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**User's Manual**

# Human Yellow Fever IgM (YF IgM) ELISA Kit

REF **DEIA-Z5092**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## Intended Use

Human Anti-Yellow fever virus Env IgM (YFV Env-IgM) tests is an indirect ELISA suitable for detecting YFV-Env IgM in Human serum or plasma. Other biological fluids, including tissue culture medium, may be validated for user in vitro research use only (RUO), not for therapeutic or diagnostic use.

## General Description

Yellow fever is a viral hemorrhagic disease spread between humans, as well as between certain other primates and humans, by the bite of yellow fever-infected mosquitoes. it is spread primarily by mosquitoes of the Aedes aegypti species. The virus is called simply Yellow fever virus (YFV) and belongs to the virus family Flaviviridae. Humans are a dead-end host, terminating the virus's life cycle and consequently suffering from much harsh symptoms than its native host. Yellow fever is a serious, potentially deadly flu-like disease spread by mosquitoes. This disease is most prevalent in tropical and subtropical areas. Yellow fever can't be passed directly from person to person through close contact. According to the recent analysis, there are an estimated 84 000-170 000 cases and up to 60 000 deaths due to yellow fever per year.

Yellow Fever Virus (YFV) is a positive- sense, ssRNA, (11 kb) encoding three structural (C, prM, E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). Env/E protein is the major inducer and target of virus neutralizing antibodies. The function of the virus E proteins is to attach the virus to receptors on host cells; they initiate the biggest immune response from the host. The M proteins appear to keep the E proteins functional during the assembly of new viruses. C proteins are found in the virus nucleocapsid.

YFV vaccine (killed virus) has been very effective and successful. Chimeric variants of YFV, that express E and prM proteins, are being used to produce vaccines against other arbovirus diseases such as dengue fever (DENV), West Nile (WNV), and Japanese encephalitis (JEV).

YFV Vaccines & Sample Testing

YFV vaccine came into use in 1938. It is on the WHO's List of Essential Medicines. YF-VAX®, Yellow Fever Vaccine, for subcutaneous use, is prepared by culturing the 17D-204 8 strain of yellow fever virus in chicken embryos. YFV vaccination provides life-long immunity and it has>90% efficacy. It has been administered to more than 600 million people worldwide. YF-17D is a live-attenuated vaccine that infects and replicates in target cells, vaccines who receive the YF-17D vaccine usually develop detectable viremia after vaccination. Therefore, antibodies to several structural (Env, prM, capsid) and non-structural proteins NS1-5 may be produced. Antibodies to NS1 are also found in natural infection.

YFV-17D, chimeric variants of YFV are being used to produce vaccines against other arbovirus diseases such as dengue fever (DENV), West Nile (WNV), and Japanese encephalitis (JEV). A new technology, called ChimeriVax has been developed to excise the specific genes that encode the E and prM proteins of the YFV and replace it with the gene for the E and prM protein of the target virus such as DENV. Recombinant RFV-17D chimeic vaccines may retain the NS1 protein so antibodies to NS1 will also be produced.

Serologically, an ELISA during the acute phase of the disease using specific IgM against yellow fever or an increase in specific IgG-titer (compared to an earlier sample) can confirm yellow fever. Together with clinical symptoms, the detection of IgM or a fourfold increase in IgG-titer is considered sufficient indication for yellow fever. Since these tests can cross-react with other flaviviruses, like dengue virus, these indirect methods

cannot conclusively prove yellow fever infection. In a differential diagnosis, infections with yellow fever must be distinguished from other feverish illnesses like malaria. Other viral hemorrhagic fevers, such as Ebola virus, Lassa virus, Marburg virus, and Junin virus, must be excluded as cause.

## Principles of Testing

The Human Anti-YFV-Env IgM ELISA kit is based on the binding of Human antibody in samples to ENV antigen coated on the plate, and virus antibody is detected by anti-Human IgM- specific antibody conjugated to HRP. After a washing step, substrate (TMB) is added and color (blue) is developed, which is directly proportional to the amount of antibody present in the sample. Stop Solution is added to terminate the reaction (converts blue to yellow color), and A450nm is then measured using an ELISA reader. The presence or concentration of antibody in samples is determined relative to supplied controls or calibrators.

## Reagents And Materials Provided

**Wash Solution Concentrate** (50x), 10ml, to be reconstituted and store as indicated.

**Sample/HRP Diluent Concentrate** (20x), 10ml, to be reconstituted and store as indicated.

**Anti-Human IgM- HRP Conjugate Concentrate** (100x), 0.11ml, to be reconstituted and store as indicated.

**YFV-ENV- coated Plate**, 8-well strips (12): Coated with YFV-ENV antigen, and post- coated with stabilizers.

**Anti-YFV Env IgM Calibrators**, 0.65 ml/vial: Four (4) vials (3 U/ml, 10 U/ml, 30 U/ml, 100 U/ml), each containing anti-YFV (NS1) IgG levels in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.

**Human Anti- YFV NS1 IgM Positive control**, 0.65 ml: 1 vial containing anti- YFV-Env IgM; diluted in buffer with protein, detergents and antimicrobial as stabilizers.

**Low NSB Sample Diluent**, 30 ml: Green Solution (1x) diluent.

**TMB Substrate**, 12 ml: Chromogenic substrate for HRP containing TMB and peroxide.

**Stop Solution**, 12 ml: Dilute sulfuric acid.

## Materials Required But Not Supplied

_ Pipettors and pipettes that deliver 100ul and 1-10ml. A multi- channel pipettor is recommended.

_ Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human IgG HRP Concentrate.

_ Distilled or deionized water to dilute reagent concentrates.

_ Microwell plate reader at 450 nm wavelength.

## Storage

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

## Specimen Collection And Preparation

### Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

### Antibody Stability and sample dilution

Initial dilution of serum into Working Sample Diluent (1X WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further test dilutions(1:100 or more) in Low NSB Sample Diluent (green solution) which provides the lowest assay background should be done the same day as the assay. Do not store test dilution.

**Example:**

Initial (1/10): 10ul serum + 90ul WSD [or 0.1ml + 0.9ml]

Further test dilution (1/0): 20 ul initial (1/10) + 180ul WSD

Note: Do not test human samples below 1:100. Final test dilution should be made in Low NSB diluent (Green solution) to suppress non-specific biding.

## Reconstitution And Storage

**Wash Solution Concentrate (50x), 10ml**: Dilute the entire volume 10ml + 490 ml with distilled or deionized water into a clean stock bottle. Label as 1X Wash Solution and store at 4°C for long term and ambient temp. for short term.

**Sample/HRP Diluent Concentrate (20x), 10ml**: Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample Diluent (WSD) and store at 2-8°C until the kit lot expires or is used up.

**Anti-Human IgM- HRP Conjugate Concentrate (100x), 0.11ml**: in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample Diluent is sufficient for 1 8- well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

## Plate Preparation

Review Calculation of Results and Limits of the Assay (above) before proceeding:

_ Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding and other matrix effects; for example, net signal for non- immune samples should be lower than the calibrator B (10 U/ml) or user specified cut-off values. This is usually 1/200 or greater dilution for sera.

_ Run a Sample Diluent Blank. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.

### Plate Set-up

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

_ Determine the number of wells for the assay run. Duplicates are recommended, including 4 Control wells and 2 wells for each sample and internal control to be assayed.

_ Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.

## Assay Procedure

_ Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul - 60 min; 4 washes]

- Add 100ul of 1X Low NSB diluent (blank), calibrators, samples and controls each to pre-determined wells.

- Tap the plate gently to mix reagents and incubate for 60 minutes.

- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul - 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgG-HRP to each well. o Incubate for 30 minutes.

- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul - 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.

- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.

- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.

- Read absorbance of the entire plate at 450nm within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

## Calculation

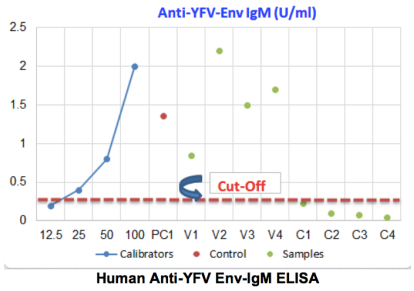
1. Subtract blank values from all values (standards, controls and samples) to calculate the net A450.
2. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
3. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis)

corresponding to the OD of the selected Index

= Total IgG Antibody Activity Units

## Typical Standard Curve

| Wells | Stds/samples | U/mL | Mean A450 | Net A450 |
|---|---|---|---|---|
| A1, A2 | Blank (0 U/ml) | 0.0 | 0.060 | - |
| B1, B2 | Calibrator A | 12.5 | 0.2 | 0.14 |
| C1, C2 | Calibrator B | 25 | 0.4 | 0.34 |
| D1, D2 | Calibrator C | 50 | 0.804 | 0.74 |
| D1, D2 | Calibrator D | 100 | 2.0 | 1.94 |



Human Anti-YFV Env-IgM ELISA

The sensitivity of the assay to detect Ant-YFV-Env IgM, from either natural infection or vaccinated animals, is controlled so that the threshold A450 for most true positives in Human serum at 1:100 are above the cut-off values.

Visual inspection of the data in the above graph shows the following:

PC1 is an internal positive control (not supplied with the kit).

V1-V4 are vaccinated Human samples.

C1-C4 are non-vaccinated control samples.

## Sensitivity

The YFV-Env antigen coating level, HRP conjugate concentration, and sample Diluent are optimized to differentiate anti-YFV-Env IgG from background (non-antibody) signal with Human serum samples at an appropriate dilution. The positive controls at 100 U/ml represent ~ 100 ng/ml Human IgG. The lowest limit of detection is about 0.3 ng of Human IgG.

## Specificity

YFV Env Antigen

YFV-Env protein (17D strain, 445-aa, EC domain) is highly conserved in various isolates of YFV (97-100%). YFV-Env has no significant sequence homology with other flaviviruses.

Recombinant, highly purified YFV-Env protein (strain 17 D, Env full length, HEK cells,>95%) is used to coat the microwells; thus the assay is specific for antibodies directed to YFV-Env. The Anti-Human IgM-HRP conjugate reacts specifically with Human IgM class antibodies; IgA, IgG and IgE antibody would not be measured above background signals.

## Precautions

Controls, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts

of water.

## Limitations

_ The assay detects IgM antibodies directed to the YFV-Env protein. It may be possible for an animal to have YFV infection without detectable antibodies specific to the YFV- Env protein.

_ Anti-YFV-Env antibody levels of an infected animal may be below detection threshold related to the time course of the infection, e.g., too early for positive titer development.