



Fecal *Helicobacter Pylori* Antigen ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Quantitative and Qualitative Detection of *Helicobacter pylori* Antigen



KT 826

EU:



I. INTENDED USE

This microplate-based ELISA (enzyme linked immunosorbent assay) kit is intended for the quantitative and qualitative detection of *Helicobacter pylori* antigen in feces. The assay is a useful tool in the detection of active *H. pylori* infection. It is for in-vitro diagnostic use.

II. SUMMARY OF PHYSIOLOGY

H. pylori (previously known as *Campylobacter pyloridis*) is a type of bacteria that infects the stomach and is a common cause of peptic ulcers. *H. pylori* bacteria can be passed from person to person through direct contact with saliva, vomit or fecal matter. *H. pylori* can also be spread through contaminated food or water.

The infection is normally acquired during childhood. *H. pylori* usually goes undiagnosed until symptoms of a peptic ulcer occur. *H. pylori* infection is quite common and is present in about half the people in the world.

III. ASSAY PRINCIPLE

This "sandwich" ELISA is designed, developed and produced for the quantitative and qualitative measurement of *H. pylori* antigen in stool specimen. The assay utilizes the microplate-based enzyme immunoassay technique by coating highly purified antibody onto the wall of microtiter wells.

Assay calibrators and extracted fecal specimen are added to microtiter wells of microplate that was coated with a highly purified monoclonal *H. pylori* antibody on its wall. During the assay, the *H. pylori* antigen will be bound to the antibody coated plate after an incubation period. The unbound material is washed away and another HRP-conjugated monoclonal antibody which specifically recognizes the protein of *H. pylori* is added for further immunoreactions. After an incubation period, the immunocomplex of "H. pylori Antibody – *H. pylori* Antigen – HRP-conjugated Anti-*H. pylori* Tracer Antibody" is formed if *H. pylori* antigen is present in the test sample. The unbound tracer antibody and other proteins in buffer matrix are removed in the subsequent washing step. HRP conjugated tracer antibody bound to the well is then 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 *H. pylori* proteins captured on the wall of each microtiter well is directly proportional to the amount of *H. pylori* antigen level in each test specimen.

IV. 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 equalize to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

1. *H. pylori* Antibody Coated Microplate (Cat. No. 30665)

One microplate with 8 x 12 strips (96 wells total) coated with highly purified *H. pylori* antibody. The plate is framed and sealed in a foil zipper 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. Anti-*H. pylori* Tracer Antibody (Cat. No. 30666)

One vial containing 12 mL ready-to-use horseradish peroxidase (HRP)-conjugated monoclonal *H. pylori* antibody in a stabilized protein matrix. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

3. ELISA HRP Substrate (Cat. No. 10020)

One bottle containing 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.

4. ELISA Stop Solution (Cat. No. 10030)

One bottle containing 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.

5. *H. pylori* Antigen Calibrator 6 (Cat. No. 30789)

1 vial containing 1.5 mL of *H. pylori* Antigen Calibrator 6. This calibrator is in a liquid bovine serum albumin-based matrix with mercury and sodium azide preservative. **Refer to vials for exact concentration.** This reagent should be stored at 2 – 8 °C and are stable until the expiration date on the kit box, -20°C for long term storage.

6. ELISA Wash Concentrate (Cat. No. 10010)

One bottle containing 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 and non-mercury based preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.

7. *H. Pylori* Concentrated Assay Buffer (Cat. No. 30669)

One bottle containing 30 mL of 4-fold concentrated buffer matrix with protein stabilizers and preservative. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box. Before use the concentrated buffer must be diluted with 90 mL of demineralized water and mixed well. Upon dilution, this yields as **1x Assay Buffer**, which serves as a Calibrator Level 1, and as a **patient sample diluent** containing a surfactant in phosphate-buffered saline with a non-azide preservative. The diluted reagent is stored at 2 – 8 °C and is stable until the expiration date on the kit box.

V. SAFETY PRECAUTIONS

The reagents must be used in a laboratory and are for professional use only. Materials sourced for reagents containing bovine serum albumin were derived in the contiguous 48 United States and 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.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

- ❖ Precision single channel pipettes capable of delivering 10 µL, 50 µL, 100 µL, and 1000 µL, etc.
- ❖ Repeating dispenser suitable for delivering 100 µL.
- ❖ Disposable pipette tips suitable for above volume dispensing.
- ❖ Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
- ❖ Disposable plastic 1000 mL bottle with cap.
- ❖ Aluminum foil.
- ❖ Deionized or distilled water.
- ❖ Plastic microtiter well cover or polyethylene film.
- ❖ ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- ❖ Spectrophotometric microplate reader capable of reading absorbance at 450/620 nm.

VII. SPECIMEN COLLECTION & STORAGE

Fresh fecal sample should be collected into a stool sample collection container. It is required to collect a minimum of 1-2 mL liquid stool sample or 1-2g solid sample. The collected fecal sample must be transported to the lab in a frozen condition (-20°C). If the stool sample is collected and tested in the same day, it is allowed to be stored at 2-8°C.

VIII. 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) Concentrated Assay Buffer (Cat. 30669) must be diluted to working solution prior use. Please see REAGENTS section for details.
- (3) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior use. Please see REAGENTS section for details.
- (4) Prepare **1:3** serially diluted calibrators using H.pylori Ag Calibrator Level 6 (30789) and **1x Assay Buffer** as the dilution buffer. Store at 2-8°C, -20°C for long term storage, Avoid more than 3x freeze thaw cycle.

Calibrator	Calibrator Volume	Volume of 1x Assay Buffer
Cal 6	<i>Calibrator Level 6</i>	-
Cal 5	0.5 mL of Cal 6	1 mL
Cal 4	0.5 mL of Cal 5	1 mL
Cal 3	0.5 mL of Cal 4	1 mL
Cal 2	0.5 mL of Cal 3	1 mL
Cal 1	<i>1x Assay Buffer</i>	-

2. Patient Sample Preparation

2.1. For manual weighing procedure only:

Patient samples need to be diluted **1:24** with 1x Assay Buffer before being measured.

- (1) Label a test tube (12x75 mm) or a 4 ml plastic vial.
- (2) With solid stool sample, take or weigh an equivalent amount (about **40mg**, size as a grain of rice) with a spatula or a disposable inoculation loop. Suspend the solid stool sample with **1 mL 1x Assay Buffer** and mix well on a vortex mixer.
- (3) Centrifuge the diluted fecal sample at 3000 rpm (800-1500 g) for 5-10 minutes. The supernatant can be directly used in the assay. As an alternative to centrifuging, let the diluted samples sit and sediment for 30 minutes and take the clear supernatant for testing.
Note: If the test procedure is performed on an automated ELISA system, the supernatant must be particle-free by centrifuging the sample.
- (4) This sample can be stored at 2-8°C up to three (3) days and below -20°C for longer storage. Avoid more than 3x freeze and thaw cycle.

2.2. Using EDI Fecal Sample Collection Devices, (Cat. KT864)

- (1) Label a Fecal Sample Collection tube
- (2) Follow the instructions on the Sample Collection Tube insert, KT864.
- (3) This sample can be stored at 2-8°C up to three (3) days and below -20°C for longer storage. Avoid more than 3x freeze and thaw cycle.
- (4) Two drops of the extracted sample is equivalent to 100 µL.

3. Assay Procedure

- (1) Place a sufficient number of *H. Pylori* monoclonal antibody-coated microwell strips (Cat. 30665) in a frame.
- (2) Test Configuration

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

- (3) Add **100 µL** of calibrators and diluted patient stool samples into the designated microwell. Mix by gently tapping the plate. Cover the plate with one plate sealer. Cover with foil or other material to protect from light. **Note: if the collection tubes from KT-864 is used, add two drops of extracted fecal sample into each well.**
- (4) Incubate plate at room temperature, static, for **1 hour**.
- (5) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well, then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (6) Add **100 µL** ready-to-use anti- *H. Pylori* Tracer Antibody (Cat. 30666). Mix by gently tapping the plate.
- (7) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
- (8) Incubate plate at room temperature for **30 minutes**.
- (9) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400

µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

- (10) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
- (11) Cover the plate with a new aluminum foil to avoid exposure to light.
- (12) Incubate plate at room temperature for **20 minutes**.
- (13) Remove the aluminum foil. Add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
- (14) Read the absorbance at 450/620 nm.

Alternative Procedure (Qualitative Measurement):

- (1) If a qualitative measurement is desired, use the H.pylori Antigen Calibrator 6 (Cat# 30789) as the positive control and 1x Assay Buffer (Cat# 30669) as the negative control.
- (2)

ROW	STRIP 1	STRIP 2	STRIP 3
A	NEG CTL	SAMPLE 3	SAMPLE 7
B	NEG CTL	SAMPLE 3	SAMPLE 7
C	POS CTL	SAMPLE 4	SAMPLE 8
D	POS CTL	SAMPLE 4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	SAMPLE 9
F	SAMPLE 1	SAMPLE 5	SAMPLE 9
G	SAMPLE 2	SAMPLE 6	SAMPLE 10
H	SAMPLE 2	SAMPLE 6	SAMPLE 10

- (3) Add **100 µL** of calibrators and diluted patient stool samples into the designated microwell. Mix by gently tapping the plate. Cover the plate with one plate sealer. Cover with foil or other material to protect from light. **Note: if the collection tubes from KT-864 is used, add two drops of extracted fecal sample into each well.**
- (4) Incubate plate at room temperature, static, for **1 hour**.
- (5) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well, then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (6) Add **100 µL** ready-to-use anti- H. Pylori Tracer Antibody (Cat. 30666). Mix by gently tapping the plate.
- (7) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
- (8) Incubate plate at room temperature for **30 minutes**.
- (9) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (10) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
- (11) Cover the plate with a new aluminum foil to avoid exposure to light.
- (12) Incubate plate at room temperature for **20 minutes**.
- (13) Remove the aluminum foil. Add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
- (14) Read the absorbance at 450nm.

IX. PROCEDURAL NOTES

1. It is recommended that all calibrators 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 amber bottles.
3. Store any unused antibody-coated strips in the foil zipper 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. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

X. INTERPRETATION OF RESULTS

Quantitative Measurement:

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the calibrator 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibrator 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.

The H. Pylori concentrations for the unknown samples are read directly from the calibrator curve using their respective corrected absorbance.

Qualitative Measurement

Visual:

1. Positive or reactive: Any sample well that is obviously more yellow than the negative control well.
2. Negative or non-reactive: Any sample well that is not obviously more yellow than the negative control well.

Note: The negative control, as well as some patient samples, may show some slight yellow color. A sample well must be obviously darker or more yellow than the negative control well, when it is interpreted as a positive result.

ELISA Reader:

1. Calculate the average absorbance for each pair of duplicate test results.
2. Calculate the cut-off

The positive cut-off and the negative cut-off are established by using following formula.

Positive Cut-Off = 1.1 x (mean extinction of negative control + 0.10)

Negative Cut-Off = 0.9 x (mean extinction of negative control + 0.10)

3. Interpret test result
 - Positive: patient sample extinction is greater than the Positive Cut-Off
 - Negative: patient sample extinction is less than the Negative Cut-Off
 - Equivocal: patient sample extinction is between the Positive Cut-Off and the Negative Cut-Off.

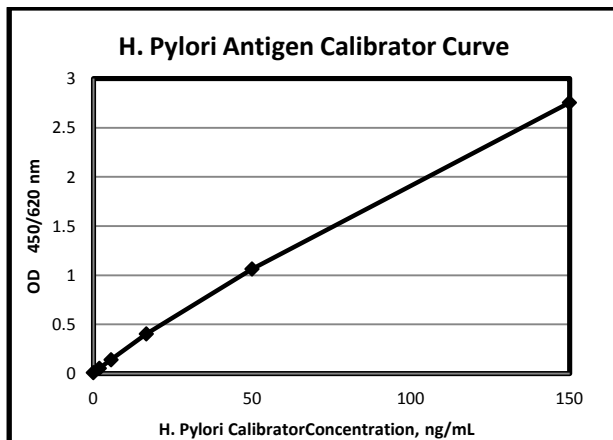
4. Assay quality control
 1. Positive control must show an average OD reading greater than 0.8.
 2. Negative control should show an average OD reading less than 0.09.

XI. EXAMPLE DATA AND CALIBRATOR CURVE

Quantitative Measurement:

A typical absorbance data and the resulting calibrator curve from Fecal H. Pylori antigen ELISA are represented. **This curve should not be used in lieu of calibrator curve run with each assay.**

Well I.D.	OD 450/620 nm Absorbance		
	Readings	Average	Corrected
0 ng/mL	0.011	0.011	0
	0.012		
1.9 ng/mL	0.056	0.055	0.044
	0.053		
5.6 ng/mL	0.140	0.142	0.131
	0.144		
16.7 ng/mL	0.407	0.405	0.394
	0.404		
50.0 ng/mL	1.116	1.065	1.054
	1.014		
150.0 ng/mL	2.752	2.757	2.746
	2.763		



Qualitative Measurement:

	OD 450 nm	Average OD 450 nm
Negative Control	0.049 0.050	0.050
Positive Control	1.332 1.376	1.354

Positive Cut-Off = 1.1 x (0.050 + 0.10) = 0.165
 Negative Cut-Off = 0.9 x (0.050 + 0.10) = 0.135

XII. EXPECTED VALUES

Quantitative Measurement:

Stool from 25 normal adults were measured with this ELISA. We found that normal people show undetectable H. pylori antigen in the extracted stool sample according to the sample collection, extraction and assay procedures described in this insert. The suggested positive cut-off for fecal H. pylori antigen is 3 ng/mL.

Qualitative Measurement:

Stool samples from 29 negative specimens and 17 positive specimens were tested with this ELISA.

Samples Epitope's ELISA	True Positive	True Negative	Total
Positive	17	0	17
Negative	0	29	29
Total	17	29	46

Sensitivity: 100% (17/17)
 Specificity: 100% (29/29)
 Accuracy: 100% (46/46)

XIII. LIMITATION OF THE PROCEDURE

- (1) The results obtained with this *H. pylori* Antigen Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves without taking other clinical findings such as stomach endoscope and biopsy, etc.
- (2) Single *H. pylori* negative results in untreated patients do not rule out *H. pylori* infection.
- (3) For unknown sample value read directly from the assay that is greater than the highest calibrator, it is recommended to measure a further diluted sample for more accurate measurement.
- (4) Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.

XIV. QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known *H. pylori* antigen levels. We recommend that all assays include the laboratory's own controls.

To order EDI H. pylori controls. Please order H. pylori control 1 (Cat# 30825), Control 2 (Cat# 30826), or Control set (Cat#30827.)

XV. PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of the Fecal H. pylori Ag ELISA as determined by the 95% confidence limit on 16 duplicate determination of zero standard is approximately 0.165 ng/mL.

Precision

The intra-assay precision is validated by measuring two samples in a single assay with 12 replicate determinations.

Mean H. Pylori antigen Value (ng/mL)	CV (%)
13.1	5.4
1.8	2.8

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

Mean H. Pylori antigen Value (ng/mL)	CV (%)
13.9	5.9
1.8	5.2

Specificity

The assay does not cross react to the following organisms: Cryptosporidium parvum, Giardia lamblia, rotavirus and adenovirus.

Linearity

Two stool samples were collected, diluted with 1x Assay Buffer and tested. The results of H. Pylori percent recovery value in ng/mL are as follows:

DILUTION	OBSERVED VALUE (ng/mL)	RECOVERY %
Neat A	77.4	-
1:2	38.1	98.4
1:4	17.5	90.4
Neat B	24.8	-
1:2	12.2	98.6
1:4	6.3	102.7

Spike Recovery

Two spiked stool samples and three assay calibrators (1.9, 16.7 and 50 ng/mL) were combined at equal volumes and tested. The results are as follows:

DILUTION	OBSERVED VALUE (ng/mL)	RECOVERY %
Neat A	0.3	-
Cal-2 1.9 ng/mL	1.1	97.2
Cal-4 16.7 ng/mL	7.1	84.2
Cal-5 50 ng/mL	20.9	83.2
Neat B	0.2	-
Cal-2 1.9 ng/mL	1.0	93.8
Cal-4 16.7 ng/mL	7.0	82.0
Cal-5 50 ng/mL	21.0	83.1

XVI. 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.

XVII. REFERENCES












1: Ungar BL, Yolken RH, Nash TE, Quinn TC. Enzyme-linked immunosorbent assay for the detection of H. Pylori in fecal specimens. J Infect Dis. 1984 Jan;149(1):90-7.

2: Rosenblatt JE, Sloan LM, Schneider SK. Evaluation of an enzyme-linked immunosorbent assay for the detection of H. Pylori in stool specimens. Diagn Microbiol Infect Dis. 1993 May-Jun;16(4):337-41.
 3: Stibbs HH, Samadpour M, Manning JF. Enzyme immunoassay for detection of H. Pylori cyst antigens in formalin-fixed and unfixed human stool. J Clin Microbiol. 1988 Sep;26(9):1665-9.
 5: Stibbs HH. Monoclonal antibody-based enzyme immunoassay for H. Pylori antigen in human stool. J Clin Microbiol. 1989 Nov;27(11):2582-8.
 8: Janoff EN, Smith PD, Blaser MJ. Acute antibody responses to H. Pylori are depressed in patients with AIDS. J Infect Dis. 1988 Apr;157(4):798-804.

This product is developed and manufactured by

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  **MDSS GmbH**
 Schiffgraben 41
 30175 Hannover, Germany

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



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Short Assay Procedure of Fecal H.Pylori Antigen ELISA

QUANTITATIVE:

- (1) Add **100 µL** of calibrators, controls and **100 µL or two drops** of patient samples into the designated microwell.
 - (2) Mix, cover and incubate the plate at room temperature **NO SHAKING for 1 hour**
 - (3) Wash each well 5 times.
 - (4) Add **100 µL** of Tracer Antibody into the designated microwell.
 - (5) Mix, cover and incubate the plate at room temperature **NO SHAKING for 30 minutes.**
 - (6) Wash each well 5 times.
 - (7) Add **100 µL** ELISA HRP Substrate into each well.
 - (8) Cover and incubate plate at room temperature for **20 minutes.**
 - (9) Add **100 µL** of ELISA Stop Solution into each of the wells.
 - (10) Read the absorbance at OD 450/620nm.
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Short Assay Procedure of Fecal H.Pylori Antigen ELISA:

QUALITATIVE:

- (1) Add **100 µL** of calibrators, controls and **100 µL or two drops** of patient samples into the designated microwell.
 - (2) Mix, cover and incubate the plate at room temperature **NO SHAKING for 1 hour**
 - (3) Wash each well 5 times.
 - (4) Add **100 µL** of Tracer Antibody into the designated microwell.
 - (5) Mix, cover and incubate the plate at room temperature **NO SHAKING for 30 minutes.**
 - (6) Wash each well 5 times.
 - (7) Add **100 µL** ELISA HRP Substrate into each well.
 - (8) Cover and incubate plate at room temperature for **20 minutes.**
 - (9) Add **100 µL** of ELISA Stop Solution into each of the wells.
 - (10) Read the absorbance at OD 450nm.
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