Pepsinogen I ELISA Assay Kit

Catalog Number:
PP131-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 16.0 (18JAN20)

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

INTENDED USE

The Eagle Biosciences Pepsinogen I ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of human pepsinogen I levels in serum. The Eagle Pepsinogen I ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

INTRODUCTION

Pepsinogen I (PGI) is a proenzyme secreted primarily by the chief and mucous neck cells of the gastric fundic glands, where it is later converted into the active digestive enzyme pepsin. PGI belongs to the aspartic proteinase family and plays a key role in protein digestion by breaking down complex proteins into peptides in the acidic environment of the stomach. Under normal physiological conditions, PGI is released into the gastric lumen, but a small amount also enters the bloodstream, where its serum concentration can be measured. PGI levels are influenced by gastric mucosal integrity, and their measurement serves as an important biomarker for assessing gastric function and pathology. Given its close association with gastric physiology, PGI is extensively studied in both clinical and research settings for its diagnostic and prognostic potential in various gastric diseases.

Clinically, PGI is most commonly used in the diagnosis and risk stratification of gastric diseases, particularly atrophic gastritis and gastric cancer. In individuals with atrophic gastritis, which is characterized by the progressive loss of gastric fundic glands, serum PGI levels decrease significantly due to reduced secretion from the damaged mucosa. This has led to the development of the serum PGI test, which, when combined with PGII and the PGI/PGII ratio, can serve as a noninvasive marker for detecting gastric mucosal atrophy and predicting the risk of gastric cancer. Low PGI levels, especially a reduced PGI/PGII ratio, have been associated with an increased risk of Helicobacter pylori-associated gastritis and intestinal-type gastric cancer. Consequently, PGI testing has been integrated into screening programs, particularly in regions with a high prevalence of gastric cancer, such as Japan and South Korea, where it aids in early detection and risk assessment.

In research settings, PGI serves as a valuable biomarker for studying gastric pathophysiology, disease progression, and treatment responses. It is often used in epidemiological studies to assess the prevalence of gastric atrophy in different populations and to investigate potential associations between PGI levels and dietary, genetic, or environmental risk factors. Furthermore, PGI is utilized in studies examining the effectiveness of interventions, such as H. pylori eradication therapy, in reversing gastric mucosal atrophy and reducing cancer risk. The role of PGI as a biomarker also extends to assessing the impact of pharmacological agents, such as proton pump inhibitors (PPIs), on gastric secretion and mucosal health. As research advances, PGI continues to be explored for its potential applications in personalized medicine and precision diagnostics, offering a noninvasive and cost-effective tool for monitoring gastric health and disease progression.

PRINCIPLE OF THE ASSAY

This Pepsinogen I ELISA is designed, developed and produced for the quantitative measurement of human pepsinogen I levels in serum samples. The assay utilizes the two-site sandwich technique with two selected monoclonal antibodies that bind to different epitopes of human pepsinogen I without any cross-reaction to pepsinogen II.

Assay standards, controls and samples containing pepsinogen I are added directly to microtiter wells of microplate that was coated with streptavidin. Simultaneously, a biotinylated antibody and a horseradish peroxidase-conjugated antibodies are added to each microwell. After the first incubation period, the wall of the microtiter well captures the biotinylated antibody as well as an immune complex in the form of "streptavidin—biotin-antibody—pepsinogen I—HRP-antibody". Unbound proteins as well as unbound

HRP-conjugated antibody in each microtiter well are removed in subsequent washing step. The microwell is incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to the pepsinogen I on the wall of the microtiter well is directly proportional to the amount of pepsinogen I concentrations for each standard of point-to-point, CubicSpline or 4-parameter plot. The concentration of pepsinogen I in test samples is determined directly from this standard curve.

LIMITATIONS RELATED TO INTENDED USE

- There is no gold standard concentration available for human pepsinogen I measurement, the values of assay standards were established by diluting a highly purified human pepsinogen protein matrix
- For unknown sample values read directly from the assay that are greater than 300 ng/mL, it is recommended to measure a further diluted sample for more accurate measurement.
- If there is not a microplate reader in your laboratory able to read beyond 2.0 at OD 450 nm, adjust the computer program for an assay without the standard level 6 from the standard set.
- Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
- Water deionized with polyester resin may inactivate the horseradish peroxidase enzyme.

PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- Practice good laboratory practices when handling kit reagents and specimens. This includes:
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
- Wear protective clothing and disposable gloves.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
- All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A standard curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (if applicable with this kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.

- The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
- This kit contains 0.5 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of saker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens, All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

Specimen Collection & Storage

Only 50 µL of human sera is required for pepsinogen I measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. However, a 10 hour fasting serum sample is recommended for the test. Samples should not be taken from those taking biotin-containing multivitamins or dietary supplements at least 48 hours prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850-1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 20°C or below until measurement. Avoid more than three freeze-thaw cycles of specimen.

Specimen Pre-Treatment
No pretreatment required

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision single channel pipettes capable of delivering 10 µL, 25 µL, 50µL, 65µL, 100 µL, and 1000 µL.
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
- Disposable plastic 1000 mL bottle with cap.
- Aluminum foil.
- Distilled water
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

REAGENTS PROVIDED

1. **Microplate**

Contents:	One streptavidin-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.

2. **Pepsinogen I Tracer Antibody (21x)**

Contents:	Concentrated HRP-conjugated anti-human tracer antibody in a stabilized protein matrix.
Format:	Concentrated; Requires Preparation
Volume:	0.6 mL/bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label. After preparation: discard immediately
Preparation of Working Solution:	**Dilute 1:21**. For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with 50 µL of the Tracer Antibody Concentrate and 50 µL of Capture Antibody Concentrate immediately before the assay is run.

3. **Tracer Antibody Diluent**

Contents:	Buffer for antibody dilution
Format	Ready to Use
Volume	12 mL/bottle
Storage	2-8°C
Stability:	Stable until the expiry date printed on the label

4. **Pepsinogen I Capture Antibody Concentrate (21x)**

Contents:	Biotinylated anti-human pepsinogen I capture antibody in a stabilized protein matrix
Format:	Concentrated; Requires Preparation
Volume:	0.6 mL/bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label. After preparation: discard immediately
Preparation of Working Solution:	**Dilute 1:21**. For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with 50 µL of the Tracer Antibody Concentrate and 50 µL of Capture Antibody Concentrate immediately before the assay is run.

5. **ELISA Wash Concentrate (30X)**

Contents:	Surfactant in a phosphate buffered saline with non-azide preservative.
Format:	Concentrated; Requires Preparation
Volume:	30 mL/bottle
Storage:	2-25°C
Stability	Stable until the expiry date printed on the label
Preparation	**Dilute 1:30**. The contents must be diluted with 870 mL distilled water and mixed well before use

6. **ELISA HRP Substrate**

Contents:	Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label.

7. **ELISA Stop Solution**

Contents:	0.5 M sulfuric acid
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-25°C
Stability:	Stable until the expiry date printed on the label

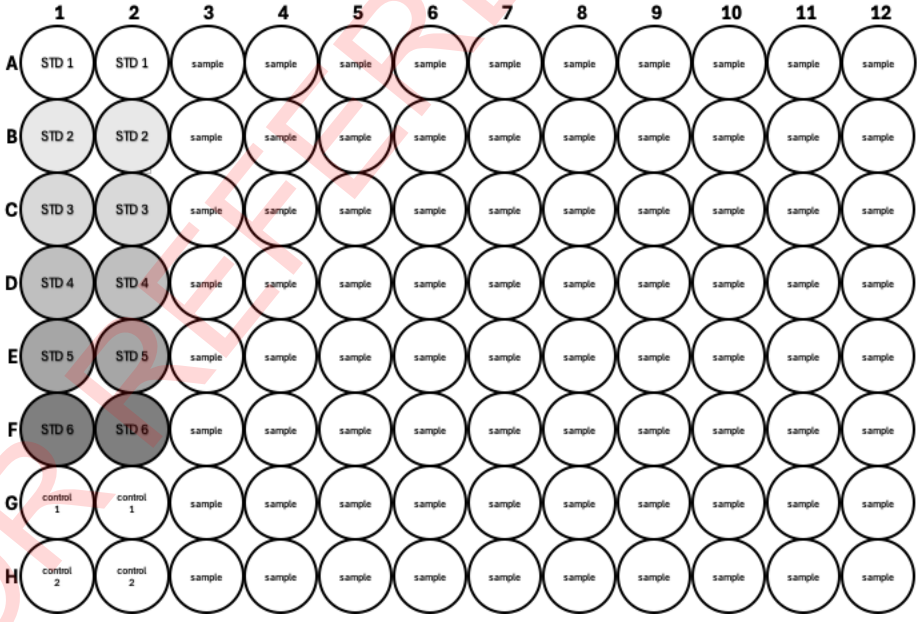
8. **Pepsinogen I Standards (Levels 1-6)**

Contents:	Six bottles of lyophilized human pepsinogen I in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentrations.
Format:	Lyophilized; constitution required
Volume:	6 vials
Storage:	2-8°C. <-20°C for long term storage after preparation. Do not exceed 3 freeze-thaw cycles.
Stability	Stable until the expiry date printed on the label
Preparation	Must be reconstituted with 0.5 mL of demineralized water, allow to sit for 10 minutes, and then mixed by inversion or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

9. **Pepsinogen I Controls**

Contents:	Lyophilized pepsinogen I in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.
Format:	Lyophilized; constitution required
Volume:	2 vials
Storage:	2-8°C. <-20°C for long term storage after preparation. Do not exceed 3 freeze-thaw cycles.
Stability:	Stable until expiry date printed on the label
Preparation	Must be reconstituted with 0.5 mL of demineralized water, allow to sit for 10 minutes, and then mixed by inversion or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

RECOMMENDED ASSAY LAYOUT*



**Layout subject to change based on standard and control quantities*

ASSAY PROCEDURE

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Place a sufficient number of microplate wells in a holder to run standards, controls and samples.
2. **Add 25 µL** of standards, controls and samples into the designated microwells. Mix by gently tapping the plate.
3. Add **100 µL** of prepared antibody working solution to each microwell.
4. Cover the plate with one plate sealer and aluminum foil. **Incubate at room temperature** (20-25°C) for **60 minutes**.
5. Remove the plate sealer. Aspirate the contents of each microwell. Wash each microwell **5 times** by dispensing 350 µL of diluted wash solution into each microwell, and completely aspirate the contents. Alternatively, an automated microplate washer can be used.
6. **Add 100 µL** of HRP Substrate into each microwell. Mix by gently tapping the plate.
7. Cover the plate with plate sealer and aluminum foil. **Incubate at room temperature** (20-25°C) for **20 minutes**.
8. Remove aluminum foil and plate sealer and add **100 µL** of Stop Solution into each well. Mix by gently tapping the plate.
9. Read the absorbance at **450 nm** within 10 minutes with a microplate reader.

CALCULATIONS

- Calculate the average absorbance for each pair of duplicate test results.
- Subtract the average absorbance of the standard level 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
- The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.
- It is recommended to use the following curve fits: (1) Point-to-point, or (2) 4-Parameter or (3) CubicSpline
- The human pepsinogen I concentrations for the controls and samples are read directly from the standard curve using their respective corrected absorbance.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known pepsinogen I levels. We recommend that all assays include the laboratory's own controls.

TYPICAL DATA

Seventy-three normal adult serum was measured with this pepsinogen I ELISA. The expected normal range is listed in the following table with different percentile cut-off and the median level of this group of population is 62.8 ng/mL

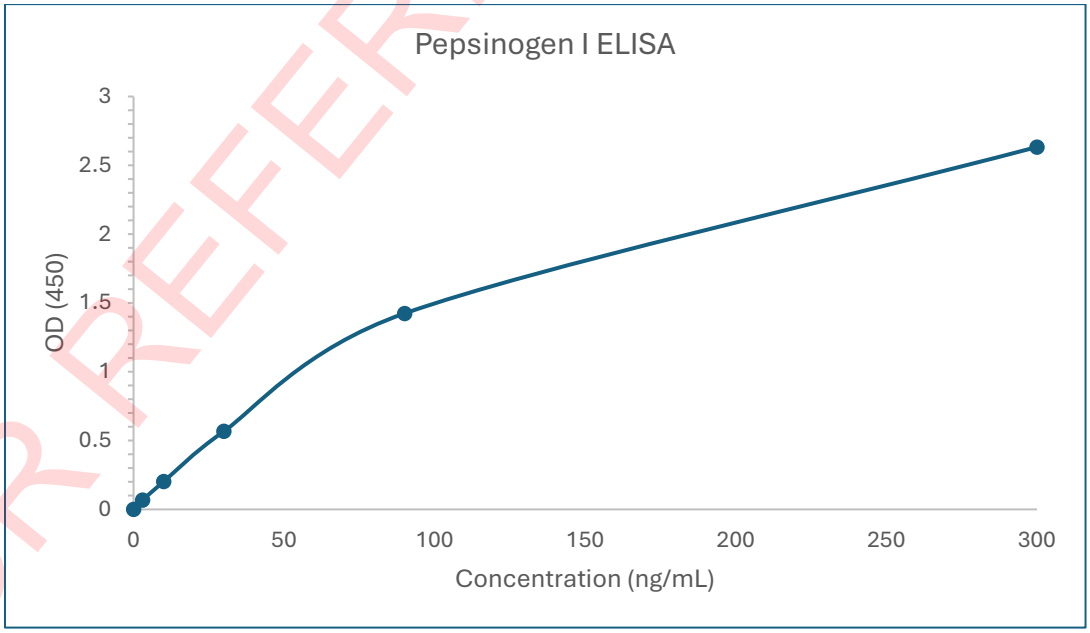
Percentile Cut-Off	Normal Range (ng/mL
95%	25-200
90%	30-150
85%	40-120
80%	40-100

TYPICAL STANDARD CURVE

A typical absorbance data and the resulting standard curve are represented.

Note: this curve should not be used in lieu of standard curve run with each assay

WELL ID	Reading Absorbance (450/620 nm)			Concentration (ng/mL)
	Readings	Average	Corrected	
Standard Level 1: 0 ng/mL	0.053	0.052	0.000	
	0.050			
Standard Level 2: 3 ng/mL	0.119	0.119	0.067	
	0.118			
Standard Level 3: 10 ng/mL	0.262	0.254	0.202	
	0.246			
Standard Level 4: 30 ng/mL	0.616	0.619	0.567	
	0.622			
Standard Level 5: 90 ng/mL	1.565	1.476	1.424	
	1.387			
Standard Level 6: 300 ng/mL	2.766	2.685	2.633	
	2.604			
Control 1	0.373	0.368	0.316	16.2
	0.363			
Control 2	1.692	1.640	1.588	118
	1.587			



PERFORMANCE AND CHARACTERISTICS

Sensitivity
The sensitivity of this pepsinogen I ELISA is 0.1 ng/mL as determined by measuring zero standard 16 times in the same assay and calculating the detection limit at 3 standard deviations above the pepsinogen I zero calibrator. The assay analytical sensitivity is approximately 0.5 ng/mL.

Specificity
This assay measures human pepsinogen I without any cross-reaction to human pepsinogen II.

Hook Effect
It was determined that this pepsinogen I ELISA did not show any high dose "hook" effect up to 10,000 ng/mL of pepsinogen I.

Reproducibility and Precision
The intra-assay precision is validated by measuring two samples in a single assay with 20 replicate determinations. The inter-assay precision is validated by measuring two samples in duplicate in 12 individual assays. The results are as follows.

	Intra-Assay		Inter-Assay	
Sample	**1**	**2**	**1**	**2**
Mean (ng/mL)	18.2	121.1	17.5	123.7
CV (%)	5.3	4.8	6.9	5.7

Linearity
Two serum samples were diluted with assay buffer and tested. The results are as follows:

Samples	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Sample 1	31.90	-	-
50%	16.21	15.95	102
25%	7.95	7.78	102
12.5%	3.73	3.99	93
6.25%	2.11	1.99	106
Sample 2	252.00	-	-
50%	125.27	126.0	99
25%	64.12	63.00	102
12.5%	31.25	31.50	99
6.25%	16.92	15.75	107

Spike Recovery
Two samples were spiked with various amounts of pepsinogen I and assayed. The results indicate below:

Samples	Observed (ng/mL)	Expected (ng/mL)	% Recovery (%)
Sample 1	18.6	-	-
10	12.6	14.3	88
30	25.1	24.3	103
90	56.2	54.3	103
Sample 2	121.1	-	-
10	61.3	65.6	93
30	70.9	75.6	94
90	104.7	105.6	99

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WARRANTY INFORMATION

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.