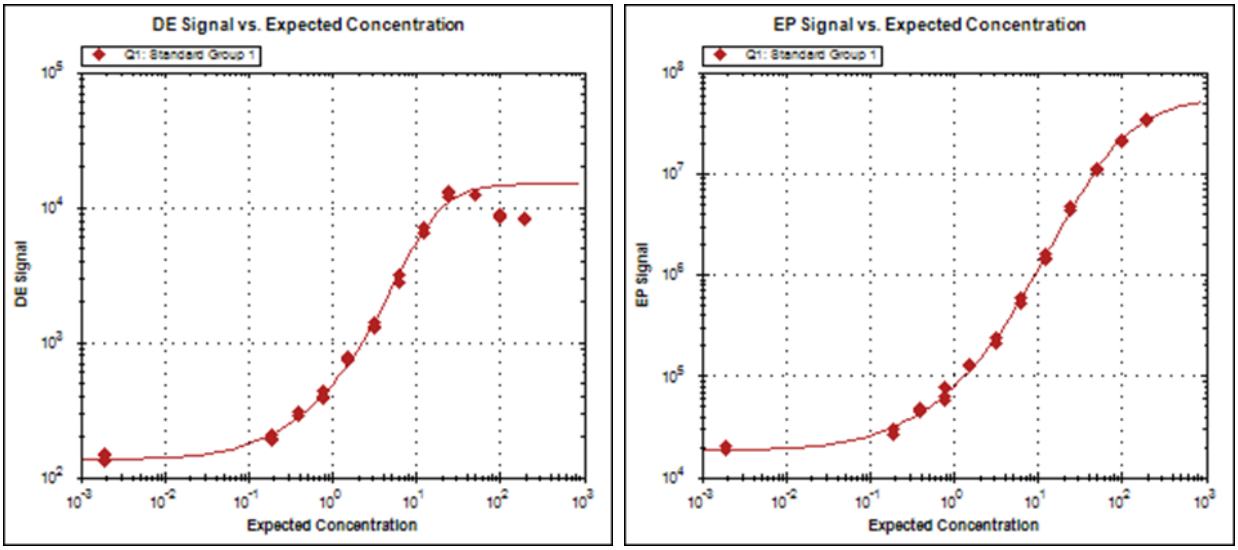
10 minutes 25°C

13. Neutralize eluted antibody**.**
14. Seal **reading plate** with pierceable foil for Erenna® or aluminum adhesive seal for SMCxPRO™.

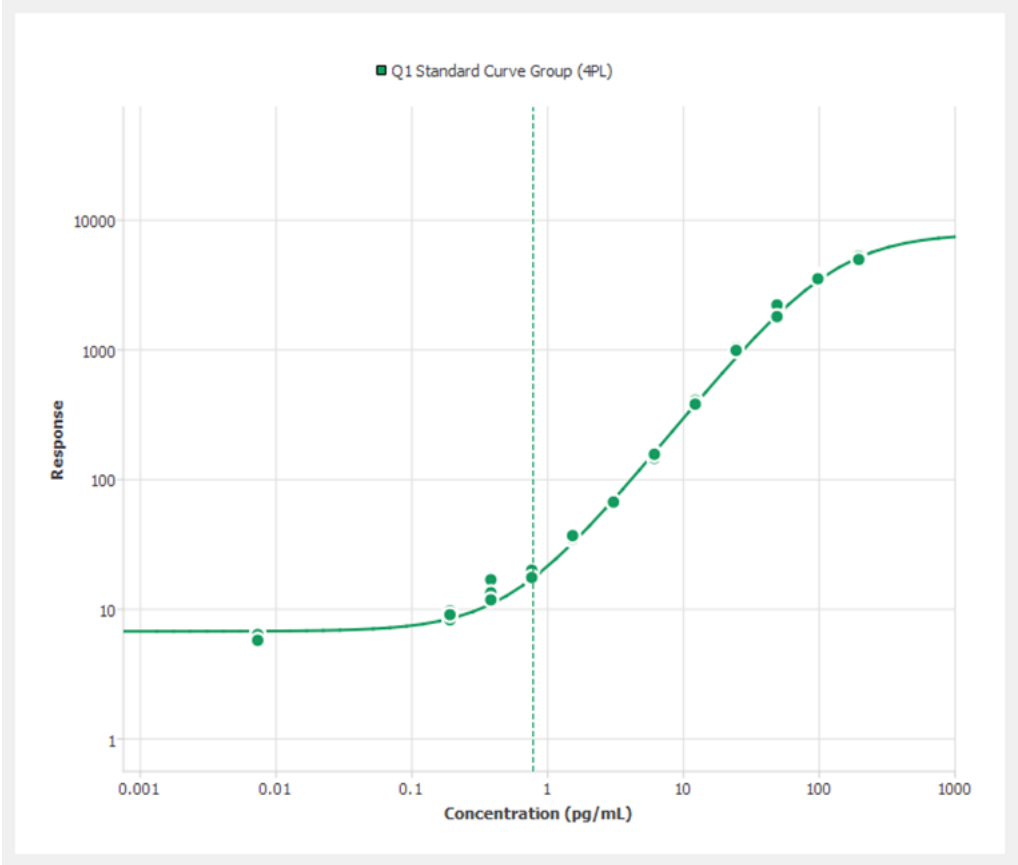
LOAD ON ERENNA® or SMCxPRO™ SYSTEM

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 glucagon is 0.781 pg/mL. The reported value is the average of multiple assays (n = 6).

B. Precision

The assay variations of EMD Millipore's SMC™ Glucagon High Sensitvity Immunoassay kit were studied using three different concentrations of glucagon (generated from serial dilutions of the SMC™ glucagon standard).

The mean intra-assay variation (n = 20) was calculated from a single assay for each of the three concentrations of glucagon standard. Mean intra-assay variation was < 15%.

The mean inter-assay variation (n = 6) was calculated from six independent assays of the same three dilutions of glucagon used to determine intra-assay variation. Mean inter-assay variation was < 20%.

C. Specificity/Cross-Reactivity

Glucagon (Human) was found to be 100% detectable.
Oxyntomodulin (Human) was found to be <0.5% detectable.
GLP-1 (Human) was not found to be detectable.
GLP-2 (Human) was not found to be detectable.
GIP (Human) was not found to be detectable.

D. Spike Recovery

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

Sample ID	Recovery %
Sample 1	85
Sample 2	87
Sample 3	85
Sample 4	88
Sample 5	84
Average	**86**

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.
		Insufficient washes—washer may need to be cleaned or reprogrammed.
	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 and Final Aspirate. Ensure that you have < 2 µL or residual remaining in the well.
	Samples may have high particulate matter or other interfering substances	Samples should be centrifuged or filtered according to the 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 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. 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.
Beads are lost during the wash	Plate washer needs optimization/cleaning	Contact Tech Support or local BCS to schedule washer programming. Refer to user guide for cleaning procedure.
	Insufficiently primed washer	Washer should be primed with wash buffer prior to running the post capture wash protocol.

TROUBLESHOOTING GUIDE (continued)

Problem	Probable Cause	Solution
Beads are lost during the wash (continued)	Beads came in contact with water	Washer should be primed with wash buffer sufficiently prior to plate wash. Viscosity of water changes the performance of the magnetic particles.
	Proper magnet was not used	Ensure that the mag plate (EMD Millipore PN 90-0003-02) was present on plate wash stage prior to running wash protocol.
Published LLoQ was not achieved	Improper dilution/reconstitution of the standard reference material	Confirm appropriate kit protocol was followed when preparing standard curve.
		Check plate washer to confirm no beads were lost during washes and that plate contains < 2 µL following the post-capture and final aspiration protocols.
		Ensure standards are prepared before starting capture incubation.
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.

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 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 2	Etc.									
E	Sample 1	Sample 2	Etc.									
F												
G												
H												

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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