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# **Human Endocab- IgM ELISA Kit**

**96 Tests**

**Catalogue Number: SLX2461Hu**

**Store all reagents at 2-8C**

**Validity Period: six months**

**For samples:**

**In serum, plasma, culture media or any biological fluid.**

**FOR RESEARCH USE ONLY !**

**NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS !**

**PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING !**

# Human Endocab- IgM ELISA Kit

**FOR RESEARCH USE ONLY**

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## Drug Names

Generic Name: **Human Endocab- IgM ELISA Kit**

## Purpose

Our Human Endocab- IgM ELISA kit is to for the qualitative determination of Endocab- IgM in Human serum, plasma, culture media or any biological fluid.

## Principle

The ELISA is based on the the qualitative enzyme immunoassay technique. The Microplate provided in this kit has been pre- coated with an antigen specific to Endocab- IgM, make it to solid-phase antigen. Samples are added to the Microplate wells and combined to the specific antigen. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific for Endocab- IgM is added to each Microplate well and incubated, so the antigen-antibody- Enzyme labeled antigen complex is formed. Following a wash to remove any unbound reagent, then the TMB substrate solution is added to each well. Only those wells that contain Endocab- IgM and HRP conjugated Zika Virus antigen will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The qualitative determination of Endocab- IgM is determined by comparing with the CUTOFF value.

## Materials provided with the kit

	Materials provided with the kit	96 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	Microplate	1	2- 8 C
5	Negative control	0.5ml ×1 bottle	2- 8 C

6	Positive control	0.5ml ×1 bottle	2-8 C
7	HRP- Conjugate reagent	10ml ×1 bottle	2-8 C
8	Sample diluent	6ml ×1 bottle	2-8 C
9	Chromogen Solution A	6ml ×1 bottle	2-8 C
10	Chromogen Solution B	6ml ×1 bottle	2-8 C
11	Stop Solution	6ml ×1 bottle	2-8 C
12	wash solution	25ml (20X) × 1bottle	2-8 C

## Sample preparation

### 1. Serum preparation

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

### 2. Plasma preparation

Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifugated for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifugated again.

### 3. Urine samples

Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

Cell samples If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X10<sup>6</sup>/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again.

### 4. Tissue samples

Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80 C for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4C. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

**Notes:**

1. Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay can not be performed immediately, samples can be stored at -20 C .Repeated freeze-thaw cycles should be avoided.
2. Our kits can not be used for samples with NaN<sub>3</sub> which can inhibit the activity of HRP.

**Procedure**

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.
2. Separately add Positive control and Negative control 50µl to the Positive and Negative well; Add testing sample 10µl then add Sample Diluent 40µl to testing sample well.
3. Add 100µl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.
4. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Solution (400µl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add chromogen solution A 50µl and chromogen solution B 50µl to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.
6. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

## Determine the result

Test effectiveness: the average value of positive control  $\geq 1.00$ ; the average value of negative control  $\leq 0.15$ .

The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15

Negative judgement: if the OD value < CUT OFF, the sample is Human Endocab- IgM negative.

Positive judgement: if the OD value  $\geq$  CUT OFF, the sample is Human Endocab- IgM positive.

## Notes

1. Store the kit at 4 ° C upon receipt. The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from Zika Virus antigen- Coated plate, reseal them in zip-lock foil and keep at 4 ° C.
2. Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.
3. In order to avoid cross-contamination, Closure plate membranes are for one-time use only.
4. Please keep Substrate away from light.
5. All the operation should be accordance with the manufacturer's instructions strictly. The results determined by the Microplate Reader.
6. All the samples, washing buffer and wastes should be treated as infectious agents.
7. Reagents from different lots should not be mixed.

## Storage and validity

1. Storage: 2-8C.
2. Duration: 6 months