EPITOPE DIAGNOSTICS, INC.

# *EDI*™ Fecal Helicobacter Pylori Antigen ELISA Kit

***Enzyme Linked Immunosorbent Assay (ELISA) for the quantitative and qualitative detection of H.pylori Antigen***

REF KT-826 CE IVD 96

## 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. This kit is for in vitro diagnostic use only.

## 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.

## 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.

## 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.

### 1. H.pylori Antibody Coated Microplate (30665)

Microplate coated with *H.pylori* antibody.
Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use.

### 2.Anti-H.pylori Tracer Antibody (30870)

HRP-conjugated monoclonal *H.pylori*antibody in a stabilized protein matrix.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

### 3. ELISA HRP Substrate (10020)

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

### 4. ELISA Stop Solution (10030)

0.5 M sulfuric acid.
Qty: 1 x 12 mL
Storage: 2 – 25°C
Preparation: Ready to Use.

### 5. H.pylori Antigen Calibrators Levels 1 to 6 (31141-31146)

Calibrator in bovine serum albumin-based matrix with a proclin preservative.
Qty: 6 x Vials
Storage: 2 – 8°C , <-20°C for long term storage
Do not exceed 3 freeze-thaw cycles.
Preparation: Ready to Use.

### 6. H.pylori Antigen Controls (31147,31148)

Calibrator in bovine serum albumin-based matrix with a proclin preservative.
Qty: 2 x Vials
Storage: 2 – 8°C , <-20°C for long term storage
Do not exceed 3 freeze-thaw cycles.
Preparation: Ready to Use.

### 7. 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.

### 8. H.pylori Concentrated Assay Buffer (30669)

Concentrated buffer matrix with protein stabilizers and preservativewhich serves as a patient sample diluent containing a surfactant in phosphate-buffered saline with a non-azide preservative.
Qty: 1 x 30 mL
Storage: 2 – 8°C
Preparation: 4X Concentrate. The contents must be diluted with 90 mL distilled water and mixed well before 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 10 µL, 25 µL, 50 µl, 65 µL, 100 µL, and 1000 µL.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with cap.
6. Aluminum foil.
7. Plastic microtiter well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

## 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 (-20ºC). If the stool sample is collected and tested in the same day, it is allowed to be stored at 2-8ºC.

## ASSAY PROCEDURE

**1. Reagent Preparation**

1. Prior to use allow all reagents to come to room temperature (20-25 ºC). Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate (10010) must be diluted to working solution prior use. Please see REAGENTS section for details.

**2.Sample Preparation**

For manual weighing procedure only:

1. Patient samples need to be diluted**1:24** with 1x Assay Buffer before being measured.
2. Label a test tube (12x75 mm) or a 4 ml plastic vial.
3. 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.
4. 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.
5. Note: If the test procedure is performed on an automated ELISA system, the supernatant must be particle-free by centrifuging the sample**.**
6. This sample can be stored at 2-8ºC up to three (3) days and below -20ºC for longer storage. Avoid more than 3x freeze and thaw cycle.

2. **Using EDI Fecal Sample Collection Devices (KT854 or KT864)**
   1. Label a Fecal Sample Collection tube
   2. Follow the instructions on the Sample Collection Tube insert, KT854 or KT864.
   3. This sample can be stored at 2-8ºC up to three (3) days and below -20ºC for longer storage. Avoid more than 3x freeze and thaw cycle.
   4. Two drops of the extracted sample is equivalent to 100 µl.
   5. *Note: KT-854 is suitable for both automated ELISA system operation and manual assay procedure. KT864 is most appropriate for manual assay use.*

**2. Quantitative Assay Procedure**

1. Place a sufficient number of microwell strips (30665) in a holder to run calibrators (31141-31146), controls (31147,31148), 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. Add **100 µL** of calibrators (31141-31146), controls (31147,31148), and samples into the designated microwells. *Note: if the collection tubes from KT-864 is used, add two drops of extracted fecal sample into each well.*
4. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **60 minutes.**
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
6. Add **100µL** of Anti-H.pylori Tracer Antibody (30666) to each well. Mix by gently tapping the plate.
7. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **30 minutes.**
8. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
9. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells. Mix by gently tapping the plate.
10. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
11. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells. Mix by gently tapping the plate.
12. Read the absorbance at **450/620 nm**within **10 minutes** with a microplate reader.

**3. Qualitative Assay Procedure**

1. Place a sufficient number of microwell strips (30665) in a holder to run positive control [H.pylori Antigen Calibrator Level 6 (31146)], negative control [diluted 1X Assay Buffer (30669)], and samples in duplicate
2. Test Configuration

| Row | Strip 1 | Strip 2 | Strip 3 |
|---|---|---|---|
| A | Negative Control (30669) | SAMPLE 3 | SAMPLE 7 |
| B | Negative Control (30669) | SAMPLE 3 | SAMPLE 7 |
| C | Positive Control (31146) | SAMPLE 4 | SAMPLE 8 |
| D | Positive Control (31146) | 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 positive control [H.pylori Antigen Calibrator Level 6 (31146)], negative control [diluted 1X Assay Buffer (30669)], and samples into the designated microwells. *Note: if the collection tubes from KT-864 is used, add two drops of extracted fecal sample into each well.*
4. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **60 minutes.**
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
6. Add **100 µL** of Anti-H.pylori Tracer Antibody (30666) to each well. Mix by gently tapping the plate.
7. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **30 minutes.**
8. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
9. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells. Mix by gently tapping the plate.
10. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
11. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells. Mix by gently tapping the plate.
12. Read the absorbance at **450nm**within **10 minutes** with a microplate reader.

## PROCEDURAL NOTES

1. It is recommended that all control 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. Store any unused antibody coated strips in the foil zip-seal bag with desiccant to protect from moisture. Exposure of the plates to humidity drastically reduces the shelf life.
3. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
4. Incubation times or temperatures other than those stated in this insert may affect the results.
5. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate readings.
6. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

## INTERPRETION OF RESULTS

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

**2. 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-offs:
   - Positive cut-off: 1.1 x (mean extinction of the negative control + 0.10).
   - Negative cut-off: 0.9 x (mean extinction of the negative control + 0.10).
3. Interpret test results
   - Positive: patient sample extinction is greater than the positive cut-off.
   - Negative: patient sample extinction is less than the negative cut-off.
   - Equivocal: sample extinction is between the positive and negative cut-off.
4. Assay Quality Control
   - Positive control must show an average OD reading greater than 0.8.
   - Negative control must show an average OD reading less than 0.18.

## LIMITATIONS 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.

## 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.

## 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)

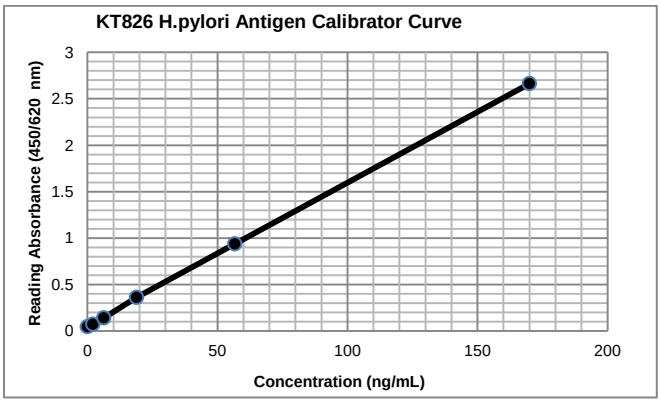
## EXAMPLE DATA

**Quantitative Measurement**

A typical absorbance data and the resulting calibration curve from Fecal H. Pylori antigen ELISA are represented.

*Note:This curve should not be used in lieu of calibration curve run with each assay.*

| Well ID | Reading Absorbance (450/620 nm) | | | Concentration (ng/mL) |
|---|---|---|---|---|
| | Readings | Average | Corrected | |
| **Calibrator Level 1: 0 ng/mL** | 0.047 | 0.047 | 0.000 | |
| | 0.047 | | | |
| **Calibrator Level 2: 2.1 ng/mL** | 0.075 | 0.073 | 0.026 | |
| | 0.071 | | | |
| **Calibrator Level 3: 6.3 ng/mL** | 0.140 | 0.142 | 0.095 | |
| | 0.144 | | | |
| **Calibrator Level 4: 18.9 ng/mL** | 0.357 | 0.363 | 0.316 | |
| | 0.368 | | | |
| **Calibrator Level 5: 56.7 ng/mL** | 0.909 | 0.939 | 0.892 | |
| | 0.968 | | | |
| **Calibrator Level 6: 170 ng/mL** | 2.682 | 2.665 | 2.618 | |
| | 2.647 | | | |
| **Control 1** | 0.076 | 0.076 | 0.029 | 2.2 |
| | 0.074 | | | |
| **Control 2** | 0.737 | 0.724 | 0.677 | 42.6 |
| | 0.711 | | | |




### Qualitative Measurement

| | Reading Absorbance (450 nm) | Average |
|---|---|---|
| **Negative Control** | 0.049<br>0.050 | 0.050 |
| **Positive Control** | 1.332<br>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

## 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 calibrator is approximately 0.165 ng/mL.

### Specificity

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

### Reproducibility and Precision

The intra-assay precision is validated by measuring two samples in a single assay with 12 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)** | 13.1 | 1.8 | 13.9 | 1.8 |
| **CV (%)** | 5.4 | 2.8 | 5.9 | 5.2 |

### Linearity

Two (2) stool samples were diluted with assay buffer and tested. The results are as follows:

| Samples | Observed (ng/mL) | Recovery (%) |
|---|---|---|
| **Sample A** | 77.4 | - |
| **50%** | 38.1 | 98.4 |
| **25%** | 17.5 | 90.4 |
| **Sample B** | 24.8 | - |
| **50%** | 12.2 | 98.6 |
| **25%** | 6.3 | 102.7 |

**Spike Recovery**
Two samples were spiked with calibrators each other in equal volume and assayed. The results indicate below:

| Samples | Observed (ng/mL) | % Recovery (%) |
|---|---|---|
| **Sample A** | 0.3 | - |
| **+ Level 2: *1.9 ng/mL*** | 1.1 | 97.2 |
| **+ Level 4: *16.7 ng/mL*** | 7.1 | 84.2 |
| **+ Level 5: *50 ng/mL*** | 20.9 | 83.2 |
| **Sample B** | 0.2 | - |
| **+ Level 2: *1.9 ng/mL*** | 1.0 | 93.8 |
| **+ Level 4: *16.7 ng/mL*** | 7.0 | 82.0 |
| **+ Level 5: *50 ng/mL*** | 21.0 | 83.1 |

## 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

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4. Kalach N, Gosset P, Dehecq E, Decoster A, Georgel AF, Spyckerelle C, Papadopoulos S, Dupont C, Raymond J. A one-step immune-chromatographic Helicobacter pylori stool antigen test for children was quick, consistent, reliable and specific. Acta Paediatr. 2017 Dec;106(12):2025-2030.
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## 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.

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


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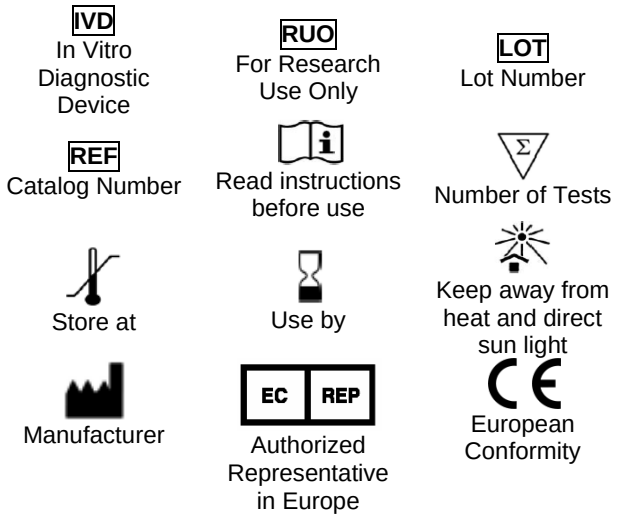
## GLOSSARY OF SYMBOLS (EN 980/ISO 15223)




## SHORT ASSAY PROCEDURE
**1. Quantitative Measurement**
1. Add **100 µL** of the calibrators, controls, and samples into the designated microwells.
2. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **60 minutes**.
3. Wash each well five times.
4. Add **100 µL** of the tracer antibody to each well.
5. Cover and incubate at **room temperature (20-25 ºC)** for **30 minutes**.
6. Wash each well five times
7. Add **100 µL** of substrate to each well.
8. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes**.
9. Add **100 µL** of the stop solution to each well.
10. Read the absorbance at **450/620 nm**.

**2. Qualitative Measurement**
1. Add **100 µL**of positive control [H.pylori Antigen Calibrator Level 6 (31146)], negative control [diluted 1X Assay Buffer (30669)], and samples into the designated microwells.
2. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **60 minutes**.
3. Wash each well five times.
4. Add **100 µL** of the tracer antibody to each well.
5. Cover and incubate at **room temperature (20-25 ºC)** for **30 minutes**.
6. Wash each well five times
7. Add **100 µL** of substrate to each well.
8. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes**.
9. Add **100 µL** of the stop solution to each well.
10. Read the absorbance at **450 nm**.