

Instruction Manual of MR UREA[®] S

(Modified Rapid Urease Test based on Urease Method)

Diagnostic Reagent for Determination of Infection with *Helicobacter pylori*

Thoroughly read this instruction manual before use of this kit

[Background of development and features]

Since isolation of helical gram-negative bacillus from gastric mucous membrane biopsy sections and its cultivation by Warren and Marshall in 1983¹⁾, many reports have been made suggesting its strong association with gastritis and duodenal diseases. Initially, it was called *Campylobacter pylori*, and since 1989, upon proposal by Goodwin, et al., it has been called *Helicobacter pylori* (*H. pylori*).

For determination of infection with *H. pylori*, there are i) culture method, ii) histological microscopic observation method, iii) urease activity detection method, and iv) antibody detection method. This diagnostic reagent allows determination of *H. pylori* at ease by means of detection of urease activity.

Features:

- (1) Urease activity can be detected in a short time.
- (2) Urease activity can be detected at high sensitivity.
- (3) Simple operation without requiring any special tool, reagent or equipment.

[Kit configuration]

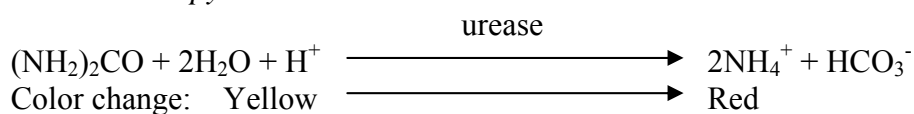
Substrate reagent (Urea and phenol red)	3 vials
Solvent (2.5 mL)	3 vials
Reaction cup with cap	30 ea.
Reaction cup holder	1 ea.
Sample labels (30 ea.)	1 sheet

[Effect]

Detection of *H. pylori* in gastric or duodenal mucous membrane tissues.

[Principle of determination]

The determination principle of this kit is based on the urease detection method. A sample, gastric or duodenal mucous membrane tissue, is put into a reaction cup containing substrate reagent dissolved by the solvent. When there is *H. pylori* in a sample, *H. pylori* derived urease hydrolyzes urea substrate and generates ammonia. When ammonia is generated, color of phenol red as pH indicator changes from yellow to red allowing visual identification of *H. pylori*.



[Preparation of reagent and operation]

1. Preparation of reagent

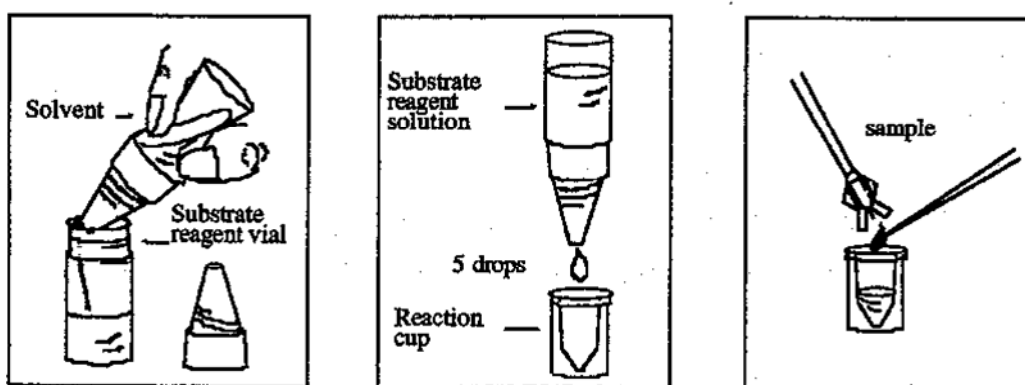
- (1) Empty one vial of the solvent (2.5 mL) into one substrate reagent vial containing urea and phenol red, and gently shake to prepare substrate reagent solution for 10 samples.

Note

The substrate reagent once reconstituted is stable for two months if stored at 2 ~ 8°C. It can be stored in aliquots with caps. Aluminum pouch containing substrate reagent vial should not be opened before preparation of it.

2. Operation

- (1) Dispense 5 drops (0.2 mL) of the substrate reagent solution in a cup provided.
- (2) After collecting a sample, quickly put it in the reaction cup and seal it with its cap. Gently shake the reaction cup and leave it to stand at 15 ~ 30°C.
- (3) Visually check change in color.

**Note**

- When substrate reagent is prepared, the nozzle of substrate reagent vial is taken off. Regarding the solvent, only a cap is taken off, and the nozzle of the vial should be closed tightly.
- The nozzle of the vial should be closed tightly, when the reagent is dispensed in a reaction cup.

[Determination]

Samples are tested positive when color of the substrate reagent solution turns red within 2 hrs, and tested negative when there is no change in color after 2 hrs.

[Operational precautions]

(1) Samples

Samples should be tissue sections as fresh as possible and should not be dried.

(2) Collection of samples

H. pylori may be unevenly present at upper body of stomach or prepyloric area of stomach. It is, therefore, desirable that samples are collected from at least these two parts.

- (3) In case of patients with intestinal metaplasia of the stomach mucosa, samples should not be collected from such lesion.

(4) Interference substance

Samples should not be washed as substance. It recommends that samples should be wiped softly to remove blood, because blood (0.5µL or more) may affect determination.

- (5) Intestinal mucosa samples where *H. pylori* colony can not be formed, they may test false negative.
- (6) Samples from patients administered with antibiotics or bismuth preparation generally tend to test negative due to their bacteria elimination effect, therefore, samples collected within 4 weeks after their administration should not be used for this test. Of patients once cleared of *H. pylori* with administration of antibiotics or bismuth preparation but infected with it again, it may be present unevenly. To avoid false negative, it is desirable to collect samples from a number of places.
- (7) Amount of *H. pylori* may decrease after administered with antibiotics or bismuth preparation. It recommends to trace the progress of the patient, and to test again.
- (8) In case of a medicine like Lansoprazole which has acteristatic action is administrated, it may test false negative. Therefore, the test should be made after 4 weeks or more than the date of their administration is stopped.
- (9) H₂ blocker preparation does not affect proliferation of *H. pylori*. However, patients administrated with a large amount of such preparation, suffering from pernicious anemia or those undergone stomach operation may test false negative by principle of this kit (color reaction of color indicator by pH value) because of change (rise) in pH on the tissue surface due to complication with anacidity. Determination should be made in combination with another method such as culture method.
- (10) Although their urease activity level is as low as 1/10 or less of *H. pylori*, co-infected with urease producing bacteria other than *H. pylori* (*P. vulgaris*, *L. fermentum*, *P. prevotii*, *E.aerofaciens*, etc.) may cause false positive through a longer reaction time, therefore, specified reaction time should be observed.
- (11) Be aware that some powder used for gloves may affect the result of the test.
- (12) When the color around a sample turns slightly red as soon as a sample is put in the reaction cup, shake the reaction cup and test it.
- (13) For confirmation of infection with *H. pylori*, other methods such as culture method, histological microscopic method, and urease breath test should also be used.
- (14) Samples once used for this test should not be used for this test or other tests.

[Specifications]

- (1) Sensitivity test
This kit tests positive when 100 µL of 0.006 unit/mL of *H. pylori* derived urease solution is used as a sample.
- (2) Specificity test
This kit tests negative when *H. pylori* negative panel samples are tested. This kit tests positive when *H. pylori* positive panel samples are tested.
- (3) Simultaneous reproducibility
When this kit tests 10 times simultaneously *H. pylori* negative panel samples, this kit tests them all negative. When this kit tests 10 times simultaneously *H. pylori* positive panel samples, this kits tests them all positive.

[Correlation]

Correlation between this kit and a kit manufactured by A company on the same principle was checked. Test of 95 samples showed correlation of 95.8 % between the two kits.

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[Cautions in handling or operation of this kit]

This kit should be used according to the instructions given in this manual. No result is guaranteed in any use or for any purpose other than those described in this manual.

1. General cautions

- (1) Do not use substrate reagent melted like syrup before addition of the buffer.
- (2) Do not use expired reagents.
- (3) Do not combine and use reagents of different manufacturing lots.

2. Cautions in operation

- (1) Handle samples as if they were capable of transmitting diseases.
- (2) Reconstituted substrate reagent should be returned to 15 ~ 30°C, if it stored at 2 ~ 8°C. If it was stored in vial, it recommends to dispense 5 drops of the substrate reagent solution in a cup provided, and to return only the number of vial for use to 15 ~ 30°C.
- (3) Avoid contact of reagents with skin or mucous membrane. If they may come in contact with skin or get in eyes or mouth, wash it off with a sufficient volume of water. Get a medical aid, if need.
- (4) Cups used for the test should be discarded as the medical waste.

3. Cautions for storage

- (1) The substrate reagent once reconstituted should be stored with cap at 2 ~ 8°C.

4. Others

- (1) Do not use the vials, cups and caps in this kit for purposes other than specified.
- (2) The clinical diagnosis should not be made by the results of this test alone but should be made in combination with supportive clinical information and other tests.

[Storage and shelf life]

This kit should be stored at room temperature in the dark. The shelf life of this kit is 18 months after the date of manufacture (use up this kit before the expiry date shown on the package).

[Package]

30 tests. Code No. 1HB1

[References]

- (1) Warren J. R., Marshall B. J.: Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* **i**: 1273-1275, 1983
- (2) Goodwin C. S. et al.: Evaluation of clinical techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa. *J. Clin. Pathol.* **38**: 1127-1131, 1985

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