User Guide

GLP-1 Total ELISA Kit

96-Well Plate

EZGLP1T-36K
EZGLP1T-36BK

Intended Use

This GLP-1 Total ELISA kit is used for the non-radioactive quantification of GLP-1 (7-36 and 9-36) in serum, plasma, and cell tissue culture. The GLP-1 sequence is highly conserved between the species, with no sequence variation occurring in mammals. One kit is sufficient to measure 39 unknown samples in duplicate.

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

This kit requires 50 µL sample volume. For mouse and rat samples, 20 µL sample volume may be used.

Principles of Assay

This assay is a Sandwich ELISA based, sequentially, on:

- capture of GLP-1 Total molecules from samples to the wells of a microtiter plate coated by a pre-titered amount of anti- GLP-1 polyclonal antibody
- 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 conjugates
- quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3’,5,5’-tetramethylbenzidine

The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured GLP-1 Total in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of GLP-1.

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
GLP-1 Total ELISA Microtiter Plate with plate sealer	-	1 plate 2 sealers	EP36
10X HRP Wash Buffer Concentrate	50 mL/each	2 bottles	EWB-HRP
GLP-1 Standard	0.5 mL/vial Lyophilized	1 vial	E8036-K
GLP-1 Quality Controls 1 and 2	0.5 mL/vial Lyophilized	1 vial each	E6036-K
Serum Matrix Solution	1.5 mL	1 vial	EMTX-PS
Assay Buffer	12 mL	1 bottle	EABGLP
GLP-1 Detection Antibody	12 mL	1 bottle	E1036
Enzyme Solution	12 mL	1 bottle	EHRP
Substrate Solution (Minimize the exposure to light)	12 mL	1 vial	ESS-TMB
Stop Solution	12 mL	1 vial	ET-TMB

Storage and Stability

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

Sodium azide has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide 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.

Hydrochloric Acid

Hydrochloric acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eye. Do not swallow or ingest.

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

Symbol Definitions

Ingredient	Cat. No.	Label	
GLP-1 Quality Controls 1 & 2	E6036-K		**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 Standard	E8036-K		**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.
Stop Solution	ET-TMB		**Warning.** May be corrosive to metals.
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
- Microtiter Plate Reader capable of reading absorbency at 450 nm and 590 nm
- Orbital microtiter plate shaker
- Absorbent paper or cloth

Sample Collection and Storage

This kit requires 50 µL sample volume. For mouse and rat samples, 20 µL of sample volume may be used. However, the sample will need to be multiplied by 2.5 for final concentration.

Preparation of Serum Samples

1. To prepare serum, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 min.
2. Promptly centrifuge the clotted blood at 2,000 to 3,000 x *g* for 15 minutes at 4 ±2 °C.
3. Transfer and aliquot serum samples in separate tubes of small quantity. Date and identify each sample.
4. Use freshly prepared serum or store samples at –20 °C for later use. Avoid multiple (> 5) freeze/thaw cycles.

Preparation of Plasma Samples

1. To prepare plasma sample, whole blood should be collected into a centrifuge tube containing enough K_3EDTA to achieve a final concentration of 1.735 mg/mL, followed by immediate centrifugation. Observe same precautions in the preparation of serum samples.
2. 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.
3. 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 to give a concentration described on the analysis sheet. Invert and mix gently until completely in solution.

2. Label five tubes as 1, 2, 3, 4, and 5. Add 200 µL Assay Buffer to each of the five tubes. Perform 3 times serial dilutions by adding 100 µL of the reconstituted standard 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).

Volume of Deionized Water to Add	Volume of Standard to Add	Standard Stock Concentration
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 5	0.2 mL	0.1 mL of Reconstituted Standard	X/3
Tube 4	0.2 mL	0.1 mL of Tube 5	X/9
Tube 3	0.2 mL	0.1 mL of Tube 4	X/27
Tube 2	0.2 mL	0.1 mL of Tube 3	X/81
Tube 1	0.2 mL	0.1 mL of Tube 2	X/243

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).

GLP-1 ELISA Assay Procedure

Pre-warm all reagents to room temperature immediately before setting up the assay.

1. Dilute the 10X concentrated HRP wash buffer 10-fold by mixing the entire contents of both buffer bottles with 900 mL de-ionized or glass distilled water.

2. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8 °C. Assemble the strips in an empty plate holder and fill each well with 300 µL diluted Wash Buffer. Decant wash buffer and remove the residual amount by inverting the plate and tapping it smartly onto absorbent towels several times. Wash assay plate using this 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.

3. Add in duplicate 50 µL Matrix Solution to Blank, Standards and Quality Control (refer to Microtiter Plate Arrangement for suggested sample order placement).

4. Add in duplicate 50 µL assay buffer to each of the Blank and sample wells.

5. Add in duplicate 50 µL GLP-1 Standards in the order of ascending concentrations to the appropriate wells. Add in duplicate 50 µL QC1 and 50 µL QC2 to the appropriate wells. Add sequentially 50 µL of the sample to the remaining wells.

   Note: For mouse and rat samples use 20 µL of sample with 30 µL of assay buffer. Sample values will need to be multiplied by 2.5 for final concentration.

6. Cover the plate with plate sealer and incubate at room temperature for 1.5 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 400-500 rpm.

7. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions 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.

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

9. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions 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.

10. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 min on the micro-titer plate shaker.

11. Remove sealer, decant solutions from the plate and tap plate to remove the residual fluid. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

12. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for approximately 5 to 20 minutes. Blue color should be formed in wells of the GLP-1 standards with intensity proportional to increasing concentrations of GLP-1 Total.

    Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

13. Remove sealer and add 100 µL Stop Solution (**Caution:** Corrosive Solution) and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units.

Assay Procedure for GLP-1 Total ELISA Kit

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6-7	Step 8	Step 8-9	Step 10	Step 11-12	Step 13-14			
Well #	Dilute both bottles of 10X HRP Wash Buffer with 900 mL de-ionized water.	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	Seal, Agitate, Incubate 1.5 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. Wash 3X with 300 µL Wash Buffer.	Substrate	Seal, Agitate, Incubate 5-20 minute at Room Temperature.	Stop Solution	Read Absorbance at 450 nm and 590 nm.
A1, B1			50 µL	50 µL	-		100 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 reconstituted Standard									
G2, H2			50 µL	-	50 µL of QC1									
A3, B3			50 µL	-	50 µL of QC2									
C3, D3			-	50 µL	50 µL of Sample									
E3, F3			-	50 µL	50 µL of Sample									
G3, H3, etc.			-	50 µL	50 µL of Sample									

Microtiter Plate Arrangement

GLP-1 Total ELISA

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

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.

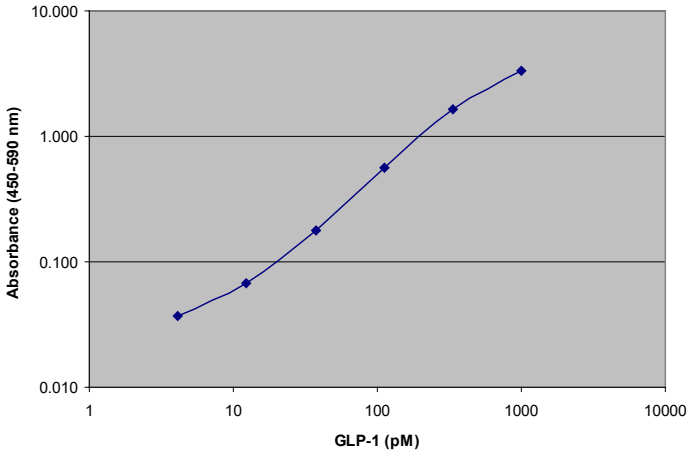
Note: When sample volumes assayed differ from 50 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (For example, if 25 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 50 µL, compensate the volume deficit with Matrix Solution with the exception of mouse and rat samples. When using 20 µL of mouse and rat samples, compensate with 30 µL of assay buffer.

Interpretation

- The assay will be considered accepted when all Quality Control values fall within the calculated QC range. If any QCs fall outside of the control range, review results with a supervisor.
- If the difference between duplicate results of a sample is > 15% CV, repeat the sample.
- The limit of sensitivity of this assay is 1.5 pM GLP-1 Total (50 µL sample size).
- The approximate range of this assay is 4.1 pM to 1000 pM GLP-1 Total (50 µL sample size). Any result greater than 1000 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 Total ELISA



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

Sensitivity

The lowest level of GLP-1 Total that can be detected by this assay is 1.5 pM using a 50 µL sample size, as derived from Statistical Ligand Immunoassay Analysis of multiple assays (n = 8) calculating the mean plus 2 standard deviations of the minimal detectable concentrations.

Specificity

The antibody pair used in this assay measures GLP-1 (7-36) and (9-36) and has no significant cross-reactivity with GLP-2, GIP, Glucagon, Oxyntomodulin.

Precision

Intra-Assay Variation

	Mean GLP-1 Levels (pM)	Intra-Assay %CV
1	32	1%
2	216	2%

Inter-Assay Variation

	Mean GLP-1 Levels (pM)	Inter-Assay %CV
1	39	<12%
2	220	<10%

The assay variations of our GLP-1 Total 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 ten separate assays with duplicate samples in each assay.

Spike Recovery of GLP-1 Total in Assay Samples

Sample	GLP-1 Added (pM)	Expected (pM)	Observed (pM)	% of Recovery
1	0	21.2	21.2	
	12.3	33.5	30.4	91%
	37.0	58.2	52.1	90%
	111	132.2	123.2	93%
2	0	7	7	
	12.3	19.3	21.2	110%
	37.0	44.0	47.9	109%
	111	118	131.9	112%
3	0	27.9	27.9	
	12.3	40.2	38.1	95%
	37.0	64.9	61.8	95%
	111	138.9	141.9	102%
4	0	25.4	25.4	
	12.3	37.7	34.2	91%
	37.0	62.4	57.3	92%
	111	136.4	131.4	96%
5	0	47.5	47.5	
	12.3	59.8	57.3	96%
	37.0	84.5	81.1	96%
	111	158.5	162.7	103%
Average				98

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

Linearity of Sample Dilution

Sample	Volume Sampled (µL)	Expected (pM)	Observed (pM)	% of Expected
1	50	57.4	57.4	100%
	25	28.7	32.7	114%
	12.5	14.4	18.3	128%
2	50	25.1	25.1	100%
	25	12.6	14.5	116%
	12.5	6.28	8.3	132%
3	50	27.2	27.2	100%
	25	13.6	13.7	101%
	12.5	6.8	7.7	113%
4	50	11.5	11.5	100%
	25	5.75	5.7	99%
	12.5	2.88	3.2	111%
5	50	35	35	100%
	25	17.5	15.9	91%
	12.5	8.75	8.5	97%
Average				107%

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, and 4 representing 50 µL, 25 µL, and 12.5 µL sample volumes assayed, respectively, were applied in the calculation of observed GLP-1 Total 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	EP36
10X HRP Wash Buffer Concentrate (50 mL)	EWB-HRP
GLP-1 ELISA Standard	E8036-K
GLP-1 Quality Controls 1 and 2	E6036-K
Matrix Solution	EMTX-PS
Assay Buffer	EABGLP
GLP-1 Total ELISA Detection Antibody	E1036
Enzyme Solution	EHRP
Substrate	ESS-TMB
Stop Solution	ET-TMB
10-pack of GLP-1 Total ELISA kits	EZGLP1T-36BK

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