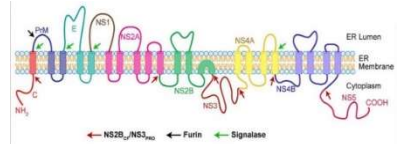


INTENDED USE

Mouse Anti-yellow fever virus NS1 IgG (YFV NS1-IgG) tests is an indirect ELISA suitable for detecting YFV-NS1 IgG in mouse serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use *in vitro research use only (RUO), not for therapeutic or diagnostic use.*

GENERAL INFORMATION

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.

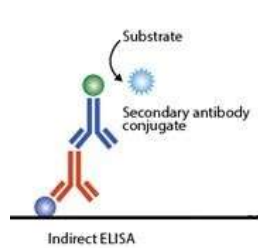


Yellow Fever Virus (YFV) is a positive-sense, ssRNA, (11 kb) encoding three

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).

PRINCIPLE OF THE TEST



The Mouse Anti-YFV-NS1 IgG ELISA kit is based on the binding of mouse antibody in samples to NS1 antigen coated on the plate, and virus antibody is detected by anti-mouse IgG-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.

KIT CONTENTS

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.

Component	Preparation Instructions
Wash Solution Concentrate (50x) Cat. #WB-50, 10 ml	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 Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up.
Anti-Mouse IgG-HRP Conjugate Concentrate (100x) Part: 530322, 0.11ml	Peroxidase conjugated anti-Mouse IgG 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.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
YFV-NS1-coated Plate	530321	8-well strips (12)	Coated with YFV-NS1 antigen, and post-coated with stabilizers.
12.5 U/ml	530323A	0.65 ml	Four (4) vials, each containing anti-YFV (NS1) IgG levels in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
25 U/ml	530323B	0.65 ml	
50 U/ml	530323C	0.65 ml	
100 U/ml	530323D	0.65 ml	
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- 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-Mouse IgG HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

ASSAY DESIGN AND SET-UP

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)** 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): **10 ul** initial (1/10) + **90ul** WSD

Assay Design

Review Calculation of Results (p5-7) 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.
- 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.

- 1st Incubation [100ul – 60 min; 4 washes]**
 - Add 100ul of 1X WSD (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.
 - 2nd Incubation [100ul – 30 min; 5 washes]**
 - Add 100ul of diluted Anti-Mouse IgG-HRP to each well.
 - 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.

PRECAUTIONS AND SAFETY INSTRUCTIONS

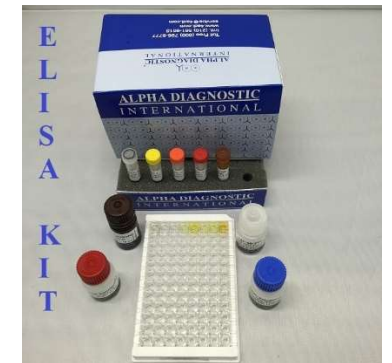
Controls, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: http://4adi.com/commerce/info/showpage.jsp?page_id=1060&cate_gory_id=2430&visit=10

Mouse Anti-Yellow Fever NS1 IgG (YFV-NS1 IgG) ELISA kit

Cat. #. 530-320-EHG, 96 Tests

For the detection of YFV-NS1 IgG in Serum, plasma or other biological fluids

For in vitro research use only (RUO), not for therapeutic or diagnostic use.



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PRODUCT SPECIFICATIONS

YFV-NS1 protein (17D strain, 352aa) is highly conserved in various isolates of YFV (92-100%). YFV-NS1 has significant sequence homology with other viruses (Wesselsbron virus, 66%; Sepik virus, 64%; Edge Hill virus, 58%; Uganda S Virus, 56%; Banzi Virus, 56%; Entebbe Bat virus, 55%; Yokose virus, 54%; Sokoluk virus, 50%; Spondweni virus, 48%). Japanese encephalitis virus, 46%, West Nile virus, 44%. It is not known if antibody to YFV-NS1 do crossreact with these viruses NS1 proteins.

Assay Sensitivity

The YFV-N1 antigen coating level, HRP conjugate concentration, and sample Diluent are optimized to differentiate anti-YFV-NS1 IgG from background (non-antibody) signal with mouse serum samples at an appropriate dilution. The positive controls at 100 U/ml represent ~ 100 ng/ml mouse IgG. The lowest limit of detection is about 0.3 ng of mouse IgG.

YFV in mice

Laboratory rodents have various degrees of susceptibility to YFV infection, with disease outcome dependent on age, viral strain, immune status of the host, inoculation route, and viral-inoculum titer. Immunocompetent infant mice are susceptible to fatal infection with intravenously or intraperitoneally injected virulent strains of YFV, including YFV-Asibi, as well as attenuated strains of YFV such as YFV-17D. However, immunocompetent adult mice are not susceptible to fatal infection with virulent or attenuated YFV delivered by intraperitoneal injection. In contrast, immunocompetent infant and adult mice are susceptible to virulent or attenuated YFV strains following olfactory bulb inoculation or direct delivery to the central nervous system (CNS) by intracerebral injection. YFV-17D can cause encephalitis after peripheral injection of adult mice lacking both alpha/beta interferon (IFN- α/β) receptors and IFN- γ receptors.

References: Krmpotic A (2003) *Microbes & Infe.* 5, 1263-1277; Reddehase MJ (2002) *J. Clin. Virol.* 25, 23-36; Rapp M, (1992) *J. Virol.* 66:4399-4406; Messerle M, (1992) *Virology* 191:355-367; Rawlinson W.D, (1996) *J. Virol.* 70:8833-8849; Dorsch-Hasler, K (1985) 82, 8325-8329

Related Items

530-200-EHG	Human Anti-YFV-Env IgG ELISA
530-205-EHM	Human Anti-YFV-Env IgM ELISA kit
530-220-EHG	Mouse Anti- YFV-Env IgG ELISA kit
530-225-EHM	Mouse Anti-YFV-Env IgM ELISA kit
530-300-EHG	Human Anti-(YFV-NS1) IgG ELISA kit
530-305-EHM	Human Anti-YFV-NS1 IgM ELISA kit
530-320-EHG	Mouse Anti-YFV-NS1 IgG ELISA kit

YFP11-A	Anti-Yellow Fluorescent Proteins (YFP) protein IgG
YFV11-M	Monoclonal Anti-Yellow Fever Virus (17D) IgG (neutralizing)
YFVNS16-R-10	Recombinant (E. coli) Yellow Fever Virus Env protein (YFV-Env/17D, full extracellular domain 445aa) (>95%, his-tag)
YFVNS16-S	Anti-Yellow Fever Virus Env protein (YFV-Env/17D) antiserum
YFVNS15-R-10	Recombinant (HEK) Yellow Fever Virus NS1 protein (YFV-NS1/17D, 779-1136aa) (>95%, his-tag)
YFVNS15-S	Anti-Yellow Fever Virus NS1 protein (YFV-NS1/17D, 779-1136aa) antiserum
YFVPR17-R-10	Yellow Fever Virus prM protein (YFV-Env/17D, 89-aa prM) (>95%, No-tag)
YFVPR17-S	Anti-Yellow Fever prM Env protein (YFV-Env/17D, 89-aa prM) antiserum

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 mouse samples.

C1-C4 are non-vaccinated control samples.

INTERPRETATION OF RESULTS (cont)

Antibody Titers from positive control dilution Curves

Positive control has been assigned an arbitrary YFV-NS1 IgG activity of 100 U/ml. Run additional controls (user supplied) and plot a standard curve to calculate unknown values.

The titer of antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method for animals immunized with YFV-NS1 antigens. Best precision can be obtained using the following guidelines:

- Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used. Prepare serial dilutions of each sample to provide a series
- that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
- A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
- The Positive, Negative and/or Internal Controls can be used to normalize inter-assay values.

Notes: The sensitivity of the assay may be increased to perhaps convert a borderline sample to a positive by using a lower dilution of the sample, e.g., 1/50. The values of negatives may increase, so an alternative threshold should be established to make sure that negative or controls samples values are <0.200)

Limits of the Assay

- The assay detects IgG antibodies directed to the YFV-NS1 protein. It may be possible for an animal to have YFV infection without detectable antibodies specific to the YFV-NS1 protein.
- Anti-YFV-NS1 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.

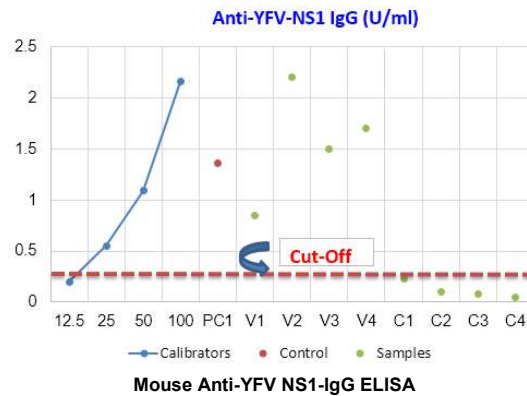
Specificity

Recombinant, highly purified YFV-NS1 protein (strain 17 D, NS1 full length, accession # P03314, HEK cells, >95%) is used to coat the microwells; thus the assay is specific for antibodies directed to YFV-NS1. The Anti-Mouse IgG-HRP conjugate reacts specifically with mouse IgG class antibodies; IgA, IgM and IgE antibody would not be measured above background signals.

INTERPRETATION OF RESULTS

Wells	Stds/sample s	U/mL	Mean A450	Net A450
A1, A2	Blank (0 U/ml)	0.0	0.012	-
B1, B2	Calibrator A	12.5	0.252	0.24
C1, C2	Calibrator B	25	0.535	0.523
D1, D2	Calibrator C	50	1.09	1.08
D1, D2	Calibrator D	100	2.2	2.19

Example:



2-soum/530-320-EHG-Mouse-Anti-YFV-NS1-IgG-ELISA-Graph

Calculations

- Subtract blank values from all values (standards, controls and samples) to calculate the net A450.
- 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).
- 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

Results

The **sensitivity** of the assay to detect Ant-YFV-NS1 IgG, from either natural infection or vaccinated animals, is controlled so that the threshold A450 for most true positives in mouse serum at 1:100 are above the cut-off values.