



# **Hu Pepsinogen II ELISA**

***KAPEPKT811***

---

**LOT** : 150811/1



# Human Pepsinogen II ELISA Kit

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

## KAPEPKT811

### IN VITRO DIAGNOSTIC

DIAsource ImmunoAssays SA - Rue du Bosquet 2, B-1348 Louvain-la-Neuve, Belgium - Tel: +32 10 84 99 11 - Fax : +32 10 84 99 91

#### INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human pepsinogen II levels in serum. Determination of human serum pepsinogen II level would allow calculation of the ratio of pepsinogen I/II, which was reported to 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 and Brunner's glands in the proximal duodenum, 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 level 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 as well as low ratio of pepsinogen I/II 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 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 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 immunocomplex 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 calibration curve is generated by plotting the absorbance versus the respective human pepsinogen II concentration for each calibrator on Point-to-Point, CubicSpline or 4-Parameter plot. The concentration of human pepsinogen II in test samples is determined directly from this calibration 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

One microplate with 12xheight 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. | | | | |--|--|--| | | | | |--|--|--| Detecting Antibody

One vial contains 0.6 mL concentrated horseradish peroxidase (HRP) conjugated anti-human pepsinogen II detecting antibody in a stabilized protein matrix. This reagent must be diluted with dilution buffer before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

#### 3. | | | | |--|--|--| | | | | |--|--|--| 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 dilution buffer before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

#### 4. | | | |--|--| | | | |--|--| Dilution buffer

One vial contains 12 mL ready to use buffer. It should be only used for detecting antibodies and capture 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. | | | | |--|--|--| | | | | |--|--|--| Washing buffer

One bottle contains 30 mL of a 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. | | | |--|--| | | | |--|--| TMB-Substrate solution

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. | | | |--|--| | | | |--|--| 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. | | | |--|--| | | | |--|--| Calibrators 0 - 5

Six vials each contain lyophilized human pepsinogen II in a bovine serum albumin based matrix with a non-azide preservative. **Refer to vials for exact concentration for each calibrator.** All the calibrators should be reconstituted with DI-water and stored at -20°C or below after the first use with up to 3 freeze cycles.

#### 9. | | | |--|--| | | | |--|--| Controls 1 - 2

Two vials each contain 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 stored at -20°C or below after the first use with up to 3 freeze cycles.

## SAFETY PRECAUTIONS

The reagents contained in this kit must be used in a professional environment and is intended for in-vitro diagnostic use.

Source material for reagents containing 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 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  $\mu\text{L}$ , 25  $\mu\text{L}$ , 100  $\mu\text{L}$ , and 1000  $\mu\text{L}$ , etc.
2. Repeating dispenser suitable for delivering 100  $\mu\text{L}$ .
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  $\mu\text{L}$  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  $-10^{\circ}\text{C}$  or below until measurement. Avoid more than three times 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) The washing buffer must be diluted to working solution prior use. Please see REAGENTS section for details.
- (3) Reconstitute all assay calibrators and controls by adding **0.5 mL** of demineralized water to the vial of calibrator level 0 and **0.5 mL** demineralized water to the vials of calibrator level 1 - 5 and control 1 & 2. Allow the calibrators 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 calibrators and controls must be stored at  $-10^{\circ}\text{C}$  or below. Do not exceed 3 freeze-thaw cycles.
- (4) Place a sufficient number of Streptavidin coated microwell strips in a holder to run human pepsinogen II calibrators, controls and unknown samples in duplicate.
- (5) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	CAL 0	CAL 4	SAMPLE 1
B	CAL 0	CAL 4	SAMPLE 1
C	CAL 1	CAL 5	SAMPLE 2
D	CAL 1	CAL 5	SAMPLE 2
E	CAL 2	C 1	SAMPLE 3
F	CAL 2	C 1	SAMPLE 3
G	CAL 3	C 2	
H	CAL 3	C 2	

- (6) Prepare working Detecting Antibody and Capture Antibody **mixture** by 1:21 fold dilution of the Pepsinogen II Detecting Antibody and the Pepsinogen II Capture Antibody with the Dilution buffer. For each strip, is required to mix 1 ml of Dilution Buffer with the addition of 50  $\mu\text{L}$  of Detecting Antibody and 50  $\mu\text{L}$  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.	Dilution buffer	Detecting Antibody	Capture Antibody
1	1 mL	50 $\mu\text{L}$	50 $\mu\text{L}$
2	2 mL	100 $\mu\text{L}$	100 $\mu\text{L}$
3	3 mL	150 $\mu\text{L}$	150 $\mu\text{L}$
4	4 mL	200 $\mu\text{L}$	200 $\mu\text{L}$
5	5 mL	250 $\mu\text{L}$	250 $\mu\text{L}$
6	6 mL	300 $\mu\text{L}$	300 $\mu\text{L}$
7	7 mL	350 $\mu\text{L}$	350 $\mu\text{L}$
8	8 mL	400 $\mu\text{L}$	400 $\mu\text{L}$
9	9 mL	450 $\mu\text{L}$	450 $\mu\text{L}$
10	10 mL	500 $\mu\text{L}$	500 $\mu\text{L}$
11	11 mL	550 $\mu\text{L}$	550 $\mu\text{L}$
12	12 mL	600 $\mu\text{L}$	600 $\mu\text{L}$

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

### 2. Manual Assay Procedure

- (1) Add **50  $\mu\text{L}$**  of calibrators, controls and patient serum samples into the designated microwell.
- (2) Add **100  $\mu\text{L}$**  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 **2 hours**.
- (5) Remove the aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350  $\mu\text{L}$  of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (6) Add **100  $\mu\text{L}$**  of TMB Substrate 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  $\mu\text{L}$  of Stop Solution into each of the wells. Mix gently.
- (10) Read the absorbance at **450 nm** within 10 minutes in a microplate reader.

### 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 mixed gently and thoroughly prior to use. Avoid foaming.

### INTERPRETATION OF RESULTS

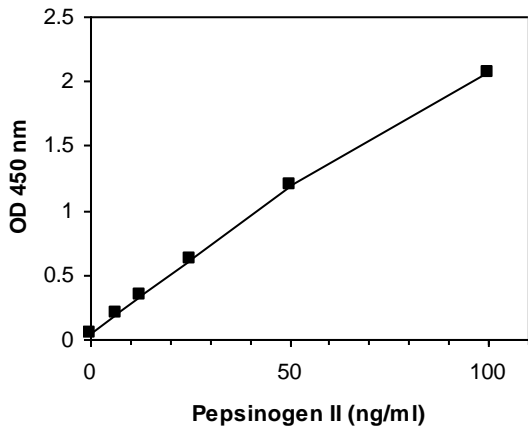
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the CAL 0 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibration curve is generated by the corrected absorbance of all calibrators 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.

The human pepsinogen II concentrations for the controls and patient samples are read directly from the calibration curve using their respective corrected absorbance.

### EXAMPLE DATA AND CALIBRATION CURVE

A typical absorbance data and the resulting calibration curve from human pepsinogen II ELISA are represented. **This curve should not be used in lieu of calibration 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	
6.3 ng/mL	0.201 0.208	0.205	0.153	
12.5 ng/mL	0.341 0.357	0.349	0.297	
25 ng/mL	0.590 0.656	0.623	0.571	
50 ng/mL	1.250 1.132	1.191	1.139	
100 ng/mL	2.064 2.074	2.069	2.017	
Control 1	0.218 0.217	0.218	0.166	6.8 ng/mL
Control 2	0.619 0.655	0.637	0.585	25.6 ng/mL



### EXPECTED VALUES

Seventy-three normal adult sera were measured 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 4.9 ng/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 recommended that each laboratory should establish their own normal range for pepsinogen II and the ratio of pepsinogen I/II based on local populations.

Patients with atrophic gastritis, as well as patients with stomach cancer would have a pepsinogen I/II level below 3. However, gastroendoscopy 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 II measurement, the values of the assay standards 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 100 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 5 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.

### PERFORMANCE CHARACTERISTICS

#### Sensitivity

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

#### Specificity

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

#### Linearity

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

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	Neat	16.2	-	-
	1:2	8.5	8.1	105
	1:4	3.9	4.1	95
	1:8	1.9	2.0	95
2	Neat	56.8	-	-
	1:2	26.7	28.4	94
	1:4	13.8	14.2	97
	1:8	6.9	7.1	97
	1:16	4.0	3.6	111

### Precision

The intra-assay precision is validated by measuring two samples in a single assay with 16-replicate determinations.

Mean Pepsinogen II Value (ng/mL)	CV (%)
8.7	3.8
33.6	7.1

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

Mean Pepsinogen II Value (ng/mL)	CV (%)
8.5	6.9
33.0	5.7

## Recovery

Two patient samples were spiked at 1:1 ratio with various amounts of human pepsinogen II and assayed. The results in the value in ng/mL are as follows:

#	Orig. Value	Amount Spiked	Observed Value	Expected Value	Recovery %
1	11.2	6.3	8.3	8.8	94
		12.5	11.4	11.9	96
		25.0	17.6	18.1	97
2	5.6	6.3	6.1	6.0	102
		12.5	9.3	9.1	102
		25.0	14.9	15.3	97




## “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.

## 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, Mochala 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-endoscopic 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, Andicochea 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. Gastroenterology 1969;57:659-669

## Pepsinogen II ELISA: Short Manual Assay Protocol

1. **50 µl Calibrators, controls and patient samples**
2. **100 µl Antibody mixture**  
  
*Incubate @ RT for 120 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**

Revision date : 2015-08-11

	<b>Used symbols</b>
	Consult instructions for use
	Storage temperature
	Use by
<b>LOT</b>	Batch code
<b>REF</b>	Catalogue number
<b>CONTROL</b>	Control
<b>I V D</b>	In vitro diagnostic medical device
	Manufacturer
	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 125I	Tracer
Ab 125I	Tracer
Ag 125I CONC	Tracer concentrated
Ab 125I CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoabsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
<b>µT</b>	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
PREC AGENT	Precipitating Agent
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor
TRAY	Incubation trays
PMSF	PMSF solution
	Protect from light
STRIP	Dot Strip
SUB	Substrate
EXTR SOLN CONC	Extraction Buffer Concentrate
CART	Cartridge
SAV HRP	Streptavidin HRP
PIPETTE	Pipette
WASH SOLN	Wash buffer