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ab222863 Glucagon-Like Peptide 2 ELISA Kit

For the quantitative measurement of Glucagon-Like 2 Peptide in serum, and plasma samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

The Glucagon-Like Peptide 2 ELISA (Enzyme-Linked Immunosorbent Assay) Kit (ab222863) kit is designed for detection of GLP-2 in plasma, and serum samples.

The kit has been validated using equine, rat, human, swine, mouse and monkey samples. This assay employs a quantitative sandwich enzyme immunoassay technique, which measures GLP-2 in approximately 4 hours. A polyclonal antibody specific for GLP-2 has been pre-coated onto a 96-well microplate with removable strips. GLP-2 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for GLP-2, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Glucagon-like peptide 2 (GLP-2) is a thirty-three-amino acid peptide that is secreted by intestinal endocrine cells alongside GLP-1. GLP-2 and GLP-1 are co-secreted from enteroendocrine L-cells located in distal intestine in response to enteral nutrient ingestion, particularly fats and carbohydrates. GLP-2 secretion is mediated by direct nutrient stimulation of the L-cells and indirect action from enteroendocrine and neural inputs. GLP-2 acts to enhance nutrient absorption by inhibiting gastric motility and secretion and by stimulating nutrient transport.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed



Add 50 μ L standard or sample to appropriate wells.

Incubate for 2 hours



Wash wells. Add 50 μ L Biotinylated Antibody to all wells.

Incubate for 1 hour



Wash wells. Add 50 μ L Streptavidin-Peroxidase Conjugate to all wells.

Incubate for 30 minutes



Wash wells. Add 50 μ L Chromogen Substrate to all wells.

Incubate for 30 minutes



Add 50 μ L Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt, apart from the Biotinylated Antibody and the Streptavidin-Peroxidase Conjugate, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-GLP-2 coated Microplate (12 x 8 wells)	96 wells	+4°C
GLP-2 Standard	1 vial	+4°C
Biotinylated GLP-2 Antibody	1 vial	-20°C
10X Diluent M Concentrate	20 mL	+4°C
20X Wash Buffer Concentrate	2 x 30 mL	+4°C
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
Chromogen Substrate	7 mL	+4°C
Stop Solution	11 mL	+4°C
Sealing Tapes	3 units	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.
- When diluting the concentrates, make sure to rinse the bottles thoroughly to extract any precipitates left in the bottle. Mix gently until the crystals have completely dissolved.

9.1 10X Diluent M Concentrate:

Dilute the Diluent M Concentrate 10-fold with reagent grade water. Store for up to 30 days at +4°C.

9.2 Biotinylated GLP-2 Antibody:

Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with Diluent M. The undiluted antibody should be stored at -20°C.

9.3 20X Wash Buffer Concentrate:

Dilute the Wash Buffer Concentrate 20-fold with reagent grade water. Mix gently until any crystals have completely dissolved. Store for up to 30 days at +4°C.

9.4 100X Streptavidin-Peroxidase Conjugate:

Spin down the Streptavidin-Peroxidase Conjugate briefly and dilute the desired amount of the conjugate 100-fold with Diluent M. The undiluted conjugate should be stored at -20°C.

9.5 Anti-GLP-2 coated Microplate (12 x 8 wells):

Ready to use 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against GLP-2. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator at +4°C.

9.6 Chromogen Substrate:

Ready to use stabilized peroxidase chromogen substrate tetramethylbenzidine.

9.7 Stop Solution:

Ready to use 0.5 N hydrochloric acid.

9.8 Sealing Tapes:

Ready to use. Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1.1 Reconstitute the GLP-2 Stock to generate a 18 ng/mL Standard #1. Aliquot stock to limit repeat freeze-thaw cycles. This solution should be stored at -20°C and used within 20 days.

10.1.2 First consult the GLP-2 Standard vial to determine the mass of protein in the vial.

10.1.3 Calculate the appropriate volume of 1X Diluent M to add when resuspending the GLP-2 Standard vial to produce a 18 ng/mL GLP-2 Standard stock by using the following equation:

CS = Starting mass of GLP-2 Standard stock (see vial label) (ng)

CF = 18 ng/mL GLP-2 Standard #1 final required concentration

VD = Required volume of 1X Diluent M for reconstitution (μL)

Calculate total required volume 1X Diluent M for resuspension:

$$(C_S / C_F) * 1,000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 46.8 ng of GLP-2 Standard in vial

C_F = 18 ng/mL GLP-2 **Standard #1** final concentration

V_D = Required volume of 1X Diluent M for reconstitution

$$(46.8 \text{ ng} / 18 \text{ ng/mL}) * 1,000 = 2,600 \text{ } \mu\text{L}$$

- 10.1.4 First briefly centrifuge the GLP-2 Standard Vial to collect the contents on the bottom of the tube.
- 10.1.5 Reconstitute the GLP-2 Standard vial by adding the appropriate calculated amount VD of 1X Diluent M to the vial to generate the 18 ng/mL GLP-2 **Standard #1**. Mix gently and thoroughly.
- 10.1 Allow the reconstituted 18 ng/mL GLP-2 Standard #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- 10.2 Label seven tubes #2 – 8.
- 10.3 Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (18 ng/ml) 1 in 2 with Diluent M to produce 9, 4.5, 2.25, 1.125, 0.563, and 0.281 ng/ml solutions. Diluent M serves as the zero standard (0 ng/ml). Using the table below as a guide, prepare serial dilutions.

Standard #	Volume to dilute (µL)	Volume Diluent M (µL)	GLP-2 (ng/mL)
1	Step 10.1		18.0
2	120 µL Standard #1	120	9.0
3	120 µL Standard #2	120	4.5
4	120 µL Standard #3	120	2.25
5	120 µL Standard #4	120	1.125
6	120 µL Standard #5	120	0.563
7	120 µL Standard #6	120	0.281
8 (Blank)	N/A	120	0.000

11. Sample Preparation

11.1 Plasma:

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Sample collection and processing should be performed as quickly as possible. Keep on ice when not in use. It is recommended that protease inhibitor cocktail is added to samples; for example: o-phenanthroline 0.44 mM, EDTA 25 mM, p-hydroxy-mercuribenzoic acid 1 mM and pepstatin A 0.12 mM. The user may need to optimize concentration of above reagents. Centrifuge samples at 3000 x g for 10 minutes. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

11.2 Serum:

Samples should be collected into a serum separator tube. Sample collection and processing should be performed as quickly as possible. Keep on ice when not in use. It is recommended that protease inhibitor cocktail is added to samples; for example: o-phenanthroline 0.44 mM, EDTA 25 mM, p-hydroxy-mercuribenzoic acid 1 mM and pepstatin A 0.12 mM. The user may need to optimize concentration of above reagents. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
 - We recommend that you assay all standards, controls and samples in duplicate.
-
- 12.1 Prepare all reagents, working standards, and samples as directed in the previous sections. The assay is performed at room temperature (20-25°C).
 - 12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
 - 12.3 Add 50 µL of GLP-2 Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
 - 12.4 Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
 - 12.5 Add 50 µL of Biotinylated GLP-2 Antibody to each well and incubate for 1 hour.
 - 12.6 Wash the microplate as described in step 12.4.
 - 12.7 Add 50 µL of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
 - 12.8 Wash the microplate as described in step 12.4.
 - 12.9 Add 50 µL of Chromogen Substrate per well and incubate in ambient light for 25 minutes or till the optimal color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
 - 12.10 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.
 - 12.11 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only.
Δ **Note:** Some unstable black particles may be generated at high

concentration points after stopping the reaction for about 15 minutes, which will reduce the readings.

12.12 Analyze the data as described below.

- 12.1.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- 12.1.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- 12.1.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

13. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

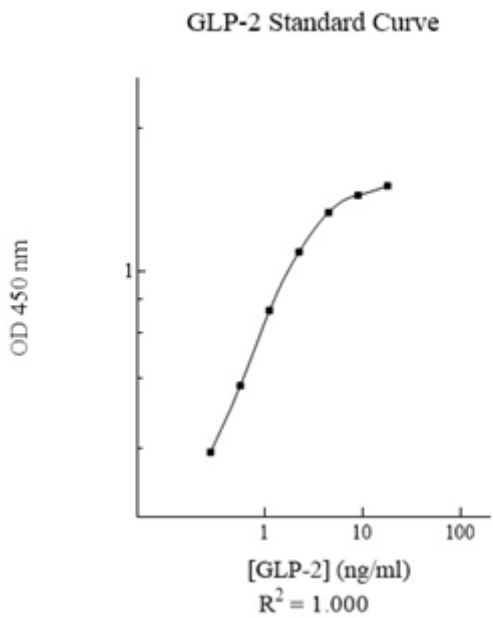


Figure 1. Example of Glucagon-Like Peptide 2 standard curve in 1X Diluent M. The standard curve was prepared as described in Section 10. Background-subtracted data values (mean +/- SD) are graphed.

14. Typical Sample Values

SENSITIVITY –

The minimum detectable dose (MDD) of Glucagon-Like Peptide 2 as calculated by 2 standard deviations from the mean of a zero standard was established to be 0.18 ng/ml.

PRECISION –

Intra-assay precision was determined by testing replicates of three plasma samples twenty times in one assay.
Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
N	20	20	20	20	20	20
CV (%)	4.1	4.0	5.1	9.8	10.1	11.6
Average CV (%)	4.4			10.5		

RECOVERY –

Standard Added Value	0.563 – 4.5 ng/ml
Recovery (%)	91 – 108 %
Average Recovery (%)	97%

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Dilution Factor	Human Plasma	Human Serum
1	90%	88%
2	110%	109%

15. Assay Specificity

This kit recognizes Glucagon-Like Peptide 2 in serum and plasma samples.

CROSS REACTIVITY –

Protein	Cross Reactivity (%)
GLP-1	<1%
GLP-2 (1-34)	100%
GLP-2 (3-33)	85%
GLP-2 (1-33)	90%

16. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are empty after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing

Unexpectedly low or high signal intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted
	Omission of step	Consult the provided procedure for complete list of steps
	Steps performed in incorrect order	Consult the provided procedure for the correct order
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance
	Wash step was skipped	Consult the provided procedure for all wash steps
	Improper wash buffer	Check that the correct wash buffer is being used
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time

Deficient Standard Curve Fit	No-optimal sample dilution	<p>Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples</p>
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions

17. Notes

Technical Support

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