# GLP-1 Total ELISA

# 96-Well Plate Assay

# Cat. # EZGLP1T-36K

**Glucagon-Like Peptide-1 (GLP-1) Total ELISA KIT**
**96-Well Plate (Cat. # EZGLP1T-36K)**




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**GLP-1 Total ELISA Kit**
**96-Well Plate (Cat.# EZGLP1T-36K)**

## I. 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 purpose only.***

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

## II. PRINCIPLES OF ASSAY

This assay is a Sandwich ELISA based, sequentially, on: 1) 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, 2) wash away of unbound materials from samples, 3) binding of a biotinylated anti- GLP-1 monoclonal antibody to the captured molecules, 4) wash away of unbound materials from samples, 5) conjugation of horseradish peroxidase to the immobilized biotinylated antibodies, 6) wash away of free enzyme conjugates, and 7) 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.

## III. REAGENTS SUPPLIED

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

### 1. GLP-1 Total ELISA Plate

Coated with pre-titered Human GLP-1 antibodies.

Quantity: 1 Strip Plate

Preparation: Ready to use.

Note: Unused strips should be resealed in the foil pouch with the dessicant provided and stored at 2-8 ℃.

### 2. Adhesive Plate Sealer

Quantity: 2 sheets

Preparation: Ready to use.

### 3. 10X HRP Wash Buffer Concentrate

10X concentrate of 50mM Tris Buffered Saline containing Tween-20.

Quantity: 2 bottles containing 50ml each

Preparation: Dilute 1:10 with distilled or de-ionized water.

### 4. GLP-1 Standard

GLP-1 standard, lyophilized

Quantity: 0.5ml upon hydration

Preparation: Reconstitute with 0.5ml distilled or de-ionized water. See insert for concentration

### 5. GLP-1 Quality Controls 1 and 2

One vial each, lyophilized, containing GLP-1 at two different levels.

Quantity: 0.5 ml/vial upon hydration.

Preparation: Reconstitute each vial with 0.5 ml de-ionized water immediately before use.

### 6. Matrix Solution

Processed serum matrix

Quantity: 1.5 ml

Preparation: Ready to use

## III. REAGENTS SUPPLIED (CONTINUED)

### 7. Assay Buffer

0.05 M phosphosaline, pH 7.4, containing 0.08% sodium azide, and 1% BSA.

Quantity: 12 ml/vial

Preparation: Ready to use.

### 8. GLP-1 Total Detection Antibody

Pre-titered detection antibody solution in buffer

Quantity: 12 ml/vial

Preparation: Ready to use.

### 9. Enzyme Solution

Pre-titered streptavidin-horseradish peroxidase conjugate in buffer.

Quantity: 12 ml/vial

Preparation: Ready to use.

### 10. Substrate

3, 3',5,5'-tetramethylbenzidine in buffer.

Quantity: 12 ml/vial

Preparation: Ready to use. Minimize the exposure to light.

### 11. Stop Solution

0.3 M HCl

Quantity: 12 ml/vial

Preparation: Ready to use.

[Caution: Corrosive Solution]

## IV. STORAGE AND STABILITY

Prior to use, all components in the kit can be stored up to 2 weeks at 2-8°C. For longer storage (> 2 weeks), freeze diluted Wash Buffer, Assay Buffer, Matrix Solution and reconstituted Standards and Controls at ≤ –20°C. Minimize repeated freeze and thaw of the Standards and Quality Controls. Unused microtiter strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8°C. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

## V. REAGENT PRECAUTIONS

### 1. Sodium Azide

Sodium azide has been added to certain reagents as a preservative at a concentration of 0.08%. Although it is at a minimum concentration, sodium azide may react with lead and copper pluming to form explosive metal azides. On disposal, flush with large volume of water to prevent azide build up.

### 2. Hydrochloric Acid

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

## VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Multi-channel Pipettes and pipette tips: 5 ~ 50 µl and 50 ~ 300 µl
2. Pipettes and pipette tips: 10µl ~ 20 µl or 20µl ~ 100 µ
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. De-ionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm and 590nm
7. Orbital Microtiter Plate Shaker
8. Absorbent Paper or Cloth

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## VII. 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.

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 ± 5°C for later use. Avoid multiple (> 5) freeze/thaw cycles.

5. To prepare plasma sample, whole blood should be collected into a centrifuge tube containing enough $K_3$ EDTA to achieve a final concentration of 1.735 mg/ml , followed by immediate centrifugation. Observe same precautions in the preparation of serum samples.

6. 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.

7. Avoid using samples with gross hemolysis or lipemia.

## VIII. REAGENT PREPARATION

### A. 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 standard should be stored in small aliquots at ≤ -20°C. Avoid multiple freeze/thaw cycles.

| Volume of Deionized Water to Add | Volume of Standard to Add | Standard Stock Concentration (pM) |
|---|---|---|
| 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** |

### B. 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. Unused portions of the reconstituted Quality Controls should be stored in small aliquots at ≤ -20°C. Avoid further freeze/thaw cycles.

## IX. GLP-1 TOTAL 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 Section X 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 to 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.

## IX. GLP-1 TOTAL ELISA ASSAY PROCEDURE (continued)

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. Read absorbance at 450 nm and 590nm 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 (Cat. # EZGLP1T-36K)

| | Step 1 | Step 2 | Step 3 | Step 4 | Step 5 | Step 6-7 | Step 8 | Step 8-9 | Step 10 | Step 11-12 | Step 12-13 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 5~25 minutes 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 QC 1 | | | | | | | | | |
| A3, B3 | | | 50 µl | -- | 50 µl of QC 2 | | | | | | | | | |
| C3, D3 | | | 50 µl | --l | 50 µl of Sample | | | | | | | | | |
| E3, F3 | | | -- | 50 µl | 50 µl of Sample | | | | | | | | | |
| G3, H3 Etc. | | | -- | 50 µl | 50 µl of Sample | | | | | | | | | |

## X. MICROTITER PLATE ARRANGEMENT

GLP-1 Total ELISA

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Blank | Tube 4 | QC2 | | | | | | | | | |
| B | Blank | Tube 4 | QC2 | | | | | | | | | |
| 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 | QC1 | Etc. | | | | | | | | | |
| H | Tube 3 | QC1 | Etc. | | | | | | | | | |

## XI. CALCULATIONS

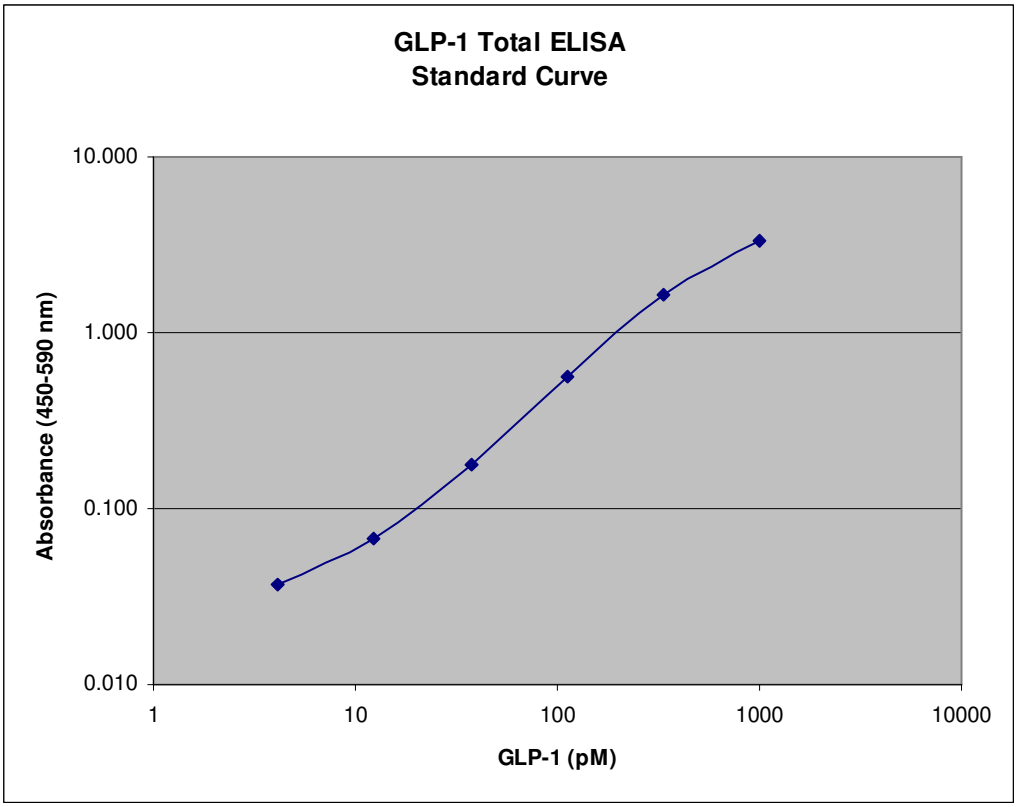
The dose-response curve of this assay fits best to a sigmoidal 4- or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function

**Note:** When sample volumes assayed differ from 50 µl, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., 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.

## XII. INTERPRETATION

1. 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.
2. If the difference between duplicate results of a sample is >15% CV, repeat the sample.
3. The limit of sensitivity of this assay is 1.5 pM GLP-1 Total (50 µl sample size).
4. 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.

## XIII. GRAPH OF TYPICAL REFERENCE CURVE




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

## XIV. ASSAY CHARACTERISTICS

### A. 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.

## XIV. ASSAY CHARACTERISTICS (continued)

### B. 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.

### C. Precision

Intra-Assay Variation

| Sample No. | Mean GLP-1 Levels (pM) | Intra-Assay %CV |
|---|---|---|
| 1 | 32 | 1% |
| 2 | 216 | 2% |

Inter-Assay Variation

| Sample No. | Mean GLP-1 Levels (pM) | Inter-Assay %CV |
|---|---|---|
| 1 | 39 | <12% |
| 2 | 220 | <10% |

The assay variations of Millipore 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.

## XIV. ASSAY CHARACTERISTICS (continued)

### D. Spike Recovery Rate of GLP-1 Total in Assay Samples

| sample No. | 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%.

## XIV. ASSAY CHARACTERISTICS (continued)

### E. Linearity of Sample Dilution

Effect of Serum Dilution

| Sample No. | Volume Sampled | Expected (pM) | Observed (pM) | % Of Expected |
|---|---|---|---|---|
| | (µL) | | | |
| 1 | 50 | 57.4 | 57.4 | 100% |
| | 25 | 28.7 | 32.7 | 114% |
| 2 | 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%.

## XVI. QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the Millipore website www.millipore.com\bmia

## XVII. TROUBLESHOOTING GUIDE

1. 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.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. 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.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. 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.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with HRP Wash Buffer or 3) overexposure to light after substrate has been added.

## XVIII. REPLACEMENT REAGENTS

| **Reagents** | **Cat. #** |
|---|---|
| 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 |

## XIX. ORDERING INFORMATION

### A. To place an order:

**For USA Customers**:

Please provide the following information to our customer service department to expedite your telephone, fax or mail order:

1. Your name, telephone and/or fax number
2. Customer account number
3. Shipping and billing address
4. Purchase order number
5. Catalog number and description of product
6. Quantity and product size

TELEPHONE ORDERS:

TOLL FREE US: (800) MILLIPORE

FAX ORDERS: (636) 441-8050

MAIL ORDERS: Millipore
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

**For International Customers:**

To best serve our international customers, it is Millipore's policy to sell our products through a network of distributors. To place an order or to obtain additional information about Millipore products, please contact your local distributor.

### B. Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to human or animals. All products are intended for *in vitro* use only.

### C. Material Safety Data Sheets (MSDS)

Material safety data sheets for Millipore products may be ordered by fax or phone. See Section A above for details on ordering.