Datasheet for ABIN415650

GLP-1 ELISA Kit

1 Image

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Overview

Quantity:	96 tests
Target:	GLP-1
Reactivity:	Mouse
Method Type:	Competition ELISA
Detection Range:	12.35-1000 pg/mL
Minimum Detection Limit:	12.35 pg/mL
Application:	ELISA

Product Details

Purpose:	The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of GLP1 in Serum,Plasma,Tissue Homogenate,Cell Lysate,Cell Culture Supernatant,Biological Fluids
Sample Type:	Cell Culture Supernatant, Cell Lysate, Plasma, Serum, Tissue Homogenate
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Glucagon Like Peptide 1 (GLP1).
Cross-Reactivity (Details):	No significant cross-reactivity or interference between Glucagon Like Peptide 1 (GLP1) and analogues was observed.
Sensitivity:	4.92 pg/mL The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined the mean O.D.

Product Details

	Value of 20 replicates of the zero standard added by their three standard deviations.
Components:	• Pre-coated, ready to use 96-well strip plate - 1 • Plate sealer for 96 wells - 4 • Standard (freeze dried) - 2 • Standard Diluent - 1×20mL • Detection Reagent A (green) - 1×120µL • Assay Diluent A (2 × concentrate) - 1×6mL • Detection Reagent B (red) - 1×120µL • Assay Diluent B (2 × concentrate) - 1×6mL • TMB Substrate - 1×9mL • Stop Solution - 1×6mL • Wash Buffer (30 × concentrate) - 1×20mL • Instruction manual - 1
Material not included:	• Microplate reader with 450nm filter. • Precision single or multi-channel pipettes and disposable tips. • Eppendorf Tubes for diluting samples. • Deionized or distilled water. • Absorbent paper for blotting the microtiter plate. • Container for Wash Solution

Target Details

Target:	GLP-1
Alternative Name:	GLP1 (GLP-1 Products)

Application Details

Application Notes:	• Limited by the current condition and scientific technology, we cannot completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit. • The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available. • Kits from different batches may be a little different in detection range, sensitivity and color developing time. • Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer. • Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.

	• There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed. • Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment. • Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended. • Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too. • Kits from different manufacturers for the same item might produce different results, since we have not compared our products with other manufacturers.
Comment:	Information on standard material: The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative. Information on reagents: The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them. Information on antibodies: The provided antibodies and their host vary in different kits.
Sample Volume:	50 µL
Assay Time:	2.5 h
Plate:	Pre-coated
Protocol:	This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific for mouse GLP1 has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled mouse GLP1 and unlabeled mouse GLP1 (Standards or samples) with the pre-coated antibody specific for mouse GLP1. After incubation the unbound conjugate is washed off.

Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of GLP1 in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of GLP1 in the sample.

- Prepare all reagents, samples and standards,
- Add 50 µL standard or sample to each well. And then add 50 µL prepared Detection Reagent A immediately. Incubate 1 hour at 37 °C,
- Aspirate and wash 3 times,
- Add 100 µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C,
- Aspirate and wash 5 times,
- Add 90 µL Substrate Solution. Incubate 15-25 minutes at 37 °C,
- Add 50 µL Stop Solution. Read at 450 nm immediately.

Reagent Preparation:

- Bring all kit components and samples to room temperature (18-25°C) before use.
- **Standard** - Reconstitute the Standard with 0.5mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 1,000pg/mL. Please prepare 5 tubes containing 0.6mL Standard Diluent and produce a triple dilution series. Mix each tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 1,000pg/mL, 333.33pg/mL, 111.11pg/mL, 37.04pg/mL, 12.35pg/mL, and the last EP tubes with Standard Diluent is the blank as 0pg/mL.
- **Assay Diluent A and Assay Diluent B** - Dilute 6mL of Assay Diluent A or B Concentrate(2×) with 6mL of deionized or distilled water to prepare 12 mL of Assay Diluent A or B. The prepared working dilution cannot be frozen.
- **Detection Reagent A and Detection Reagent B** - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with working Assay Diluent A or B, respectively (1:100).
- **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600 mL of Wash Solution (1×).
- **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

- Making serial dilution in the wells directly is not permitted.
- Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly.
- Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µl for once pipetting.
- The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
- If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals have completely dissolved.

	• Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.
Sample Collection:	**Serum:** Allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000 × g. Assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles. **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles. **Tissue Homogenates:** The preparation of tissue homogenates will vary depending upon tissue type. For this assay, rinse tissues in ice-cold PBS (0.02mol/L,pH 7.0-7.2) to remove excess blood thoroughly and weigh before homogenization. Mince the tissues to small pieces and homogenize them in 5-10 mL of PBS with a glass homogenizer on ice (Micro Tissue Grinders work, too). Sonicate the resulting suspension with an ultrasonic cell disrupter or subject it to two freeze-thaw cycles to further break the cell membranes. Centrifugate the homogenates for 5 minutes at 5000 × g. Remove the supernate and assay immediately or aliquot and store at -20°C **Cell Lysate:** Cells must be lysed before assaying according to the following directions. Adherent cells should be detached with trypsin and then collected by centrifugation (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS. Resuspend cells in PBS (1×) and subject them to ultrasonication for 4 times (or Freeze cells at -20 °C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times.) Centrifuge at 1500 × g for 10 minutes at 2 - 8°C to remove cellular debris. **Cell Culture Supernatant:** Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles. **Biological Fluids:** Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.
Sample Preparation:	**Notes**:

- The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
- Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
- Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.
- If the humidity level in the laboratory does not reach 60%, a humidifier is recommended to be used.

Assay Procedure:

- 1. Determine wells for diluted standard, blank and sample. Prepare 5 wells for standard, 1 well for blank. Add 50 µL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50 µL of Detection Reagent A to each well immediately. Shake the plate gently. Cover with a Plate sealer. Incubate for 1 hour at 37 °C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
- 2. Aspirate the solution and wash with 350 µL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
- 3. Add 100 µL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37 °C after covering it with the Plate sealer.
- 4. Repeat the aspiration/wash process for five times as conducted in step 2.
- 5. Add 90 µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15 - 25 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
- 6. Add 50 µL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
- 7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct

measurement at 450 nm immediately.

Note:

- 1. Assay preparation: Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at -20 °C until the kits expiry date.
- 2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- 3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
- 4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading.
- 5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
- 6. TMB Substrate is easily contaminated. Please protect it from light.

Calculation of Results:

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between GLP1 concentration in the sample and the assay signal intensity. Low levels of GLP1 result in a high O.D value, while a high concentration of GLP1 results in a low signal. Average the duplicate readings for each standard, control, and samples. Create a standard curve on log-log graph paper, with GLP1 concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis. Using some plot software, such as curve expert 1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

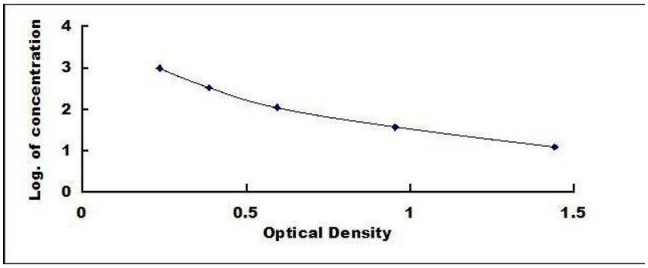
In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the log of concentration of the standard (Y-axis), although concentration is indeed the independent variable while O.D. value is the dependent variable. The O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). This curve is provided for demonstration

Application Details

	only. The customers should establish their own standard curve for each test conducted.
Assay Precision:	**Intra-assay Precision (precision within an assay):** Three samples with low, medium and high levels of the target antigen were tested twenty times on one plate, respectively. **Inter-assay Precision (precision between assays):** Three samples with low, medium and high levels of the target antigen were tested on three different plates, eight replicates in each plate. **CV (%)** = SD/mean X 100 • Intra-assay: CV less than 10 % • Inter-assay: CV less than 12 %
Restrictions:	For Research Use only

Handling

Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Handling Advice:	To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
Storage:	4 °C/-20 °C
Storage Comment:	• For unopened kit: All the reagents should be kept according to the labels on vials. The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon receipt while the others should be at 0 °C. • For opened kit: When the kit is opened, the remaining reagents still need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal. Note: It is highly recommended to use the remaining reagents within 1 month provided this is within the expiration date of the kit. • For ELISA kit, 1 day storage at 30 °C can be considered as 2 months at 0 °C, which means 3 days at 30 °C equaling 6 months at 0 °C.
Expiry Date:	6 months



ELISA

Image 1.