Epitope Diagnostics, Inc.

EDI™ Human Pepsinogen I ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the measurement of Human Pepsinogen I Levels in Serum

REF KT 810   EU: IVD C E Σ 96 ☀ +2↗+8°C

INTENDED USE
This ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human pepsinogen I levels in serum. Determination of human serum pepsinogen I level would be a useful tool in the aid of diagnosing the functional states of acid-secreting gastric mucosa.

SUMMARY OF PHYSIOLOGY
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 and mucous neck cells, but also by clear mucous cells of antrum, etc. The clinical applications of measuring pepsinogen I and pepsinogen II are a 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 serum pepsinogen I levels falling to less than 20 ng/ml was highly specific for severe atrophic gastritis. It is also observed that serum pepsinogen I levels fell with increasing severity of mucosal damage in atrophic gastritis. The diagnostic sensitivity and specificity of serum pepsinogen I level for advanced atrophic corpus gastritis are about 92% and 90% respectively. On the other hand, the decrease in serum pepsinogen I levels in patients with pernicious anemia and atrophic gastritis was found to be associated with normal or raised pepsinogen II 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 I levels were found in patients with gastric cancer, with a threefold higher incidence. Other studies have concluded that low serum pepsinogen I levels may identify persons at increased risk for intestinal types of stomach cancer.

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

ASSAY PRINCIPLE
This ELISA is designed, developed and produced for the quantitative measurement of human pepsinogen I 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 I without any cross-reaction to human pepsinogen II.

Assay standards, controls and patient serum samples containing human pepsinogen I are added directly to microtiter wells of microplate that was coated with streptavidin. Simultaneously, a biotinylated antibody and a horseradish peroxidase-conjugated

antibody are added to each well. After the first incubation period, the wall of microtiter well captures the biotinylated antibody as well as an immuno 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 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 I on the wall of the microtiter well is directly proportional to the amount of pepsinogen I in the sample. A standard curve is generated by plotting the absorbance versus the respective human pepsinogen I concentration for each standard on Point-to-Point, CubicSpline or 4-Parameter plot. The concentration of human pepsinogen I 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 this expiration date.

Prior to use allow all reagents to come to room temperature.
Reagents from different kit lot numbers should not be combined or interchanged.

1. **Streptavidin Coated Microplate (Cat. No. 10040)**
One microplate with 12 x eight strips (96 wells total) coated with streptavidin. The plate is framed and sealed in a foil Ziploc bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **Pepsinogen I Tracer Antibody (Cat. No. 30061)**
One vial contains 0.6 mL concentrated horseradish peroxidase (HRP)-conjugated anti-human pepsinogen I tracer antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **Pepsinogen I Capture Antibody (Cat. No. 30062)**
One vial contains 0.6 mL concentrated biotinylated anti-human pepsinogen I capture antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. **Tracer Antibody Diluent (Cat. No. 30017)**
One vial contains 12 mL ready-to-use buffer. It should be only used for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. **ELISA Wash Concentrate (Cat. No. 10010)**
One bottle contains 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and

mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide preservative. The diluted solution should be stored at room temperature and is stable until the expiration date on the kit box.

6. ELISA HRP Substrate (Cat. No. 10020)
One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

7. ELISA Stop Solution (Cat. No. 10030)
One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

8. Pepsinogen I Standards (Cat. No. 30053 – 30058)
Six vials each contain lyophilized human pepsinogen I in a bovine serum albumin-based matrix with a non-azide preservative. **Refer to vials for exact concentration for each standard.** All the standards should be reconstituted with DI-water and stored at -20°C or below after the first use with up to 3 freeze cycles.

9. Pepsinogen I Controls (Cat. No. 30059 – 30060)
Two vials each contain lyophilized human pepsinogen I in a bovine serum albumin-based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control.** Both controls should be reconstituted with DI-water and stored at -20°C or below after the first use with up to 3 freeze cycles.

SAFETY PRECAUTIONS
The reagents must be used in research laboratory and is for research use only. The source material for reagents containing bovine serum 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 are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. 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
1. Precision single channel pipettes capable of delivering 20 µL, 25 µL, 100 µL, and 1000 µL, etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass tubes.
5. Disposable plastic 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

SPECIMEN COLLECTION
Only 50 µL of human serum is required for human 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. 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.

ASSAY PROCEDURE
1. 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 (Cat. 10010) must be diluted to working solution prior use. Please see REAGENTS section for details.
(3) Reconstitute all assay standards (Cat. 30053-30058) and controls (Cat. 30059-30060) by adding **0.5mL** of demineralized water to the vial of standard level 1 and **0.5mL** demineralized water to the vials of standard levels 2 - 6 and controls 1 & 2. Allow the standards and controls to sit undisturbed for 10 minutes, and then mix well by gentle vortexing. Make sure that all solid is dissolved completely prior to use. These reconstituted standards and controls must be stored at - 10°C or below. Do not exceed 3 freeze-thaw cycles.
(4) Place a sufficient number of streptavidin coated microwell strips (Cat. 10040) in a holder to run human pepsinogen I standards, controls and unknown samples in duplicate.
(5) Test Configuration

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

(6) Prepare working Tracer Antibody and Capture Antibody mixture by 1:21 fold dilution of the Pepsinogen I Tracer Antibody (Cat. 30061) and the Pepsinogen I Capture Antibody (Cat. 30062) with the Tracer Antibody Diluent (Cat. 30017). For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with the addition of 50 µL of Tracer Antibody and 50 µL Capture Antibody in a clean test tube or vial. Following is a table that outlines the relationship of strips used and antibody mix prepared.

Strip no.	Tracer Antibody Diluent	Tracer Antibody	Capture Antibody
1	1 mL	50 µL	50 µL
2	2 mL	100 µL	100 µL
3	3 mL	150 µL	150 µL
4	4 mL	200 µL	200 µL
5	5 mL	250 µL	250 µL
6	6 mL	300 µL	300 µL
7	7 mL	350 µL	350 µL
8	8 mL	400 µL	400 µL
9	9 mL	450 µL	450 µL
10	10 mL	500 µL	500 µL
11	11 mL	550 µL	550 µL
12	12 mL	600 µL	600 µL

***Note:** this antibody mix should be freshly prepared right before running the assay.*

2. Manual Assay Procedure
(1) Add **25 µL** of standards, controls and patient serum samples into the designated microwell.
(2) Add **100 µL** of above antibody mixture to each well
(3) Mix gently and cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
(4) Incubate plate at room temperature for **1 hour**.
(5) Remove the aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(6) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(7) Cover the plate with one new plate sealer and also with aluminum foil to avoid exposure to light.
(8) Incubate plate at room temperature for **20 minutes.** *(This incubation period may be reduced to 8 – 15 min if a lower OD reading is demanded to fit to the plate readers specification.)*
(9) Remove the aluminum foil and plate sealer. Add 100 µL of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(10) Read the absorbance at **450 nm** within 10 minutes in a microplate reader.

3. Assay Procedure on Automated ELISA System
(1) Add **25 µL** of standards, controls and patient serum samples into the designated microwell.
(2) Add **100 µL** of above antibody mixture to each well
(3) Incubate plate with initial shaking for 1 minutes and further incubation at **37ºC** for **45 minutes**.
(4) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents.
(5) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(6) Incubate plate at **37ºC** for **15 minutes.**
(7) Add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(8) 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 standards, 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 mixed gently and thoroughly prior to 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 STD 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. 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.
4. It is recommended to use following curve fits: (1) Point-to-Point, or (2) 4-Parameter or (3) CubicSpline.
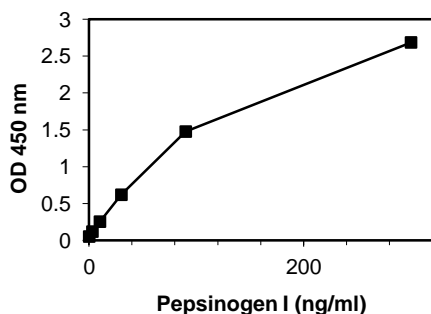
The human pepsinogen I concentrations for the controls and patient samples are read directly from the standard curve using their respective corrected absorbance.

EXAMPLE DATA AND STANDARD CURVE
A typical absorbance data and the resulting standard curve from human pepsinogen I ELISA are represented. **This curve should not be used in lieu of standard curve run with each assay.**

Well I.D.	OD 450 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.053 0.050	0.052	0.000	
3 ng/mL	0.119 0.118	0.119	0.067	
10 ng/mL	0.262 0.246	0.254	0.202	
30 ng/mL	0.616 0.622	0.619	0.567	
90 ng/mL	1.565 1.387	1.476	1.424	
300 ng/mL	2.766 2.604	2.685	2.633	
Control 1	0.373 0.363	0.368	0.316	16.2 ng/mL
Control 2	1.692 1.587	1.640	1.588	118 ng/mL

Pepsinogen I Standard Curve

EXPECTED VALUES

Seventy-three normal adult sera were measured with this human 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

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

Patients with atrophic gastritis, as well as patients with stomach cancer would have a pepsinogen I level below 20 ng/mL. However, gastroendoscope and tissue biopsy should be used as final and confirmative diagnostic method.

LIMITATION OF THE PROCEDURE
1. Since 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 I 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 standard level 6 from the standard 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 I levels. We recommend that all assays include the laboratory's own human serum based pepsinogen I controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of this human 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 standard. 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.

Linearity

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

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	Neat	31.90	-	-
	1:2	16.21	15.95	102
	1:4	7.95	7.78	102
	1:8	3.73	3.99	93
	1:16	2.11	1.99	106
2	Neat	252.00	-	-
	1:2	125.27	126.00	99
	1:4	64.12	63.00	102
	1:8	31.25	31.50	99
	1:16	16.92	15.75	107

Precision

The intra-assay precision is validated by measuring two samples in a single assay with 20 replicate determinations.

Mean Pepsinogen I Value (ng/mL)	CV (%)
18.2	5.3
121.1	4.8

The inter-assay precision is validated by measuring two samples in duplicate in 12 individual assays.

Mean Pepsinogen I Value (ng/mL)	CV (%)
17.5	6.9
123.7	5.7

Recovery

Two patient samples were spiked with various amounts of human pepsinogen I and assayed. The results in the value of ng/mL are as follows:

#	Orig. Value	Amount Spiked	Observed Value	Expected Value	Recovery %
1	18.6	10	12.6	14.3	88
		30	25.1	24.3	103
		90	56.2	54.3	103
2	121.1	10	61.3	65.6	93
		30	70.9	75.6	94
		90	104.7	105.6	99

"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.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable

for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

1. Kuipers EJ. In through the out door: serology for atrophic gastritis. Eur J Gastroenterol Hepatol. 2003 Aug;15(8):877-9.
2. Miki K, Morita M, Sasajima M, Hoshina R, Kanda E, Urita Y. Usefulness of gastric cancer screening using the serum pepsinogen test method. Am J Gastroenterol. 2003 Apr;98(4):735-9.
3. Miki K. [Serum pepsinogen I/II ratio test] Nippon Rinsho. 2003 Jan;61(1):92-5. Japanese.
4. So JB, Yeoh KG, Moochala S, Chachlani N, Ho J, Wong WK, Mack P, Goh PM. Serum pepsinogen levels in gastric cancer patients and their relationship with Helicobacter pylori infection: a prospective study. Gastric Cancer. 2002;5(4):228-32.
5. Korstanje A, den Hartog G, Biemond I, Lamers CB. The serological gastric biopsy: a non-endoscopical diagnostic approach in management of the dyspeptic patient: significance for primary care based on a survey of the literature. Scand J Gastroenterol Suppl. 2002;(236):22-6. Review.
6. Sipponen P, Harkonen M, Alanko A, Suovaniemi O. Diagnosis of atrophic gastritis from a serum sample. Clin Lab. 2002;48(9-10):505-15. Review.
7. Tabata H, Fuchigami T, Kobayashi H, Sakai Y, Nakanishi M, Tomioka K, Nakamura S, Matsumoto T, Fujishima M. Difference in degree of mucosal atrophy between elevated and depressed types of gastric epithelial tumors. Scand J Gastroenterol. 2001 Nov;36(11):1134-40.
8. Varis K, Sipponen P, Laxen F, Samloff IM, Huttunen JK, Taylor PR, Heinonen OP, Albanes D, Sande N, Virtamo J, Harkonen M. Implications of serum pepsinogen I in early endoscopic diagnosis of gastric cancer and dysplasia. Helsinki Gastritis Study Group. Scand J Gastroenterol. 2000 Sep;35(9):950-6.
9. Fernandez R, Vizoso F, Rodriguez JC, Merino AM, Gonzalez LO, Quintela I, Andicoechea A, Truan N, Diez MC. Expression and prognostic significance of pepsinogen C in gastric carcinoma. Ann Surg Oncol. 2000 Aug;7(7):508-14.
10. Kalinovskii VP, Gamaiunova VB, Shumakov AP, Khanson KP. [Radioimmunoassay of serum pepsinogen I in chronic gastritis and stomach cancer] Vopr Onkol. 2000;46(2):153-5. Russian.
11. Shumakov AR, Fedorov SN, Kalinovskii VP, Khanson KP. [Evaluation of pepsinogen A expression in stomach cancer] Vopr Onkol. 1999;45(3):238-40. Russian.
12. Kitahara F, Kobayashi K, Sato T, Kojima Y, Araki T, Fujino MA. Accuracy of screening for gastric cancer using serum pepsinogen concentrations. Gut. 1999 May;44(5):693-7.
13. Samloff IM and Taggart RT. Pepsinogens, pepsins, and peptic ulcer. Clinical and Investigative Medicine 1987;10:215-221.
14. Samloff IM. Slow moving protease and the seven pepsinogens. Electrophoretic demonstration of the existence of eight proteolytic fractions in human gastric mucosa. Gastroentrology 1969;57:659-669.

CE

EC | REP

MDSS GmbH
Schiffgraben 41
30175 Hannover,
Germany

	Manufacturer		No. of tests
REF	Catalog Number		Keep away from heat and direct sun light
CONC	Concentrate		Store at
IVD	In Vitro Diagnostic Device		Use by
	Read instructions before use	LOT	Lot No.
EC REP	Authorized Representative In Europe		

Pepsinogen I ELISA: Short Manual Assay Protocol

1. **25 µl Calibrators, controls and patient samples**

2. **100 µl Antibody mixture**

Incubate @ RT for 60 min

Wash 5 x

3. **100 µl TMB Substrate**

Incubate @ RT for 20 min

4. **100 µl Stop Solution**

5. **Read absorbance at 450 nm**

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.   www.epitopediagnostics.com

This product is developed and manufactured by
Epitope Diagnostics, Inc.
San Diego, CA 92121, USA