DRG® Human Pepsinogen I ELISA (EIA-4604)

Revised 20 Nov. 2010 rm (Vers. 2.1) **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 I levels in serum.

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 serum samples containing human pepsinogen I 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 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. 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 I Tracer Antibody**
   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**
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**
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 I Standards**
Six vials each contains lyophilized human pepsinogen I 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 I Controls**
Two vials each contains 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 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 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, 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 – 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.

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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
0.5 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 I 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 I Tracer Antibody and the Pepsinogen I 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 **25 µL** of standards, controls and 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 **1 hour**.
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

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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.

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.

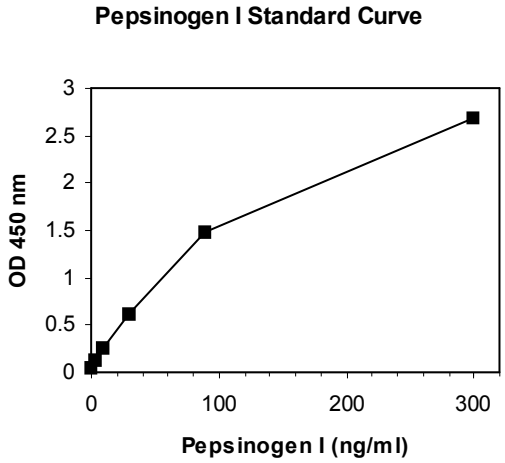
The human pepsinogen I concentrations for the controls and samples are read directly from the standard curve using their respective corrected absorbance. If log-log grapgy paper or computer assisted data reduction program utilizing logarithmic transformation are used, sample having corrected absorbance between the second standard and the next highest standard should be calculated by the formula:

$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance (2}^{nd}\text{ STD)}} \times \text{Value of the 2}^{nd}\text{ STD}$$

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	0.128	
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



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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.

Short Assay Procedure

1. Add 25 µL of standards, controls and 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 1 hour.
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

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