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 CE IVD

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. This kit is for in vitro diagnostic use only.

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 calibrators, 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 microwell. 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 microwell 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 calibration curve is generated by plotting the absorbance versus the respective human pepsinogen I concentration for each calibrator on Point-to-Point, CubicSpline or 4-Parameter plot. The concentration of human pepsinogen I 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 (10040)

Microplate coated with streptavidin.

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

2. Pepsinogen I Tracer Antibody (30061)

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 (30017) and mixed well before use.

3. Tracer Antibody Diluent (30017)

Buffer for antibody dilution according to the assay procedures. .

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

4. Pepsinogen I Capture Antibody (30062)

Biotinylated anti-human pepsinogen I capture 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 (30017) and mixed well before use.

5. ELISA Wash Concentrate (10010)

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 (10020)
Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use

7. ELISA Stop Solution (10030)
0.5 M sulfuric acid
Qty: 1 x 12 mL
Storage: 2 – 25°C
Preparation: Ready to Use

8. Pepsinogen I Calibrators Levels 1 to 6 (30053 - 30058)
Lyophilized human pepsinogen I in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.
Qty: 6 x vials
Storage: 2 – 8°C , <-20°C for long term storage
Do not exceed 3 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 I Controls (30059 - 30060)
Lyophilized human pepsinogen I 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 3 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 reagents are for in vitro diagnostic 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
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 & STORAGE
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. Samples should not be taken from patients taking biotin-containing multivitamins or dietary supplements at least 48 hours prior to specimen collection. 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 (10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.
3. Reconstitute all assay calibrators level 1 to level 6 (30053 - 30058) and controls (30059 - 30060) by adding 0.5 mL of deminerialized 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 may be stored at 2 – 8°C for up to 3 days or at –20°C or below for long-term storage. Do not exceed 3 freeze-thaw cycles.

2. Manual Assay Procedure
1. Place a sufficient number of microwell strips (10040) in a holder to run calibrators (30053 – 30058), controls (30059 - 30060), 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	SAMPLE 4
H	Calibrator Level 4	Control 2	SAMPLE 4

3. Prepare the antibody working solution by 1:21 fold dilution of the tracer antibody (30061) and capture antibody (30062) with the diluent (30017). For each strip, it is required to mix **1 mL** of the diluent (30017) with **50 µL** of the tracer antibody (30061) and **50 µL** capture antibody (30062) in a clean test tube.
Note: This antibody working solution should be freshly prepared.

4. Add **25 µL** of calibrators (30053 – 30058), controls (30059 - 30060), and samples into the designated microwells. Mix by gently tapping the plate.
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 ºC)** for **60 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 (10010) into each microwell, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
8. Add **100 µL** of substrate (10020) into each microwell. Mix by gently tapping the plate.
9. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
10. Remove the aluminum foil and plate sealer and add **100 µL** of Stop Solution (10030) 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.

3. Automated Assay Procedure

1. Prepare the antibody working solution by 1:21 fold dilution of the tracer antibody (30061) and capture antibody (30062) with the diluent (30017). For each strip, it is required to mix **1 mL** of the diluent (30017) with **50 µL** of the tracer antibody (30061) and **50 µL** capture antibody (30062) in a clean test tube.
   Note: This antibody working solution should be freshly prepared.
2. Add **25 µL** of calibrators (30053 – 30058), controls (30059 - 30060), 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 **37ºC** for **45 minutes.**
5. Aspirate the contents of each microwell. Wash each microwell **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each microwell, and then completely aspirate the contents.
6. Add **100 µL** of substrate (10020) into each microwell.
7. Incubate plate at **37ºC** for **15 minutes.**
8. Add **100 µL** of Stop Solution (10030) into each of the microwells.
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 mixed gently and thoroughly prior to use. Avoid foaming.

INTERPRETION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the calibrator 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 I 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 I measurement, the values of assay calibrators 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 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 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.

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.

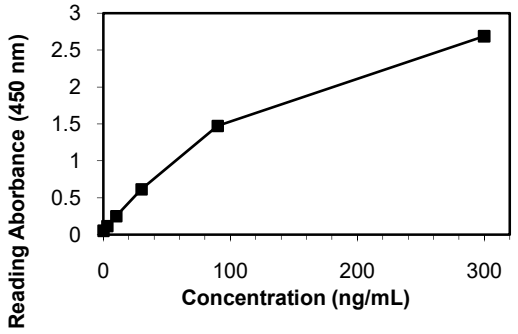
EXAMPLE DATA

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 ID	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: 3 ng/mL	0.119	0.119	0.067	
	0.118			
Calibrator Level 3: 10 ng/mL	0.262	0.254	0.202	
	0.246			
Calibrator Level 4: 30 ng/mL	0.616	0.619	0.567	
	0.622			
Calibrator Level 5: 90 ng/mL	1.565	1.476	1.424	
	1.387			
Calibrator Level 6: 300 ng/mL	2.766	2.685	2.633	
	2.604			
Control 1	0.373	0.368	0.316	16.2
	0.363			
Control 2	1.692	1.640	1.588	118
	1.587			

Example Data



PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of this human pepsinogen I 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 deviations above the pepsinogen I zero calibrator. The assay analytical sensitivity is approximately 0.5 ng/mL.

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.

Reproducibility and Precision

The intra-assay precision is validated by measuring two samples in a single assay with 20 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)	18.2	121.1	17.5	123.7
CV (%)	5.3	4.8	6.9	5.7

Linearity

Two human serum samples were diluted with assay buffer and assayed. The results are as follows:

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Sample 1	31.90	-	-
50%	16.21	15.95	102
25%	7.95	7.78	102
12.5%	3.73	3.99	93
6.25%	2.11	1.99	106
Sample 2	252.00	-	-
50%	125.27	126.00	99
25%	64.12	63.00	102
12.5%	31.25	31.50	99
6.25%	16.92	15.75	107

Spike Recovery

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

Samples	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Sample 1	18.6	-	-
10	12.6	14.3	88
30	25.1	24.3	103
90	56.2	54.3	103
Sample 2	121.1	-	-
10	61.3	65.6	93
30	70.9	75.6	94
90	104.7	105.6	99

Specificity

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

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.

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.

This product is developed and manufactured by

Epitope Diagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, US

Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

EC	REP

MDSS GmbH
Schiffgraben 41,
30175 Hannover, Germany

GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD In Vitro Diagnostic Device	RUO For Research Use Only	LOT Lot Number
REF Catalog Number	 Read instructions before use	 Number of Tests
 Store at	 Use by	 Keep away from heat and direct sun light
 Manufacturer	EC REP Authorized Representative in Europe	CE European Conformity

SHORT ASSAY PROCEDURE

1. Manual Assay Procedure

1. Add **25 µL** of calibrators, controls, and samples into the designated microwells.
2. Add **100 µL** of antibody working solution into the designated microwells.
3. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **60 minutes**.
4. Wash each microwell five times.
5. Add **100 µL** of substrate to each microwell.
6. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes**.
7. Add **100 µL** of the stop solution to each microwell.
8. Read the absorbance at **450 nm**.

2. Automated Assay Procedure

1. Add **25 µL** of calibrators, controls, and samples into the designated microwells.
2. Add **100 µL** of antibody working solution to each microwell.
3. Incubate plate with initial shaking for 1 minutes and further incubation at **37ºC** for **45 minutes.**
4. Wash each microwell five times.
5. Add **100 µL** of substrate into each microwell.
6. Incubate plate at **37ºC** for **15 minutes.**
7. Add **100 µL** of Stop Solution into each of the microwells.
8. Read the absorbance at **450 nm.**