This kit employs a competitive inhibition immunoassay technique to quantify Human GLP-2 in biological matrices.

Human GLP-2 ELISA Kit
Competitive Inhibition ELISA
Catalog EL-213-7121602

INTRODUCTION

GLP-2 is a 33-amino-acid peptide hormone produced, together with glucagon and GLP-1, from the proteolytic processing of proglucagon, the product of the GCG gene. It is co-secreted with GLP-1 by enteroendocrine L-cells concentrated in the distal small intestine and colon whenever dietary fat or carbohydrate reaches the gut lumen. Acting on receptors distributed from the stomach to the colon, GLP-2 drives expansion of the intestinal mucosa by increasing crypt cell proliferation and villus height while limiting enterocyte loss, and it tightens the gut barrier by reducing mucosal permeability and boosting nutrient uptake capacity. Because of this trophic, gut-restricted activity, GLP-2 has become a marker and research tool for studying digestive disorders such as inflammatory bowel disease, where intestinal barrier function and nutrient absorption are compromised.

PRINCIPLE OF THE ASSAY

This kit utilizes a competitive inhibition enzyme immunoassay format to measure Human GLP-2. Microtiter plate wells are pre-coated with Human GLP-2, and standards or samples are introduced simultaneously with a biotin-conjugated antibody specific to Human GLP-2. Free GLP-2 present in the standard or sample competes with the plate-bound GLP-2 for a limited supply of this antibody, meaning that higher analyte concentrations result in less antibody binding to the plate. Streptavidin-conjugated Horseradish Peroxidase (HRP) is then added to each well and allowed to incubate, linking enzymatic activity to the plate-bound biotin-antibody complex. TMB substrate solution is subsequently added to initiate the colorimetric reaction, which is terminated by the addition of a sulphuric acid stop solution. Absorbance is measured spectrophotometrically at 450 nm (± 10 nm), and sample GLP-2 concentrations are determined by comparing sample optical density values against the standard curve, where signal is inversely proportional to analyte concentration.

MATERIALS AND STORAGE

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Each kit includes:	Units
Pre-Coated Microplate	12 strips x 8 wells
Standard (Lyophilized)	2 vials
Biotinylated-Conjugate (100×)	60 µL
Streptavidin-HRP (100×)	120 µL
Standard/Sample Diluent Buffer	20 mL
Biotinylated-Conjugate Diluent	10 mL
HRP Diluent	12 mL
Wash Buffer (25×)	20 mL
TMB Substrate Solution	10 mL
Stop Reagent	6 mL
Plate Covers	2 pieces
Do not mix or substitute reagents with those from other lots.	

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

SAFETY PRECAUTIONS

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective

clothing when handling any patient sera or serum based products.

- The kit reagents contain antimicrobial agents, acid and 3,3’,5,5’-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
- Disposal must be performed in accordance with local regulations.
- Only trained laboratory personnel should execute this test.

This kit employs a competitive inhibition immunoassay technique to quantify Human GLP-2 in biological matrices.

Human GLP-2 ELISA Kit
Competitive Inhibition ELISA
Catalog EL-213-7121602

PREPARATION OF REAGENTS

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

1. **Wash Buffer (1X) Preparation: Dilute the 25X wash buffer concentrate 1:25 with double distilled water.**
2. **Detection Reagent (1X) Preparation: Dilute the biotinylated antibody and streptavidin-HRP concentrates 100-fold with Biotinylated-Conjugate Diluent and HRP Diluent, respectively, immediately before use.**
3. **Preparation of Calibrators: Prepare calibrators by serial dilution from a 10000 down to 156.25 (plus a 0 blank), per the source manual.**

Sol'n ID	Source	Source Vol (µL)	Cal. Diluent (µL)	Final Vol (µL)	Final Conc. (ng/mL)
Std 1	Stock Standard	500	500	1000	10000
Std 2	Std 1	500	500	1000	5000
Std 3	Std 2	500	500	1000	2500
Std 4	Std 3	500	500	1000	1250
Std 5	Std 4	500	500	1000	625
Std 6	Std 5	500	500	1000	312.5
Std 7	Std 6	500	500	1000	156.25
Blank	Std 7	500	500	1000	0

SPECIMEN STORAGE

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

ASSAY PROCEDURE

1.After the kit is equilibrated at room temperature, add 50 µL of Standard Working Solution (gradually dilution refers to Reagent Preparation) or 50 µL of sample to each well, immediately add 50 µL of 1× Biotinylated-Conjugate Working Solution to each well, mix well, incubate at 37°C for 60 minutes.

2.Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 µL 1× Streptavidin-HRP Working Solution to each well, incubate at 37°C for 60 minutes.

3.Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 µL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark .

4.Add 50 µL Stop Reagent to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

CALCULATIONS AND RESULTS

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.
3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

PERFORMANCE CHARACTERISTICS

Precision: Intra-assay CV < 8%. Inter-assay CV < 10%.

Detection Limit: 50.2 pg/mL

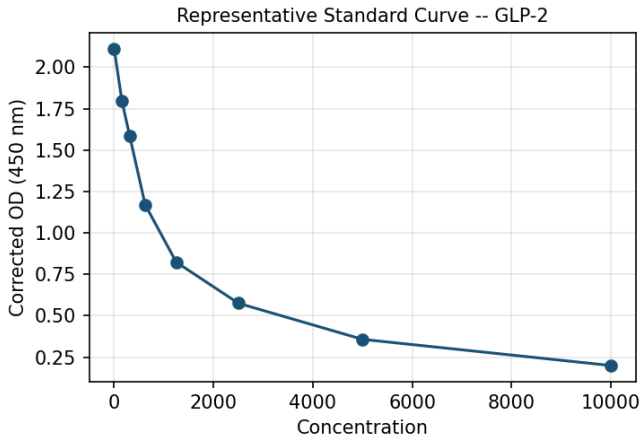
Recovery: Serum (n = 5): 85-99% (avg 92%); EDTA plasma (n = 5): 87-99% (avg 93%); Heparin plasma (n = 5): 93-107% (avg 100%)

Specificity: This assay has high sensitivity and excellent specificity for detection of Human GLP2. No significant cross-reactivity or interference between Human GLP2 and analogues was observed.

This kit employs a competitive inhibition immunoassay technique to quantify Human GLP-2 in biological matrices.

Human GLP-2 ELISA Kit
Competitive Inhibition ELISA
Catalog EL-213-7121602

SAMPLE STANDARD CURVE



Representative standard curve shown for illustration only. This curve should not be used to calculate unknown sample concentrations -- run a new standard curve with each assay.

ORDERING INFORMATION

Please visit *www.affinityimmuno.com* to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.