User Guide

High Sensitivity GLP-1 Active Chemiluminescent ELISA Kit

96-Well Plate

EZGLPHS-35K
EZGLPHS-35BK

Intended Use

This High Sensitivity GLP-1 Active ELISA kit is used for the non-radioactive quantification of GLP-1 (7-36) in serum, plasma, and cell culture. The GLP-1 sequence is highly conserved between the species, with no sequence variation occurring in mammals. One kit is sufficient to measure 38 unknown samples in duplicate.

This kit is for Research Use Only. Not for Use in Diagnostic Procedures.

Principles of Assay

This assay is a Sandwich ELISA based on:

- Capture of GLP-1 Active molecules from samples by a specific anti-GLP-1 polyclonal antibody and immobilization of the resulting complex to the wells of a microtiter plate coated by a pre-titered amount of anchor antibodies
- Wash away of unbound materials from samples
- Binding of a biotinylated anti-GLP-1 monoclonal antibody to the captured molecules
- Wash away of unbound materials from samples
- Conjugation of horseradish peroxidase to the immobilized biotinylated antibodies
- Wash away of free enzyme
- Quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in a luminometer at ~425 nm in the presence of a chemiluminescent substrate

The enzyme activity is measured by the increased relative light units (RLU). Since the increase in RLU is directly proportional to the amount of captured GLP-1 Active in the unknown sample, the concentration of GLP-1 Active can be derived by the interpolation from a reference curve generated in the same assay with reference standards of known concentrations of GLP-1 Active.

Reagents Supplied

Each kit is sufficient to run one 96-well plate and contains the following reagents:

Note: Store all reagents at 2-8 °C.

Reagents Supplied	Volume	Quantity	Catalogue Number
Microtiter Plate with 2 plate sealers Coated with pre-titered anchor antibodies	-	1 plate 2 sealers	EPDARW
10X HRP Wash Buffer Concentrate 10X concentrate of 50 mM Tris Buffered Saline containing Tween®-20	50 mL each	2 bottles	EWB-HRP
GLP-1 Standard	0.5 mL	1 vial	E8035-HS
GLP-1 Quality Controls 1 and 2	0.5 mL	1 vial each	E6035-HS
Serum Matrix Solution	1.5 mL	1 vial	EMTX-SM
Assay Buffer 0.05 M phosphosaline, pH 7.4, containing 0.08% sodium azide, and 1% BSA	12 mL	1 bottle	EABGLP
GLP-1 Capture Antibody	3 mL	1 bottle	E1035-CHS
GLP-1 Detection Antibody	12 mL	1 bottle	E1035-DHS
Enzyme Solution Pre-titered streptavidin-horseradish peroxidase conjugate in buffer	12 mL	1 bottle	EHRP-6
Substrate Solution A Stable Peroxide Minimize the exposure to light.	6 mL	1 bottle	ESS-A
Substrate Solution B Luminol/Enhancer Minimize the exposure to light.	6 mL	1 bottle	ESS-B
Mixing Bottle For mixing Substrate Solution A and Substrate Solution	-	1 bottle	-

Storage and Stability

Recommended storage for kit components is 2-8 °C.

All components are shipped and stored at 2-8 °C. Reconstituted standards and controls can be frozen for future use but repeated freeze/thaw cycles should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

Reagent Precautions

Sodium azide or Proclin™ has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide and Proclin™ may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Note: See Full Labels of Hazardous components on next page.

Symbol Definitions

Ingredient	Cat. No.	Label	
GLP-1 HS ELISA Quality Controls 1 & 2	E6035-HS		**Warning**. Harmful if swallowed. Causes serious eye irritation. Toxic 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.
GLP-1 HS ELISA Standard	E8035-HS		**Warning.** Harmful if swallowed. Causes serious eye irritation. Toxic 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.
Substrate Solution B	ESS-B		**Danger.** Causes serious eye irritation. May damage fertility or the unborn child. Obtain special instructions before use. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Get medical advice/attention.
10X HRP Wash Buffer Concentrate	EWB-HRP		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

Materials Required (Not Provided)

- Multi-channel pipettes and pipette tips: 5-50 µL and 50-300 µL
- Pipettes and pipette tips: 10-20 µL or 20-100 µL
- Buffer and Reagent Reservoirs
- Vortex mixer
- De-ionized water
- Luminometer capable of measuring Reflective Light Units at ~425 nm
- Orbital microtiter plate shaker
- Absorbent paper or cloth
- DPP-IV inhibitor for sample preparation

Sample Collection and Storage

Preparation of Serum Samples

1. To prepare serum, whole blood is directly drawn into a Vacutainer® serum tube that contains no anti-coagulant. Immediately (< 30 seconds) after collection, add appropriate amount of DPP-IV inhibitor according to manufacturer's instructions. Invert tube to mix. (If using Cat. No. DPP4, add 10 µL DPP-IV inhibitor per milliliter of blood.) Let blood clot at room temperature for 30 minutes.

2. Promptly centrifuge the clotted blood at 2,000 to 3,000 x *g* for 15 minutes at 4 ±2 °C.

Preparation of Plasma Samples

1. To prepare plasma sample, whole blood should be collected into a Vacutainer® EDTA-plasma tube. Immediately (< 30 seconds) after collection, add appropriate amount of DPP-IV inhibitor. Invert tubes to mix followed by immediate centrifugation. Observe same precautions in the preparation of serum samples.

2. Specimens can be stored at 4 °C if they will be tested within 3 hours. For longer storage, specimens should be stored at –80 °C. Avoid multiple (> 3) freeze/thaw cycles. Aliquot samples before freezing if necessary.

3. If heparin is to be used as anti-coagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.

4. Avoid using samples with gross hemolysis or lipemia.

GLP-1 Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the GLP-1 Standard with 0.5 mL distilled or de-ionized water. Invert and mix gently, let sit for 5 minutes then mix well.

2. Label six tubes as 1, 2, 3, 4, 5 and 6. Add 200 µL Assay Buffer to each of the six tubes. Perform 3-fold serial dilutions by adding 100 µL of reconstituted Standard to Tube 6, mix well and transfer 100 µL from Tube 6 to Tube 5, mix well and transfer 100 µL from Tube 5 to Tube 4, mix well and transfer 100 µL from Tube 4 to Tube 3, mix well and transfer 100 µL from Tube 3 to Tube 2, mix well and transfer 100 µL from Tube 2 to Tube 1. Mix well.

   Note: Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of reconstituted standard should be stored in small aliquots at ≤ –20 °C. Avoid multiple freeze/thaw cycles (> 2).

Tube #	Volume of Deionized Water to Add	Volume of Standard to Add	Standard Stock Concentration
Reconstituted Standard	0.5 mL	0	X (refer to analysis sheet for exact concentration)

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration (pM)
Tube 6	0.2 mL	0.1 mL of Reconstituted Standard	X/3
Tube 5	0.2 mL	0.1 mL of Tube 6	X/9
Tube 4	0.2 mL	0.1 mL of Tube 5	X/27
Tube 3	0.2 mL	0.1 mL of Tube 4	X/81
Tube 2	0.2 mL	0.1 mL of Tube 3	X/243
Tube 1	0.2 mL	0.1 mL of Tube 2	X/729

GLP-1 Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each GLP-1 Quality Control 1 and Quality Control 2 with 0.50 mL distilled or de-ionized water and gently invert to ensure complete hydration. Mix well. Unused portions of the reconstituted Quality Controls should be stored in small aliquots at ≤ –20 °C. Avoid further freeze/thaw cycles (> 2).

Preparation of Substrate Solution

Prior to use, mix the entire content of Substrate Solution A (6 mL) and Substrate Solution B (6 mL), or at a 1:1 ratio in the mixing bottle provided, and mix thoroughly. The working solution is stable for ~8 hours at room temperature in dark. Avoid prolonged exposure to the sun or any other intense light source. Short-term exposure to typical laboratory lighting will not harm the working solution. Any remaining working substrate solution should be discarded after use and should not be re-used.

High Sensitivity GLP-1 Active Elisa Assay Procedure

Warm all reagents to room temperature before setting up the assay.

1. Add 300 µL diluted Wash Buffer to each well of the plate. Decant wash buffer and remove the residual volume by inverting the plate and tapping it smartly onto absorbent towels several times. Repeat procedure 2 additional times. Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.

2. Add 50 µL of appropriate Matrix Solution to Blank, Standards and Quality Control wells (refer to Microtiter Plate Arrangement for suggested sample order placement). When assaying serum or plasma, use EMTX-SM. When assaying tissue culture or other supernatant, use proper control culture medium as the matrix solution.

3. Add 50 µL Assay Buffer to each of the Blank and Sample wells.

4. Add 50 µL Standards or Controls to the appropriate wells.

5. Add 50 µL of Sample to the appropriate wells.

6. Add 20 µL GLP-1 Capture Antibody to each well.

7. Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 400-500 rpm.

8. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

9. Add 100 µL Detection Antibody to each well. Re-cover plate with sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400-500 rpm.

10. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

11. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

12. Remove sealer, decant reagents from the plate and tap plate to remove the residual volume. Wash wells 6 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

13. Add 100 µL of working Substrate Solution to each well and shake on plate shaker for 1 minute.

14. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Measure relative light units at ~425 nm in a luminometer plate reader within 5 minutes after adding the substrate solution if comparisons of standard curve signals between assays are important. Longer periods between adding the substrate and evaluating the plate may result in significantly decreased signal intensity. However, the calculated sample results will not be affected even if the reading time is delayed to 25 minutes after substrate addition.

Assay Procedure for High Sensitivity GLP-1 Active ELISA Kit

	Step 1	Step 2	Step 3	Step 4-5	Step 6	Step 7-8	Step 9	Step 9-10	Step 11	Step 11-12	Step 13-14		
Well #	Wash plate 3X with 300 µL 1X Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels	Matrix Solution	Assay Buffer	Standards/QCs /Samples	Capture Antibody	Seal, Agitate, Incubate 2 hrs at Room Temperature. Wash 3X with 300 µL Wash Buffer.	Detection Antibody	Seal, Agitate, Incubate 1 hour at Room Temperature. Wash 3X with 300 µL Wash Buffer.	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature. 6X with 300 µL Wash Buffer.	Substrate	Seal, Agitate, Incubate 1 minute at Room Temperature.	Read Reflective Light Units (RLU) at ~425 nm.
A1, B1		50 µL	50 µL	--	20 µL		100 µL		100 µL		100 µL		
C1, D1		50 µL	-	50 µL of Tube 1									
E1, F1		50 µL	-	50 µL of Tube 2									
G1, H1		50 µL	-	50 µL of Tube 3									
A2, B2		50 µL	-	50 µL of Tube 4									
C2, D2		50 µL	-	50 µL of Tube 5									
E2, F2		50 µL	-	50 µL of Tube 6									
G2, H2		50 µL	-	50 µL of Tube 7									
A3, B3		50 µL	-	50 µL of QC1									
C3, D3		50 µL	-	50 µL of QC2									
E3, F3		--	50 µL	50 µL of Sample 1									
G3, H3		--	50 µL	50 µL of Sample 2									

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Microtiter Plate Arrangement

High Sensitivity GLP-1 Active ELISA

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

Calculations

The dose-response curve of this assay fits best to a sigmoidal 4- or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function.

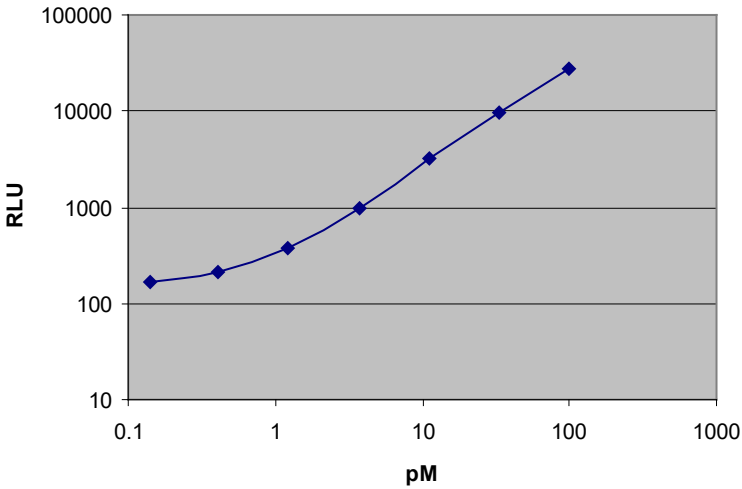
Note: When sample volumes assayed differ from 50 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (For example, if 25 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 50 µL, compensate the volume deficit with Matrix Solution.

Interpretation

- The assay will be considered accepted when all Quality Control values fall within the calculated QC range.
- If the difference between duplicate results of a sample is > 15% CV, repeat the sample.
- The limit of sensitivity of this assay is 0.14 pM (50 µL sample size).
- The approximate range of this assay is 0.14 pM to 100 pM (50 µL sample size). Any result greater than 100 pM in a 50 µL sample should be diluted using Matrix Solution and the assay repeated until the results fall within range.

Graph of Typical Reference Curve

High Sensitivity GLP-1 Active ELISA



Typical Standard Curve, not to be used to calculate data.

Specificity

The lowest level of GLP-1 Active that can be detected by this assay is 0.14 pM using a 50 µL sample size.

GLP-1(7-36)	100%
GLP-1(7-37)	72%
GLP-1(1-36)	<3%
GLP-1(1-37)	<2%
GLP-1(9-36)	*ND
GLP-2	*ND
GIP	*ND
Glucagon	*ND
Oxyntomodulin	*ND

*ND – Not detected

Precision

Intra-Assay Variation

	Mean GLP-1 Levels (pM)	Intra-Assay %CV
1	4.5	3%
2	22.1	6%

Inter-Assay Variation

	Mean GLP-1 Levels (pM)	Intra-Assay %CV
1	4.5	13%
2	21.4	10%

The assay variations of our High Sensitivity GLP-1 Active ELISA kits were studied on two samples at two levels on the GLP-1 standard curve. The mean intra-assay variation was calculated from results of eight determinations of the indicated samples. The mean inter-assay variations of each sample were calculated from results of eight separate assays with duplicate samples in each assay.

Spike Recovery of GLP-1 Active in Assay Samples

Sample	GLP-1 Added (pM)	Expected (pM)	Observed (pM)	Recovery
	1.2	2.2	1.9	86%
1	3.7	4.7	4.2	89%
	11.1	12.8	11.8	98%
	1.2	2.4	2.1	91%
2	3.7	4.8	4.0	83%
	11.1	12.2	10.9	89%
	1.2	1.7	1.5	89%
3	3.7	4.2	3.4	82%
	11.1	11.6	10.1	88%
	1.2	2.0	1.8	92%
4	3.7	4.5	3.9	89%
	11.1	11.9	11.3	96%
	1.2	2.8	2.6	93%
5	3.7	5.3	4.7	90%
	11.1	12.7	12.8	101%
Average				90

Varying amounts of human GLP-1 Active were added to individual human serum and plasma samples and the resulting GLP-1 Active content of each sample was assayed by GLP-1 Active ELISA. The recovery = [(Observed GLP-1 Active / (spiked GLP-1 Active concentration + Basal GLP-1 Active level)] x 100%.

Linearity of Sample Dilution

Sample	Volume (µL)	Expected (pM)	Observed (pM)	Expected
1	50	6.9	6.9	-
	25	3.5	3.4	100%
	12.5	1.7	1.5	87%
	6.3	0.9	0.8	89%
2	50	8.2	8.2	-
	25	4.1	4.5	109%
	12.5	2.1	2.2	106%
	6.3	1.0	1.1	108%
3	50	9.3	9.3	-
	25	4.7	4.9	106%
	12.5	2.3	2.3	98%
	6.3	1.2	1.1	91%
4	50	7.4	7.4	-
	25	3.7	3.2	88%
	12.5	1.9	1.7	91%
	6.3	0.9	0.8	82%
5	50	11.4	11.4	-
	25	5.7	5.4	94%
	12.5	2.9	2.4	84%
	6.3	1.4	1.5	105%
Average				96

Five human serum and plasma samples with the indicated sample volumes were assayed. Required amounts of serum matrix were added to compensate for lost volumes below 50 µL. The resulting dilution factors of neat, 2, 4 and 8 representing 50 µL, 25 µL, 12.5 µL and 6.3 µL sample volumes assayed, respectively, were applied in the calculation of observed GLP-1 Active concentrations.
% expected = (observed/expected) x 100%.

Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert, or available at our website SigmaAldrich.com.

Troubleshooting

- To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
- Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
- Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
- Avoid cross contamination of any reagents or samples to be used in the assay.
- Make sure all reagents and samples are added to the bottom of each well.
- Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
- Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
- High signal in background or blank wells could be due to:
    - cross well contamination by standard solution or sample, or
    - inadequate washing of wells with Wash Buffer, or
    - overexposure to light after substrate has been added

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

Replacement Reagents

Reagents	Catalogue Number
Microtiter Plates	EPDARW
10X HRP Wash Buffer Concentrate	EWB-HRP
GLP-1 ELISA Standard	E8035-HS
GLP-1 Quality Controls 1 and 2	E6035-HS
Matrix Solution	EMTX-SM
Assay Buffer	EABGLP
GLP-1 Active ELISA Capture Antibody	E1035-CHS
GLP-1 Active ELISA Detection Antibody	E1035-DHS
Enzyme Solution	EHRP-6
Substrate Solution A	ESS-A
Substrate Solution B	ESS-B
10-pack of High Sensitivity GLP-1 Active Chemiluminescent ELISA Kits	EZGLPHS-35BK

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