EliKine™ Mouse GLP-1 Competitive ELISA Kit

Cat #: KTE7019 Size: 48 T/96 T

	Mouse GLP-1 Competitive ELISA Kit		
REF	**Cat #:** KTE7019	LOT	**Lot #:** Refer to product label
	Detection range: 24.69 pg/mL-2,000 pg/mL		**Sensitivity:** 8.23 pg/mL
	Precision: Intra-assay Precision: The CV (%) < 10%. Inter-assay Precision :The CV (%) < 12%		**Recovery:** The recovery ranged from 98% to 116% with an overall mean recovery of 106%.
	Specificity: EliKine™ Mouse GLP-1 Competitive ELISA Kit has high sensitivity and excellent specificity for detection of Mouse GLP-1. No significant cross-reactivity or interference between Mouse GLP-1 and analogues was observed.		
	Applicable samples: Serum, Plasma, Tissue homogenate, Cell lysates, and Cell culture supernatant		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

GLP-1 is a peptide hormone released from intestinal L-cells upon nutrient consumption. It bindsthe GLP-1 receptorin the pancreas and displays various antidiabetic effects by potentiating glucose-induced secretion of insulin from pancreatic β-cells, increasing insulin expression. EliKine™ Mouse GLP-1 Competitive ELISA Kit uses a competitive method to quantify mouse GLP-1 in a sample. Mouse specific antibody against glucagon-like peptide 1(GLP-1) was coated on the enzyme label plate, and the antigen to be tested (sample or standard) and biotin-labeled antigen were added to the well of the enzyme label plate at the same time. The antigen to be tested and the biotin-labeled antigen were competitively bound to the specific antibody. After washing, the proprietary streptavidin-HRP conjugate was added to the wells. After washing and removing any unbound streptavidin-HRP, adding HRP Substrate (TMB), TMB turns blue under the catalysis of HRP, and turns yellow after adding stop solution. Measure the OD value with a microplate reader at 450 nm wavelength. The Mouse GLP-1 concentration is inversely proportional to the OD450 nm value.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Mouse GLP-1 Microplate	48 wells	96 wells	4℃
Mouse GLP-1 Standard (lyophilized)	1	2	4℃
Sample Diluent (5×)	3.5 mL	7 mL	4℃
Assay Buffer (5×)	3.5 mL	7 mL	4℃
Biotin Conjugated Mouse GLP-1 (100×)	30 μL	60 μL	4℃
Streptavidin-HRP (100×)	60 μL	120 μL	4℃, protected from light

HRP Substrate (TMB)	5 mL	10 mL	4℃
Stop Solution	25 mL	50 mL	4℃
Wash Buffer (20×)	1	2	RT

Materials Required but Not Supplied

- Microplate reader capable of measuring absorbance at 450 nm
- Multi channel pipette or automated microplate washer
- Incubator, refrigerated centrifuge, homogenizer (for tissue samples)
- Precision pipettes, disposable pipette tips
- Deionized water

Reagent Preparation

1×Sample Diluent: Sample Diluent (5×) equilibrate to room temperature and dilute with deionized water 1:5 to obtain the 1× Sample Diluent before use. Mix gently to avoid foaming. Store at 4℃. This solution is stable for 30 days. If your samples need to be diluted, 1×Sample Diluent is used for dilution of standard and samples.

1×Assay Buffer: Assay Buffer (5×) equilibrate to room temperature and dilute with deionized water 1:5 to obtain the 1×Assay Buffer before use. Mix gently to avoid foaming. Store at 4℃. This solution is stable for 30 days. 1×Assay Buffer is used for dilution of Biotin Conjugated Mouse GLP-1 (100×) and Streptavidin-HRP (100×).

Mouse GLP-1 Standard: Reconstitute the Mouse GLP-1 standard in 250 µL of 1×Sample Diluent for a concentration of 2,000 pg/mL. Allow the standard to sit for a minimum of 15 min with gentle shake prior to making dilutions.

1×Biotin Conjugated Mouse GLP-1: Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated detect antibody solution with 1×Assay buffer in a clean plastic tube as needed according to the standards and samples. 1× Biotin Conjugated Mouse GLP-1 should be used within 30 min.

1×Streptavidin-HRP: Mix well prior to making dilutions. Make a 1:100 dilution of Streptavidin-HRP (100×) with 1×Assay Buffer in a clean plastic tube as needed according to the standards and samples. 1×Streptavidin-HRP should be used within 30 min.

HRP Substrate (TMB): Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Stop Solution: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃

1×Wash Buffer: Wash Buffer (20×) equilibrate to room temperature and dilute with deionized water 1:20 to obtain the 1×Wash buffer before use. Mix gently to avoid foaming. Store at room temperature. Please note that 1×Wash buffer is stable for 30 days.

Standard Curve Setting: dilute 2,000 pg/mL standard with 1×Sample Diluent to 666.67, 222.22, 74.07, 24.69 and 0 pg/mL of Mouse GLP-1 standard just as below.

Num.	Volume of Standard	Volume of 1×Sample Diluent (µL)	The Concentration of Standard（pg/mL）
Std.1	250 µL of 2,000 pg/mL	0	2,000
Std.2	80 µL of Std.1 (2,000 pg/mL)	160	666.67
Std.3	80 µL of Std.2 (666.67 pg/mL)	160	222.22
Std.4	80 µL of Std.3 (222.22 pg/mL)	160	74.07
Std.5	80 µL of Std.4 (74.07 pg/mL)	160	24.69
Std.6	0	160	0

Note：Always prepare a fresh set of standards per use.

Sample Preparation

1. Cell culture supernatants: Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
2. Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
3. Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of

collection. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

4. Tissue homogenates: It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolyzed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a homogenizer on ice, such as 0.1g tissue sample corresponding to 0.9 mL of PBS, the specific volume can be adjusted according to the needs of the experiment, and make a record. It is recommended to add a protease inhibitor to PBS) to the homogenizer and grind thoroughly on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic for 2 min (power 20% or 200 W, ultrasonic for 3 s, interval 7 s, repeat 12 times). The homogenates are then centrifuged for 10 min at 10,000 g at 4℃. Take the supernatant, and put it on ice for testing.

5. Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1,000 g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 μL of pre-cooled PBS to keep the cells suspended. Use an ultrasonic for 2 min (power 20% or 200 W, ultrasonic for 3 s, interval 7 s, repeat 12 times) until the cells are fully lysed. Centrifuge for 10 min at 10,000 g at 4℃. Take the supernatant, and put it on ice for testing.

Note: Do not use grossly hemolyzed or lipemic specimens. If samples are to be used within 24 h, they may be stored at 2 to 8℃. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal. The strips used for testing are equilibrated to room temperature before use.

2. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250 μL) using a multi channel pipette or automated microplate washer, and let it stand for 1-2 min, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.

3. Add 50 μL of diluted standard or sample per well. It is recommended that all Standards and Samples be added in duplicate to the microplate. Then, add 50 μL of 1×Biotin Conjugated Mouse GLP-1 to each well to ensure continuous sampling without interruption and the sampling process is completed within 15 min. The NSB well (non-specific binding well) is added with 50 μL 1×Sample Diluent and 50 μL 1×Assay Buffer, cover with the plate cover provided. Incubate for 1 h at 37℃.

4. Repeat the wash process for five times as in step 2.

5. Add 100 μL of 1×Streptavidin-HRP to each well. Cover the plate and incubate for 30 min at 37℃. Avoid placing the plate in direct light.

6. Repeat the wash process for five times as in step 2.

7. Add 90 μL of HRP Substrate (TMB) to each well. Cover the plate and incubate for 3-5 min at 37℃. Protect from light.The reaction is fast, and it can be terminated when the standard curve S5 and S6 wells are obviously blue when observed by the naked eye.

8. Add 50 μL of Stop solution to each well. Stop Solution should be added to the plate in the same order as TMB. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm.

Data Analysis

1. Average the duplicate readings for each standard and sample and subtract the NSB wells optical density (O.D.).

2. Drawing of standard curve: With the standard solution concentration as the x-axis and the mean absorbance for each standard as the y-axis, draw the standard curve. A computer software can be used to create a standard curve.

Note: If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data

Typical standard curve（$R^2\geqslant0.99$）

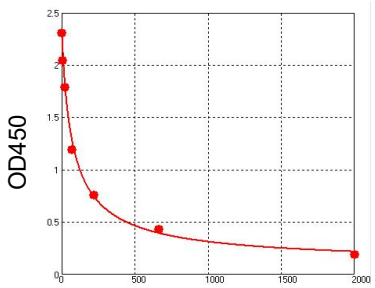

Figure1. Standard Curve of Mouse GLP-1 in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Precautions

1. If Sample Diluent (5×) and Assay Buffer (5×) appears to turn yellow or a small amount of precipitation, etc., it is caused by the serum contained in the reagent. Please centrifuge to remove the precipitate, which will not affect normal use.
2. Do not mix or substitute reagents with those from other lots or sources.
3. 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.
4. To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.
5. Stop Solution has certain Corrosive. Please take protective measures when operating.

FAQ

Problem	Cause	Suggested Solution
Poor standard curve	Inaccurate Pipetting.	Check pipettes
	Improper standard dilution.	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too short.	Ensure sufficient incubation times; increase to 2 or 3 h standard/ sample incubation
	Inadequate reagent volumes or improper dilution.	Check pipettes and ensure correct preparation.
	Incubation times with TMB too short.	Ensure sufficient incubation time until blue color develops prior addition of Stop solution.
High background /Large CV	Plate is insufficiently Washed.	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed
	Contaminated Wash Buffer.	Make fresh Wash Buffer
Low sensitivity	Improper storage of the ELISA kit.	Store your reconstituted standards at -20℃ (avoid repeated freeze-thaw cycles), all other assay components 4℃. Keep TMB Development Solution protected from light
	Stop Solution.	Stop Solution should be added to each well before measurement.

Recommended Products

Catalog No.	Product Name
KTE9009	EliKine™ Rat GLP-1 Competitive ELISA Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.