Pepsinogen II ELISA Assay Kit

Catalog Number:

PP231-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

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INTENDED USE

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

INTRODUCTION

Pepsinogen consists of a single polypeptide chain of 375 amino acids with an average molecular weight of 42 kDa. Pepsinogen I is synthesized at gastric chief cells and mucous neck cells, while pepsinogen II is produced not only by gastric chief cells, mucous neck cells, but also by clear mucous cells of antrum and Brunner's glands in the proximal duodenum, etc. The clinical applications of measuring pepsinogen I and pepsinogen II are of useful aid in diagnosing severe atrophic gastritis and stomach cancer. It was suggested that the measurement of serum pepsinogens served as a "serological biopsy" for predicting the presence of atrophic gastritis or superficial gastritis.

Atrophic Gastritis: It was found that a serum pepsinogen II level falling to less than 20 ng/ml was highly specific for severe atrophic gastritis. It is also observed that serum pepsinogen II levels fell with increasing severity of mucosal damage in atrophic gastritis. The diagnostic sensitivity and specificity of serum pepsinogen II level for advanced atrophic corpus gastritis are about 92% and 90% respectively. On the other hand, the decrease in serum pepsinogen II levels in patients with pernicious anemia and atrophic gastritis was found to be associated with normal or raised pepsinogen I levels. Therefore, a pepsinogen I/pepsinogen II ratio is significantly lower than those with superficial gastritis or normal remnant mucosa.

Stomach Cancer: Low serum pepsinogen II levels were found in patients with gastric cance, with a threefold higher incidence. Other studies have concluded that low serum pepsinogen II levels may identify persons at increased risk for intestinal types of stomach cancer.

Duodenal Ulcer: A low serum pepsinogen II level can exclude a diagnosis of duodenal ulcer. Although a high pepsinogen II level has less clinical useful for establishing the diagnosis of a duodenal ulcer, the combination of hypergastrinemia and a highly elevated serum pepsinogen II strongly suggests the possibility of the Zollinger-Ellison syndrome.

PRINCIPLE OF THE ASSAY

The Eagle Biosciences Human Pepsinogen II ELISA Assay Kit is designed, developed and produced for the quantitative measurement of human pepsinogen II level in serum sample. The assay utilizes the two-site "sandwich" technique with two selected monoclonal antibodies that bind to different epitopes of human pepsinogen II without any cross-reaction to human pepsinogen I.

Assay calibrators, controls and patient serum samples containing human pepsinogen II is added directly to microtiter wells of microplate that was coated with a streptavidin. Simultaneously, a biotinylated antibody and a horseradish peroxidase conjugated antibody is added to each well. After the first incubation period, on the wall of microtiter well captures the biotinylated antibody as well as an immuno complex in the form of "streptavidin – biotin-antibody – pepsinogen II – HRP-antibody". Unbound proteins as well as unbound HRP conjugated antibody in each microtiter well are removed in the subsequent washing step. The well 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 II on the wall of the microtiter well is directly proportional to the amount of pepsinogen II in the sample. A standard curve is generated by plotting the absorbance versus the respective human pepsinogen II concentration for each standard on Point-to-Point, CubicSpline or 4-Parameter plot. The concentration of human pepsinogen II in test samples is determined directly from this standard curve.

REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until the expiration date. Allow all reagents to come to room temperature prior to use. Reagents from different kit lot numbers should not be combined or interchanged.

1. Streptavidin Coated Microplate

Microplate coated with streptavidin

Qty:	1 x 96 well microplate
Storage:	2 – 8 °C
Preparation:	Ready to Use.

2. Pepsinogen II Tracer Antibody

HRP-conjugated anti-human tracer antibody in a stabilized protein matrix

Qty:	1 x 0.6 mL
Storage:	2 – 8 °C
Preparation:	21X Concentrate. The contents must be diluted with tracer antibody diluent and mixed well before use.

3. Tracer Antibody Diluent

Buffer for antibody dilution according to the assay procedures.

Qty:	1 x 12 mL
Storage:	2 – 8 °C
Preparation:	Ready to Use.

4. Pepsinogen II Capture Antibody

Biotinylated anti-human pepsinogen II capture antibody in a stabilized protein matrix.

Qty:	1 x 0.6 mL
Storage:	2 – 8 °C
Preparation:	Ready to Use.

5. ELISA Wash Concentrate

Surfactant in a phosphate buffered saline with non-azide preservative

Qty:	1 x 30 mL
Storage:	2 – 25 °C
Preparation:	30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

6. ELISA HRP Substrate

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty:	1 x 12 mL
Storage:	2 – 8 °C
Preparation:	Ready to Use.

7. ELISA STOP SOLUTION

0.5M sulfuric acid

Qty:	1 x 12 mL
Storage:	2 – 25 °C
Preparation:	Ready to Use.

8. Pepsinogen II Calibrators Levels 1 to 6

Lyophilized human pepsinogen II in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty:	6x vials
Storage:	2 – 8 °C, < -20°C for long term storage. Do not exceed three freeze-thaw cycles
Preparation:	Must be reconstituted with 0.5 mL of demineralized water, allowed to sit for 10 minutes, and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

9. Pepsinogen II Controls

Lyophilized human pepsinogen II in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty:	2 x vials
Storage:	2 – 8 °C, < -20°C for long term storage. Do not exceed three freeze-thaw cycles
Preparation:	Must be reconstituted with 0.5 mL of demineralized water, allowed to sit for 10 minutes, and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

SAFETY PRECAUTIONS

The Human Pepsinogen II ELISA Assay Kit reagents are for Research Use Only. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision single channel pipettes capable of delivering 20 µL, 25 µL, 100 µL, and 1000 µL etc.
- 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 caps.
- Aluminum foil.
- Deionized or 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 450nm.

SPECIMEN COLLECTION

Only 100 µL of human serum is required for human pepsinogen II 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. 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 repeated more than three times freezing and thawing of specimen.

ASSAY PROCEDURE

Reagent Preparation

1. Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.
3. Reconstitute all assay calibrators level 1 to level 6 and controls by adding **0.5 mL** of demineralized water to each vial. Allow the calibrators and controls to sit undisturbed for 10 minutes, and then mix well by inversions or gentle vortexing. One must make sure that all solid is dissolved completely prior to use. These reconstituted calibrators and controls must be stored at 2 – 8°C for up to three days or at - 20°C or below for long-term storage. Do not exceed 3 freeze-thaw cycles.

Manual Assay Procedure

1. Place a sufficient number of streptavidin coated microwell strips in a holder to run calibrators, controls and samples in duplicate.
2. Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	CALIBRATOR LEVEL 1	CALIBRATOR LEVEL 5	SAMPLE 1
B	CALIBRATOR LEVEL 1	CALIBRATOR LEVEL 5	SAMPLE 1
C	CALIBRATOR LEVEL 2	CALIBRATOR LEVEL 6	SAMPLE 2
D	CALIBRATOR LEVEL 2	CALIBRATOR LEVEL 6	SAMPLE 2
E	CALIBRATOR LEVEL 3	CONTROL 1	SAMPLE 3
F	CALIBRATOR LEVEL 3	CONTROL 1	SAMPLE 3
G	CALIBRATOR LEVEL 4	CONTROL 2	
H	CALIBRATOR LEVEL 4	CONTROL 2	

3. Prepare the antibody working solution by 1:21 fold dilution of the Tracer Antibody and the Capture Antibody with the Diluent. For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with the addition of 50 µL of the Tracer Antibody and 50 µL Capture Antibody in a clean test tube or vial. *Note: This antibody working solution should be freshly prepared.*
4. Add **50 µL** of calibrators, controls, and samples into the designated microwell.
5. Add **100 µL** of antibody working solution to each microwell
6. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25°C)** for **120 minutes**.
7. 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, then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
8. Add **100 µL** of Substrate into each of microwell. Mix by gently tapping the plate.
9. Cover the plate with one new plate sealer and also with aluminum foil. Incubate plate at **room temperature (20-25°C)** for **20 minutes.**
10. Remove the aluminum foil and plate sealer and add **100 µL** of ELISA Stop Solution into each of the microwells. Mix by gently tapping the plate.
11. Read the absorbance at **450 nm** within **10 minutes** with a microplate reader.

Assay Procedure on Automated ELISA System

1. Prepare the antibody working solution by 1:21 fold dilution of the tracer antibody and capture antibody with the diluent. For each strip, it is required to mix **1 mL** of the diluent with **50 µL** of the tracer antibody and **50 µL** capture antibody in a clean test tube. *Note: this antibody working solution should be freshly prepared.*
2. Add **50 µL** of calibrators, controls, and samples into the designated microwells.
3. Add **100 µL** of antibody working solution to each microwell
4. Incubate plate with initial shaking for 1 minutes and further incubation at **37°C** for **60 minutes**.
5. Aspirate the contents of each microwell. Wash each microwell 5 times by dispensing 350 µL of diluted wash solution into each well and then completely aspirating the contents.
6. Add **100 µL** of Substrate into each microwell.
7. Incubate plate at **37°C** for **18 minutes**
8. Add **100 µL** of Stop Solution into each of the wells.
9. Read the absorbance at 450 nm

Note: The above automated ELISA procedure has been performed on DS2 system. A satisfactory patient sample correlation was observed between the manual and automated assay procedures (r = 0.943, slope = 1.0958). One may adjust the procedure according to different automated ELISA system used in each laboratory.

PROCEDURAL NOTES

1. It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original bottles and avoid unnecessary exposure to the light.

3. Store any unused antibody coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading
7. All reagents should be mix gently and thoroughly prior use. Avoid foaming.

INTERPRETATION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the LEVEL 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator 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.
4. It is recommended to use following curve fits: (1) Point-to-Point, or (2) 4-Parameter or (3) CubicSpline.
5. The human pepsinogen II concentrations for the controls and patient samples are read directly from the calibration curve using their respective corrected absorbance.

LIMITATIONS OF THE PROCEDURE

1. Since there is no Gold Standard concentration available for human pepsinogen II measurement, the values of assay calibrators were established by diluting a highly purified human pepsinogen II in a protein matrix.
2. For unknown sample value read directly from the assay that is greater than 300 ng/mL, it is recommended to measure a further diluted sample for more accurate measurement.
3. 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 calibrator level 6 from the calibrator set.
4. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
5. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known pepsinogen II levels. We recommend that all assays include the laboratory's own human serum based pepsinogen II controls in addition to those provided with this kit.

EXPECTED VALUES

Sevently-three normal adult sera were measure with this human pepsinogen II 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 4.9ng/mL.

Percentile Cut-off	Normal Range (ng/mL)
95%	2.3 – 20
90%	2.5 – 15
85%	3.0 – 12
80%	3.0 – 11

The ratio of pepsinogen I/II is calculated from the same group of normal population.

Percentile Cut-off	Normal Range (ng/mL)
95%	3 – 32
90%	4 – 25
85%	4 – 24
80%	6 – 22

It is highly recommend that each laboratory should establish their own normal range for pepsinogen I/II based on local populations.

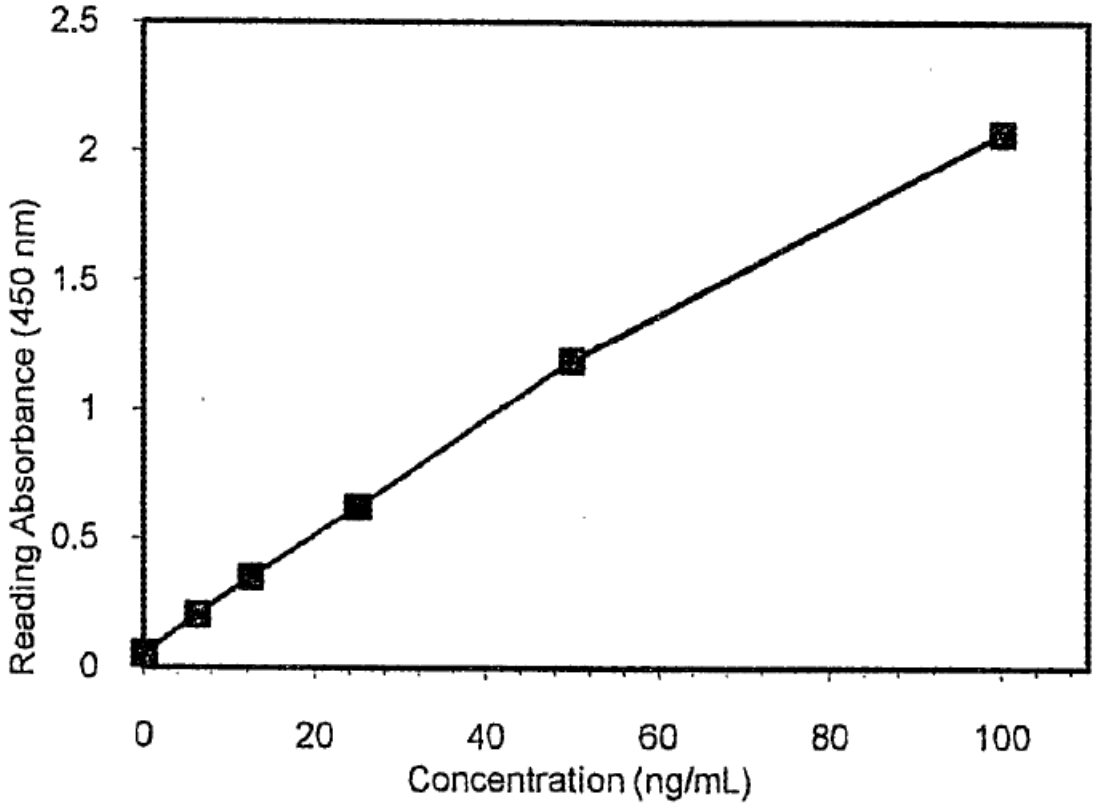
Subjects with atrophicgastritis, as well as subjects with stomach cancer would have a pepsinogen I/II level below 3 ng/mL.

EXAMPLE DATA

A typical absorbance data and the resulting calibration curves from are represented. *Note: This curve should not be used in lieu of calibrator curve run with each assay.*

Microwell I.D.	Reading Absorbance (450 nm)			Concentration ng/mL
	Readings	Average	Corrected	
Calibrator Level 1: 0 ng/mL	0.053	0.052	0.000	
	0.050			
Calibrator Level 2: 6.3 ng/mL	0.201	0.205	0.153	
	0.208			
Calibrator Level 3: 12.5 ng/mL	0.341	0.349	0.297	
	0.357			
Calibrator Level 4: 25 ng/mL	0.590	0.623	0.571	
	0.656			
Calibrator Level 5: 50 ng/mL	1.250	1.191	1.139	
	1.132			
Calibrator Level 6: 100 ng/mL	2.064	2.069	2.017	
	2.074			
Control 1	0.218	0.218	0.166	6.8
	0.217			
Control 2	0.619	0.637	0.585	25.6
	0.655			

Example Data



PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of this Human Pepsinogen II ELISA Assay Kit 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 deviation above the pepsinogen II zero standard. Whereas the assay analytical sensitivity is approximately 0.5 ng/mL.

Hook Effect

It was determined that this pepsinogen II ELISA did not show any high dose "hook" effect up to 1,000 ng/mL of pepsinogen II.

Reproducibility and Precision

The intra-assay precision is validated by measuring two samples in a single assay with 16 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)	8.7	33.6	8.5	33.0
CV (%)	3.8	7.1	6.9	5.7

Linearity

Two human serum samples spiked with pepsinogen II were diluted with assay buffer and assayed. The results in the value of ng/mL are as follows:

Sample	OBSERVED VALUE (ng/mL)	EXPECTED VALUE (ng/mL)	RECOVERY %
		-	-
Sample 1	16.2		
50%	8.5	8.1	105
25%	3.9	4.1	95
12.5%	1.9	2.0	95
		-	-
Sample 2	56.8		
50%	26.7	28.4	94
25%	13.8	14.2	97
12.5%	6.9	7.1	97
6.25%	4.0	3.6	111

Spike Recovery

Two patient samples were spiked with various amounts of human pepsinogen II and assayed. The results are as follows:

Samples	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Sample 1	8.3	-	-
6.3	11.4	8.8	94
12.5	17.6	11.9	96
25.0	6.1	18.1	97
Sample 2	9.3	-	-
6.3	14.9	6.0	102
12.5	8.3	9.1	102
25.0	11.4	15.3	97

Specificity

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

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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.