DRG® H. Pylori IgM ELISA (EIA-2111)

CE

Revised 21 Apr. 2011 rm (Vers. 5.1) **USA:RUO**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

PRINCIPLE OF THE TEST

Purified H. pylori antigen is coated on the surface of microwells. Diluted serum sample is added to the wells, and the H. pylori IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away. Enzyme conjugate is added, which binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

REAGENTS

Materials provided with the kit

- **Purified H. pylori anatiegen coated microtiter plate, 96 wells.**
- **Enzyme Conjugate Reagent (red color), 13 ml.**
- **Sample Diluent (blue color), 22 ml.**
- **Low Control, 100 µl**
- **Calibrator, H. Pylori IgM EIA Index = 1, 100 µl**
- **High Control 100 µl**
- **Wash Buffer Concentrate (20x), 50 ml**
- **TMB Reagent (One-Step), 11 ml**
- **Stop Solution (1N HCl) 11 ml**

Materials required but not provided

- Distilled water.
- Precision pipettes: 5 µl, 100 µl and 200 µl.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.

STORAGE OF TEST KITS AND INSTRUMENTATION

Unopened test kits should be stored at 2-8° C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

Warnings and Precautions

1. CAUTION: This kit contains human material. The course material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Dilute 1 volume of Wash Buffer (20×) with 19 volumes of distilled water.
   For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash buffer is stable for 1 month at 2-8° C. Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Prepare **1:40 dilution** of test samples, low control, high control, and calibrator by adding 5 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well for 10 seconds.
4. Incubate at room temperature for 30 minutes.
5. At the end of the incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 4 times with diluted wash buffer (1x) and then one time with distilled water. (Please do not use tap water.)
6. Dispense 100 µl of enzyme conjugate to each well. Mix gently for 10 second.
7. Incubate at room temperature for 30 minutes.
8. Remove enzyme conjugate from all wells. Rinse and flick the microtiter wells 4 times with diluted wash buffer (1x) and then one time with distilled water.
9. Add 100 µl of TMB Reagent to each well. Mix gently for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Add 100 µl of Stop Solution to each well including the 2 blanks.
12. Mix gently for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
13. Read the optical density at 450 nm **within 15 minutes** with a microtiter plate reader.

Important Note:
The wash procedure is critical. Insufficient washing will result in improper color development.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate calibrator value x_c.
2. Calculate the mean of duplicate High Control, Low Control and samples.
3. Calculate the H. pylori IgM EIA Index of each determination by dividing the mean values of each sample by calibrator mean value, x_c.

Limitations of the Procedure

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory price.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

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