



**This kit employs a competitive inhibition immunoassay technique to quantify Human ProIAPP (amylin/IAPP) in biological matrices.**

## **Human amylin/IAPP ELISA Kit Competitive Inhibition ELISA Catalog EL-213-01**

### **INTRODUCTION**

Amylin, encoded by IAPP, is a 37-amino-acid peptide hormone of the calcitonin family that is co-secreted with insulin from pancreatic beta cells in response to meals. Acting mainly through calcitonin receptor complexes paired with receptor-activity-modifying proteins, it slows gastric emptying, suppresses inappropriate glucagon release after eating, promotes satiety, and selectively blunts insulin-stimulated glucose uptake in skeletal muscle without affecting fat cell metabolism. Circulating amylin is measurably diminished in type 1 diabetes and in advanced type 2 diabetes as beta-cell mass declines, yet within the pancreas itself the peptide has a tendency to misfold and accumulate as islet amyloid, a process thought to further accelerate beta-cell loss in type 2 disease. The hormone also has reported effects on bone remodeling and blood pressure regulation, along with an intrinsic antimicrobial activity, reflecting functions that extend beyond glucose control alone.

### **PRINCIPLE OF THE ASSAY**

This kit utilizes a competitive inhibition enzyme immunoassay format to measure Human ProIAPP. Microtiter plate wells are pre-coated with Human ProIAPP antigen. During the assay, standards or samples are added simultaneously with a biotin-conjugated antibody specific to Human ProIAPP; free ProIAPP present in the standard or sample competes with the plate-bound antigen for a limited supply of this detection antibody. Streptavidin-conjugated Horseradish Peroxidase (HRP) is then added to each well and allowed to incubate, linking enzymatic activity to antibody captured on the plate. Following incubation, TMB substrate solution is added to initiate the colorimetric enzyme-substrate reaction. The reaction is stopped by addition of a sulfuric acid stop solution, and absorbance is measured spectrophotometrically at 450 nm ( $\pm 10$  nm). Because free analyte in the sample competes with plate-bound antigen for antibody binding, the resulting signal is inversely proportional to ProIAPP concentration; sample concentrations are determined by comparing optical density values against the standard curve.

### **MATERIALS AND STORAGE**

Store kit components at  $-20^{\circ}\text{C}$  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 $\times$ )                               | 60 $\mu\text{L}$    |
| Streptavidin-HRP (100 $\times$ )                                     | 120 $\mu\text{L}$   |
| Standard/Sample Diluent Buffer                                       | 20 mL               |
| Biotinylated-Conjugate Diluent                                       | 10 mL               |
| HRP Diluent                                                          | 12 mL               |
| Wash Buffer (25 $\times$ )                                           | 20 mL               |
| TMB Substrate Solution                                               | 10 mL               |
| Stop Reagent                                                         | 6 mL                |
| Plate Covers                                                         | 2 pieces            |
| <i>Do not mix or substitute reagents with those from other lots.</i> |                     |

### **MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED**

- Precision pipettes calibrated to deliver 5-1000 $\mu\text{L}$
- Multi-channel pipette calibrated to deliver 50-200 $\mu\text{L}$
- 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.

# Human amylin/IAPP ELISA Kit

## Competitive Inhibition ELISA

### Catalog EL-213-01

#### 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 2000 down to 31.25 (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           | 2000                |
| Std 2    | Std 1          | 500             | 500               | 1000           | 1000                |
| Std 3    | Std 2          | 500             | 500               | 1000           | 500                 |
| Std 4    | Std 3          | 500             | 500               | 1000           | 250                 |
| Std 5    | Std 4          | 500             | 500               | 1000           | 125                 |
| Std 6    | Std 5          | 500             | 500               | 1000           | 62.5                |
| Std 7    | Std 6          | 500             | 500               | 1000           | 31.25               |
| 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

- 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.
- The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.
- 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:** 8.9 pg/mL

**Recovery:** Serum (n = 5): 87-99% (avg 93%); EDTA plasma (n = 5): 80-95% (avg 87%); Heparin plasma (n = 5): 87-99% (avg 93%)

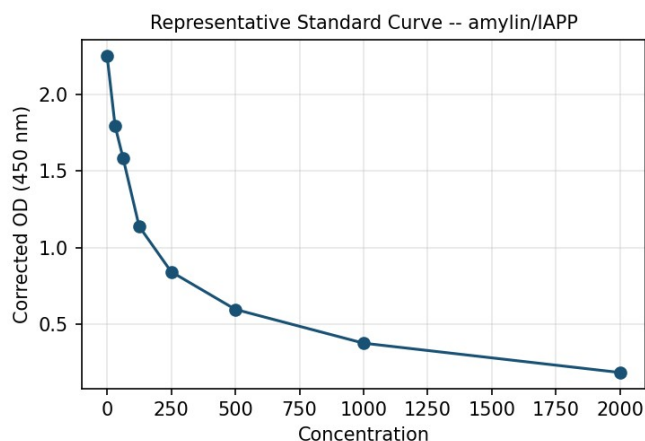
**Specificity:** This assay has high sensitivity and excellent specificity for detection of Human ProIAPP. No significant cross-reactivity or interference between Human ProIAPP and analogues was observed.



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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](http://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.