DRG® Human Pepsinogen II ELISA (EIA-4603)

Revised 20 Nov. 2010 rm (Vers. 3.1) **RUO in the USA**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is intended for determination of human pepsinogen II levels in serum.

ASSAY PRINCIPLE

This ELISA 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 standards, 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, Cubic Spline 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 this expiration date.

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

1. **Streptavidin Coated Microplate**
   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 II Tracer Antibody**
One vial contains 0.6 mL concentrated horseradish peroxidase (HRP) conjugated anti-human pepsinogen II 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 II Capture Antibody**
One vial contains 0.6 mL concentrated biotinylated anti-human pepsinogen II 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**
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**
One bottle contains 20 mL of 30 fold concentrate.
Before use the contents must be diluted with 580 mL of distilled water and mix 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**
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**
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 II Standards**
Six vials each contains lyophilized human pepsinogen II in a bovine serum albumin based matrix with a non-azide preservative.
Refer to vial 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 II Controls**
Two vials each contains lyophilized human pepsinogen II 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 store at -20°C or below after the first use with up to 3 freeze cycles.

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SAFTY PRECAUTIONS

The reagents must be used in research laboratory and is for research use only. Source material from which reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from donor health 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 potential 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 sever 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 lease 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 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, it is recommended drawing a 10 hour fasting serum sample 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 – 1500 x g 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 –20C 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. Regents 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 standards and controls by adding
1 mL of demineralized water to the vial of **standard level 1** and
0.5 mL demineralized water to the vials of **standard level 2 – 6** and **control 1 & 2**.
Allow the standards and controls to sit undisturbed for 10 minutes, and then mix well by gentle vortexing. One must 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

Assay Procedure

1. Place a sufficient number of Streptavidin coated microwell strips in a holder to run human pepsinogen II standards, controls and unknown samples in duplicate.
2. 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	

3. Prepare working Tracer Antibody and Capture Antibody mixture by 1:21 fold dilution of the Pepsinogen II Tracer Antibody and the Pepsinogen II Capture Antibody with the Tracer Antibody Diluent.
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.

4. Add **50 µL** of standards, controls and patient serum samples into the designated microwell.
5. Add **100 µL** of above antibody mixture to each well
6. Mix gently and cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
7. Incubate plate at room temperature for **2 hours**.
8. 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.
9. Add **100 µL** of ELISA HRP Substrate into each of the wells.
10. Cover the plate with one new plate sealer and also with aluminum foil to avoid exposure to light.
11. 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.)*
12. Remove the aluminum foil and plate sealer.
    Add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently.
13. Read the absorbance at **450 nm** within 10 minutes in a microplate reader

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 mix gently and thoroughly prior use. Avoid foaming.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. DRG Instruments GmbH DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall DRG Instruments GmbH 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 / Literature

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

Short Assay Procedure

1. Add 50 µL of standards, controls and patient serum samples into the designated microwell.
2. Add 100 µL of antibody mixture to each well.
3. Mix, cover and incubate the plate at room temperature for 2 hours.
4. Wash each well 5 times.
5. Add 100 µL of ELISA HRP Substrate into each of the wells.
6. Cover and incubate plate at room temperature for 20 minutes.
7. Add 100 µL of ELISA Stop Solution into each of the wells.
8. Read the absorbance at 450 nm.