Erenna®

Total GLP-1 Immunoassay Kit Instructions

Microparticle Assay

Catalog # 03-0025-06

Immunoassay kit for the quantitative determination of **Total Glucagon-like peptide-1 (GLP-1)** in human EDTA plasma

FOR RESEARCH USE ONLY

NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC PROCEDURES

Manufactured & Distributed by:

Singulex®

1701 Harbor Bay Parkway
Suite 200
Alameda, CA 94502
United States of America

Ph: (510) 995-9000
Fax: (510) 995-9090
info@singulex.com
www.singulex.com

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INTRODUCTION

The Erenna® Total GLP-1 Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure Total GLP-1 in human EDTA plasma samples. A capture antibody specific for human Total GLP-1 has been pre-coated onto paramagnetic microparticles (MPs). The user pipettes MPs, standards, and samples into uncoated microplate wells. During incubation, the Total GLP-1 present in the sample binds to the capture antibody on the coated MPs. Unbound molecules are washed away during the subsequent buffer exchange and wash steps. Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to Total GLP-1 that has been captured onto the MPs. During the following wash step the MPs are transferred to a clean plate. Elution buffer is then added and incubated. The elution buffer dissociates the bound protein sandwich from the MP surface, releasing the labeled antibodies. These antibodies are separated during transfer to a final microplate. The plate is loaded into the Erenna® System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of Total GLP-1 present in the sample when captured. The amount of Total GLP-1 in unknown samples is interpolated from a standard curve.

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MATERIALS

The Erenna® Total GLP-1 Immunoassay kit includes all reagents listed in Table 1: Reagents Provided. Additional reagents and supplies may be required to run this immunoassay, as listed in APPENDIX B: Additional Supplies Required and below in the section titled, General Supplies Required but Not Provided. All reagents supplied are for Research Use Only.

Reagents Provided

Item #	Description	Shipping Conditions	Storage Conditions	Component Part No.	Packaging Details
1	**Total GLP-1 Coated Beads Vial 1**	With cold pack	2-8°C	02-0477-01	1 x 500 µL
2	**Total GLP-1 Coated Beads Vial 2**	With cold pack	2-8°C	02-0716-00	1 x 500 µL
3	**Total GLP-1 Standard Diluent**	With cold pack	2-8°C	02-0291-04	1 x 20 mL
4	**GLP-1 Assay Buffer**	With cold pack	2-8°C	02-0287-01	1 x 15 mL
5	**Total GLP-1 Standard**	On dry ice	≤ -20°C	02-0478-00	1 x 20 µL
6	**Total GLP-1 Detection Antibody**	With cold pack	2-8°C	02-0292-00	1 x 20 µL
7	**Erenna® Total GLP-1 Immunoassay Kit Instructions**	N/A	Ambient	05-0331-06	1
8	**10X Wash Buffer**	With cold pack	2-8°C	02-0001-03	1 x 30 mL
9	**Elution Buffer B**	With cold pack	2-8°C	02-0211-02	1 x 5 mL
10	**Buffer D**	With cold pack	2-8°C	02-0359-00	1 x 3 mL

Table 1: Reagents Provided

Storage Instructions

The **Erenna® Total GLP-1** Immunoassay Reagent Kit should be stored at 2–8°C. The Standard analyte should be stored at ≤ -20°C. Proper kit performance can only be guaranteed if the materials are stored properly.

General Supplies Required

- De-ionized or distilled water
- 12-channel pipettes capable of transferring 20 µL- 250 µL
- 8-channel pipette capable of transferring 10 µL
- Micro-centrifuge tubes
- Micro-centrifuge
- Container capable of holding 300 mL
- 500 mL graduated cylinder
- Rotisserie rotator for microparticle resuspension
- If using an automated plate washer additional **10X Wash Buffer** may be needed.
- Two 96-well polypropylene plates labeled **Plate 1** and **Plate 2** (Axygen P-96-450V-C)
- One 384-well polypropylene plate labeled **Plate 3** (Nunc 264573)

TECHNICAL HINTS DUE TO HIGH SENSITIVITY

- Wipe down bench and pipettes with 70% isopropanol before use.
- Quickly spin concentrated standard before opening vials.
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Use filter tips while transferring concentrated standard.
- Use a 12-channel reservoir for preparing standards.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.

ADDITIONAL SAMPLE INFORMATION

- The Erenna® Total GLP-1 Immunoassay validation data has been compiled using human EDTA plasma.
- Ensure sample is clear of precipitants and other visible particulate matter before testing with the Erenna® Total GLP-1 Immunoassay.

PRECAUTIONS

- Use caution when handling biological samples; wear protective clothing and gloves.
- Components of this reagent kit contain approximately 0.1% sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.

ASSAY PREPARATION

Reagent Preparation

1. Warm the following reagents to room temperature prior to use: **Standard Diluent, Assay Buffer**, **Coated Beads**, **Elution Buffer B**, **Buffer D**, **Detection Antibody** and **10X Wash Buffer**.
2. Store the **Detection Antibody** away from light until ready to use.
3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
    a. Pour the contents of the 30 mL bottle of 10X Wash Buffer into a container capable of holding at least 300 mL
    b. Add 270 mL of deionized water
    c. Mix thoroughly by gentle inversion or with a clean, sterile stir bar
4. Mix **Total GLP-1 Coated Beads** (coated microparticles) on a rotisserie spin rotator, or manually by repeat inversion, for 10-20 minutes until all MPs are completely resuspended.

Sample Preparation

1. Prepare samples by one of the following methods:
    a. If using a filter plate with prefilter (Pall PN: 5041): Stack the Pall 5041 filter plate on top of a 96-well receptacle plate. Place 200 µL of sample into a filter plate well and spin for ≥ 10 minutes at 1,100 x *g*.
    b. If using a microcentrifuge: Centrifuge samples at >13,000 x *g* for 10 minutes immediately prior to use. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer.
2. Dilute the clarified samples 1:5 using the **Standard Diluent** (e.g., for triplicates, transfer 80 µL of clarified sample to the sample preparation plate and add 320 µL **Standard Diluent**). Mix thoroughly before transferring to assay plate.

Note: During data analysis, the interpolated value of the diluted sample needs to be multiplied by 5 in order to calculate the correct concentration of the analyte in the sample.

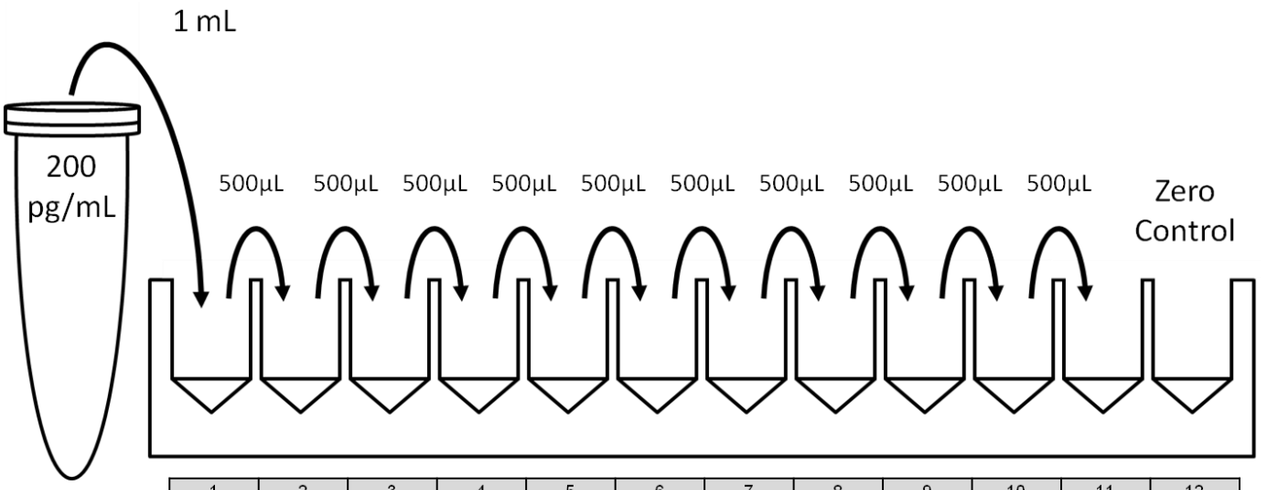
Initial Standard Stock Preparation

1. Quick spin and pipette mix the **Total GLP-1 Standard Analyte** vial in a mini-centrifuge prior to opening. Use care when opening this concentrated standard vial to prevent loss of materials and contamination of specimens or plates with aerosols.
2. Refer to the standard value assignment for the starting concentration of the **Total GLP-1 Standard Analyte** in the vial.
3. To make your **Analyte Working Stock**, perform the necessary serial dilutions to achieve the final working concentration of 200 pg/mL in a 1 mL final volume. Ensure that all pipetting steps transfer ≥10 µL of liquid to achieve the best precision.

TOTAL GLP-1 ASSAY PROCEDURE

Standard Curve

Prepare the standard curve in a 12-channel reservoir dilution plate. Perform 1:2 serial dilutions of the **Analyte Working Stock** for dilutions 2 through 11, to achieve a curve from 200 pg/mL to 0.10 pg/mL. Well 12 will not contain any standard analyte and will be run as your zero standard. 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. Add 1000 µL of the 200 pg/mL **Analyte Working Stock** from standard preparation into well 1.
3. Mix well 1 thoroughly then transfer 500 µL from well 1 into well 2. Change tips. Continue serial dilutions from well 2 stopping at well 11. **Use a fresh tip with each transfer**.

Target Capture

1. Pipette 100 µL per well of Standards or diluted Samples to **Plate 1** (96-well polypropylene).
2. Mix microparticles (MPs) by gentle inversion until all MPs are completely resuspended.
3. Immediately before adding to the assay plate, add the vial of **Total GLP-1 Coated Beads Vial 1** and **Total GLP-1 Coated Beads Vial 2** to 10.0 mL of the supplied **Assay Buffer**. Mix by gentle inversion. Ensure that all beads have been transferred.
4. Pipette 100 µL per well of the **Total GLP-1 Coated Beads** into **Plate 1**.
5. Cover **Plate 1** with an Axyseal plate cover.
6. Incubate for 2 hours at 25°C on Boekel Scientific, The Jitterbug™ setting 5.
7. Approximately 10 minutes prior to the end of Target Capture incubation, dilute concentrated **Total GLP-1 Detection Antibody** 1:10 by adding 10 µL of the detection antibody to 90 µL of **Assay Buffer**. Further dilute the **Total GLP-1 Detection Antibody** by adding 30 µL of the 1:10 dilution to 2970 µL of **Assay Buffer**. Filter the final diluted detection antibody using the syringe with a 0.2 µm filter into a clean tube.
8. When incubation is complete, carefully remove temporary plate cover to avoid splashing.

Post-Capture Wash

1. **Plate Washer**
    a. BioTek; Post Capture Wash (POSTCAP)
    b. HydroFlex; Post Capture Wash (PCW)
2. **Manual Post-Capture Wash Protocol**
    a. Place **Plate 1** onto magnet (Dynal MPC® - 96S)
    b. Wait 2 minutes for MPs to settle (ensure all MPs are amassed as a pellet by magnet)
    c. Aspirate the supernatant (MPs remain visible)
    d. Add 200 µL of **Wash Buffer**
    e. Wait ≥ 1 minute, to be sure that the MPs remain amassed
    f. Aspirate buffer
3. If using automation please contact your technical service representative, techsupport@singulex.com, for the appropriate automation procedure.

Detection

1. Immediately remove **Plate 1** from the magnet and add 20 µL per well of **Total GLP-1 Detection Antibody**.
2. Cover **Plate 1** with an Axyseal plate cover.
3. Incubate for 1 hour at 25°C on Jitterbug setting #5.
4. Carefully remove Axyseal plate cover to avoid splashing.

Pre-Transfer Wash

1. **Plate Washer**
    a. BioTek; 4 cycle Pre-Transfer (4CYCPRE)
    b. HydroFlex; 4 cycle Pre-Transfer (*4cyPrTra)*
2. **Manual Pre-Transfer Wash Protocol**
    a) Place **Plate 1** onto magnet
    b) Add 100 µL of **Wash Buffer** to each well of **Plate 1**
    c) Wait 2 minutes
    d) Aspirate the supernatant and discard into waste, change tips
    e) Add 200 µL of **Wash Buffer** to each well
    f) Wait ≥ 1 minute, to be sure that the MPs remain amassed, do not suspend or remove MP from the magnet during this time
    g) Aspirate buffer from each well, discard into waste and change tips
    h) Repeat steps e – g three more times for a total of four washes
    i) Add 200 µL of **Wash Buffer** to each well of **Plate 1**
    j) Remove **Plate 1** from magnet
3. If using automation please contact your technical service representative, techsupport@singulex.com, for the appropriate automation procedure.

Plate Transfer

1. **Manual Plate transfer Protocol**
    a) Prepare manual transfer station:
        a. Open multichannel pipette tip box
        b. Set manual 12 channel pipette to 100 µL
        c. Fill reservoir with **Wash Buffer**
        d. Plate **Plate 2** on a magnet
    b) Put clean tips onto 12 channel manual pipette.
    c) In the first row of **Plate 1**, pipette up and down 10 times to resuspend MPs gently to minimize bubbles.
    d) Transfer 200 µL (100 µL x 2) of suspended MPs from Row A of **Plate 1** to Row A of **Plate 2**.
    e) Change tips.
    f) Aspirate 100 µL of **Wash Buffer** from reservoir and dispense into Row A of **Plate 1**.
    g) Pipette up and down 10 times to resuspend any remaining MPs, then transfer 100 µL of suspended MPs to Row A of **Plate 2**.
    h) Change Tips.
    i) Repeat steps c-h for remaining 7 rows.
    j) Inspect **Plate 1** for any remaining MPs
        a. Yes, MPs are present in **Plate 1**:
            - Add 100 µL of **Wash Buffer** to wells containing MPs
            - Gently mix by pipetting to re-suspend the MP pellet.
            - Transfer the contents of each well containing MPs to **Plate 2** on magnet.
        b. No, MPs are not present in **Plate 1.**
    k) Discard Plate 1
    l) Magnetized MP pellet should be visible in **Plate 2.**
2. If using automation please contact your technical service representative, techsupport@singulex.com, for the appropriate automation procedure

Final Aspiration

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

Elution

1. Immediately remove **Plate 2** from the magnet.
2. Add 10 µL **Elution Buffer B** per well.
3. Cover **Plate 2** with an AxySeal plate cover.
4. Incubate plate for 10 minutes at 25°C on Jitterbug setting 5.
5. Add 10 µL per well of **Buffer D** to assay **Plate 3** (384-well polypropylene plate, Nunc, PN 264573) using a 12-channel manual P20.
6. Place **Plate 2** on bar magnet bed, remove AxySeal plate cover, and allow MPs to form a tight pellet for 2 minutes
7. Set manual 8 channel pipette to 15 µL and put 8 tips onto the pipettor. Transfer eluate to **Plate 3** by **columns**, avoiding the pelleted MPs.
8. Cover **Plate 3** with a Universal Plate Cover and spin plate for 5 minutes at RT, approximately 1,100 x *g*.
9. Cover **Plate 3** with Heat Sealing Foil, according to manufacturer instructions for the heat sealer.

Run on Erenna Immunoassay System

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

APPENDIX A: ERENNA® Quick Assay Guide

1. Prepare all reagents, standard curve, and samples as instructed.
2. Add 100 µL of Standard/ diluted Samples and 100 µL of **Coated Beads** to **Plate 1**.
3. Cover and incubate for 2 hours at 25°C on Jitterbug (setting 5).

2 Hours
25°C

4. Perform Post-Capture Wash (**Plate 1**).
5. Remove from magnet and add 20 µL of **Detection Antibody** per well.
6. Cover and incubate for 60 minutes at 25°C on Jitterbug (setting 5).

60 Minutes
25°C

1. Perform Pre-Transfer Post-Detection Wash (**Plate 1**).
2. Perform Manual Plate Transfer (**Plate 2).**
3. Perform Final Aspiration **(Plate 2).**
4. Remove from magnet and add 10 µL of **Elution Buffer B** to each well.
5. Cover and incubate at 25°C for 10 minutes on Jitterbug (setting 5).

10 Minutes
25°C

1. Add 10 µL **Buffer D** per well to **Plate 3.**
2. Transfer contents of **Plate 2** to **Plate 3**.
3. Cover and centrifuge for 5 minutes at 1,100 x *g*.
4. Cover assay **Plate 3** with pierceable plate seal cover.

LOAD ON ERENNA® SYSTEM

APPENDIX B: Additional Supplies Required (not provided)

Description	Mfr Supplier	Component Part Numbers	Product Uses	Packaging Detail
Erenna® 10X Systems Buffer	Singulex	02-0111-00	Systems (Analysis) Buffer, fluid used to run Erenna System	1 L (10 L mixed)
Erenna® 10X Wash Buffer	Singulex	02-0001-01	Wash buffer used for manual and automation wash protocols	1 L (10 L mixed)
Reservoirs for 12-Channel Pipetters	VWR	80092-466	Standard Curve	10/pkg
96-Well V-Bottom polypropylene Plate, 480 µL	Axygen	P-96-450V-C or P-96-450V-C-S	Assay Plate 1 and Plate 2, Receptacle plates	10 plates/unit 5 units/case
8-Well Low Profile Reservoir	VWR	12000-732	Transfer of Reagents	Variable
384-Well Round Bottom Polypropylene Plate, 120 µL	Nunc	264573	Assay Pate 3, analysis plate	20/pk or 120/cs
Syringe (5 mL)	VWR	66064-772 (or equivalent)	To filter diluted detection antibody	100 units/pk
0.2 µm Syringe Filter	Pall	4187	To filter diluted detection antibody	50/pk
AcroPrep™ 96-well Filter Plate (Supor Membrane)	Pall	5041	Alternate sample preparation	10/pkg
Universal Plate Cover	Nunc	253623	Cover the plate	25 units/pk
AxySeal—PCRSP Plate sealing film series	Axygen	PCR-SP	Sealing plates during incubation/ mix/store	100 films/ case
Dynal MPC® - 96S	Dynal™	120.27	Rare Earth Magnet to capture MP during wash	1 plate
Sphere Mag Plate -SBS Foot Print	Singulex	45-0036-00	Sphere magnet used to capture MPs during automated washes	1 plate
Centrifuge w/ Plate Rotor	- - -	- - -	Remove MP via filter plate ~1,100 xg	1
Vacuum Pump	Welch	2511B-01	Degassing systems buffer	1
Microplate Incubator / Shaker	Boekel Scientific	130000 The Jitterbug™	Incubating plate	1
Heat Sealing Plate Foil	Singulex	01-0216-00 or equivalent	Sealing plate for analysis on Erenna	- - -
Heat Sealer with adjustable temperature and time	FluidX	XTS-384 or equivalent	Sealing plate for analysis on Erenna	1
Universal Adapter for SBS format plates	FluidX	42-1001 or equivalent	Required for proper sealing on XTS-384	1

CONTACT INFORMATION

To reach Singulex, Inc. reagent technical support, call **(510) 995-9000 ext. 3**, or in the U.S. you may call us toll-free at (888) 603-3033.

You can also send us an e-mail at **techsupport@singulex.com**

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Singulex®

1701 Harbor Bay Parkway, Suite 200
Alameda, CA 94502
United States of America

Phone: (510) 995-9000
Fax: (510) 995-9090
www.singulex.com
email: info@singulex.com