



Human anti-myocardial antibody (AMA) ELISA Kit

Catalog No. CSB-E08337h

(96 T)

- This immunoassay kit allows for the in vitro semi-quantitative determination of **human anti-myocardial antibody (AMA)** concentrations in **serum**.
- **Expiration date** six months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with myocardial antigen. Sample Diluent and Samples are then added to the appropriate microtiter plate wells and incubated. Then add Horseradish Peroxidase (HRP)-conjugated anti-human IgG and incubated. Then substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of $450 \text{ nm} \pm 2 \text{ nm}$. The concentration of anti-myocardial antibody AMA in the samples is then determined by comparing the O.D. of the samples to the standard curve.

SPECIFICITY

This assay recognizes human anti-myocardial antibody AMA , no significant cross-reactivity or interference was observed.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Sample Diluent	1 x 12 ml
HRP-conjugate	1 x 12 ml
Positive Control	1 x 300 μ l
Negative Control	1 x 300 μ l
Wash Buffer	2 x 15 ml (30xconcentrate)
Substrate A	1 x 6 ml
Substrate B	1 x 6 ml
Stop Solution	1 x 6 ml

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit, provided it is stored as prescribed above. Refer to the package label for the expiration date.
2. Opened test plate should be stored at 2-8°C in the aluminum foil bag with desiccants to minimize exposure to damp air. The kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 15 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 450 ml of Wash Buffer.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

- An incubator which can provide stable incubation conditions up to $37^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20°C . Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples be assayed in duplicate. All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.

1. Set three Negative Control wells, two Positive Control wells and one Blank well.
2. Add 100 μl of Sample Diluent per well, not to Blank well.
3. Add 10 μl of **diluted Sample**, **Positive Control** and **Negative Control** per well. Cover with the adhesive strip. Incubate for 45 minutes at 37°C .

4. Aspirate each well and wash, repeating the process five times for a total of five washes. Wash by filling each well with **Wash Buffer** (350µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, stay for 30 seconds. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100µl of **HRP-conjugate** to each well, not to Blank well. Cover the microtiter plate with a new adhesive strip. Incubate for 30 minutes at 37° C.
6. Repeat the aspiration and wash five times as before.
7. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well. Incubate for 10 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
8. Add 50µl of **Stop Solution** to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

For calculation the valence of anti-myocardial antibody AMA , compare the sample well with control.

(1) Negative Control OD values must no more than 0.1.

- If one of the Negative Control OD values high than 0.1, discard it.
- If more than two Negative Control OD values high than 0.1, repeat the test.
- If the average value of $OD_{\text{negative}} < 0.05$, caculate it as 0.05.

(2) Positive Control OD Values must no less than 0.45.

- If one of the Positive Control OD values less than 0.45, discard it.
- If the two Positive Control OD value less than 0.45, repeat the test.

(3) A cutoff value was defined as the average Negative Control value plus 0.2.

While $OD_{\text{sample}} < \text{Cutoff Value}$: Negative

While $OD_{\text{sample}} \geq \text{Cutoff Value}$: : Positive

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- Centrifuge vials before opening to collect contents.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless or light blue until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless or light blue to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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