This kit employs a competitive inhibition immunoassay technique to quantify Oxyntomodulin (OXM) in biological samples.

Human Oxyntomodulin ELISA Kit
Competitive Inhibition ELISA
Catalog EL-213-15

INTRODUCTION

Oxyntomodulin is a 37-residue peptide generated when the proglucagon precursor, encoded by the GCG gene, is cleaved in intestinal L cells; its sequence duplicates the 29 amino acids of glucagon but carries an additional eight-residue extension at the carboxy terminus that distinguishes it from its parent hormone. Released after meals alongside GLP-1, oxyntomodulin acts as a dual agonist at both the glucagon receptor and the GLP-1 receptor, slowing gastric emptying, damping gastric acid output, and blunting appetite. These combined actions on food intake and energy expenditure have made oxyntomodulin and its analogs of interest for weight-management and metabolic research. Because it shares nearly its entire sequence with glucagon, reliable measurement depends on antibodies that recognize its unique C-terminal extension rather than the glucagon core it has in common with other proglucagon-derived peptides.

PRINCIPLE OF THE ASSAY

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

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.
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- Only trained laboratory personnel should execute this test.

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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 500 down to 7.82 (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	500
Std 2	Std 1	500	500	1000	250
Std 3	Std 2	500	500	1000	125
Std 4	Std 3	500	500	1000	62.5
Std 5	Std 4	500	500	1000	31.25
Std 6	Std 5	500	500	1000	15.63
Std 7	Std 6	500	500	1000	7.82
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: 2.82 ng/mL

Recovery: Serum (n = 5): 95-103% (avg 99%); EDTA plasma (n = 5): 92-102% (avg 97%); Heparin plasma (n = 5): 81-95% (avg 100%)

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

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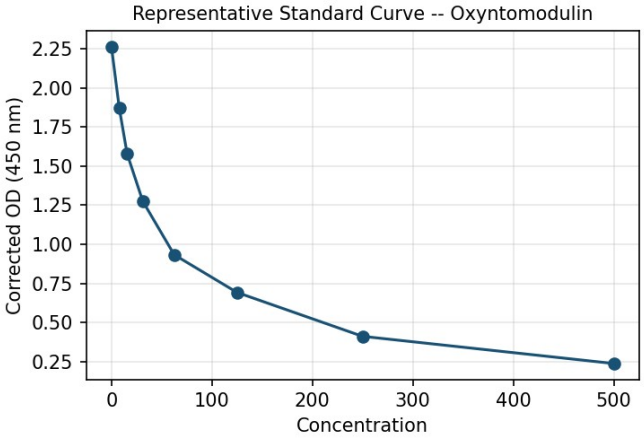
SAMPLE STANDARD CURVE



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.