Size : 137 x 218 mm

Qualisa™
Microwell Enzyme Immunoassay | DEN IgG
DENGUE IgG

Enzyme linked Immunosorbent Assay (ELISA) for Qualitative Determination of Dengue IgG antibody in Human Serum/Plasma.

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Enzyme Linked Immunosorbent Assay for the Qualitative detection of IgG antibody to Dengue in human serum or plasma. For in Vitro Diagnostic Use only.

INTRODUCTION

Dengue fever (DF) is an acute, self limiting, viral disease that is characterized by fever, headache, body pains, rash, lymphadenopathy and prostration. In its most severe form, dengue hemorrhagic fever (DHF), infected patients will experience severe fever and renal failure leading to the often fatal dengue shock syndrome (DSS). It is estimated that approximately two billion people are at risk for DF world wide, and that over one million people per year are infected. This, combined with the hundreds of thousands of cases of DSS, make dengue the most important arbovirus disease in the world.

PRINCIPLE OF THE ASSAY

Dengue antigen is coated on the microwells. Diluted samples along with positive and negative controls are added to the coated wells and incubated. Dengue Virus specific IgG antibodies if present bind to the antigen on the microwells. The wells are washed to remove unbound components and Agglutinating sera for Human IgG conjugated to horseradish peroxidase (HRPO) is added. After a short incubation the wells are washed again and bound enzyme is detected by adding substrate. The reaction is stopped after specified time with an acid and absorbance is determined for each well at 450nm and 630nm with an ELISA reader.

MATERIALS AND COMPONENTS

A. Materials provided with the test kit

1. Coated Microwells: Microwells coated with Dengue antigen.
2. Sample Diluent: Buffered solution containing stabilizing proteins and preservatives. Ready to use.
3. Negative Control: Ready to use.
4. Positive Control: Ready to use.
5. Washing Concentrate (20X): Buffer containing Surfactants.
6. Enzyme Conjugate (50X): Goat anti-human IgG-HRPO conjugate. To be diluted 50 times with conjugate diluent.
7. Conjugate diluent: Buffered solution containing stabilizing proteins and preservatives. Ready to use.
8. Substrate: Solution containing Tetremethyl benzidine (TMB) and hydrogen peroxide. Ready to use.
9. Stop Solution: Diluted acid. Ready to use.
10. Microwell holder.
11. Instructions for use.
12. ELISA protocol sheet.
13. Plate sealer.

B. Materials required but not provided

1. Manual or automatic pipette
2. Pipette tips
3. Absorbent sheets
4. ELISA washer
5. ELISA reader
6. Pipetting troughs or boats
7. Biohazard waste container
8. Timer
9. Disposable gloves
10. Disinfectant

STORAGE AND STABILITY

1. Store the kit at 2 - 8 °C.
2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

SAMPLE COLLECTION & PREPARATION

1. Collect Blood specimen by venipuncture according to standard procedure.
2. Serum or plasma can be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing particulate matter should be clarified by centrifugation prior to use.

PRECAUTIONS

1. Bring all the reagents & specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. Use Protective clothing when handling samples.
5. Do not mix components of one kit with another.
6. Do not use kit after expiry date.
7. Always use a new tip for each specimen & reagent.

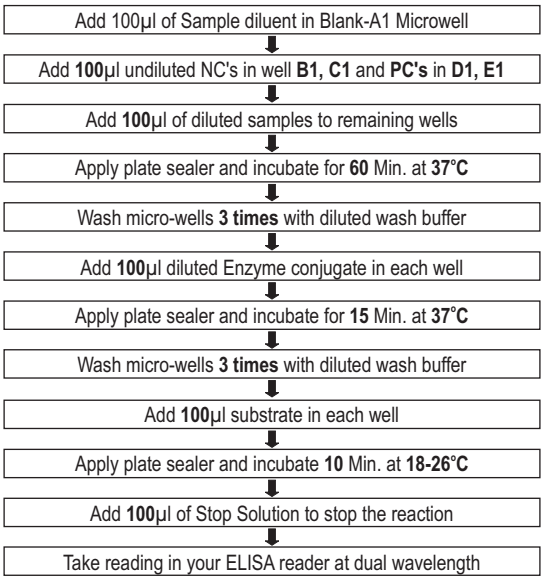
REAGENT PREPARATION

1. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95ml of distilled or deionized water).
2. Dilute conjugate 50 times (for example add 20µl concentrated conjugate to 980µl of conjugate diluent).

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
50x conjugate	20 µl	40µl	60µl	80µl	100µl	120µl	140µl	160µl	180µl	200µl	220µl	240µl
Conjugate diluent	980µl	1960µl	2940µl	3920µl	4900µl	5880µl	6860µl	7840µl	8820µl	9800µl	10780µl	11760µl

TEST PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Preparation for Sample Dilution : 1:100 dilutions by adding 5µl of the test sample to 500µl of sample diluent. Mix well.
3. For the reagent blank, dispense 100µl of sample diluent in A1 well position, followed by negative control and positive control (ready to use, do not dilute). Dispense 100µl of diluted sera into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 60 minutes at 37°C.
4. Wash each well three times by filling approximately 350µl diluted wash buffer & blot dry.
5. Dispense 100µl of diluted enzyme conjugate to each well and incubate for 15 minutes at 37°C.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Dispense 100µl of TMB Chromogenic Substrate to each well and incubate for 10 minutes at room temperature, away from direct light.
8. Add 100µl of Stop Solution to stop the reaction.
9. Read O.D. at 450 - 630 nm with an ELISA reader.



The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.100.
2. The O.D. value of the Negative control should be less than 0.150.
3. The O.D. value of the Positive control should be more than 0.600.

CALCULATION OF RESULTS

1. Calculate the mean absorbance of the negative control.
2. Cut-off value (COV):
   COV=Av.NC+0.600.
3. IgG index value can be calculated by dividing the sample absorbance by the COV.

$$\text{IgG Index Value} = \frac{\text{Absorbance of Sample}}{\text{COV}}$$

INTERPRETATION OF THE RESULT

IgG Index Value	Result
IgG Index value	<0.9 Negative
IgG Index value	0.9-1.5 Grey zone
IgG Index value	>1.5 Positive

PERFORMANCE CHARACTERISTICS

Four hundred and seventy five specimens- out of which twenty five positive and four fifty negative specimens were tested with **Qualisa™ Dengue IgG** and compared with a commercially available ELISA kit utilizing similar principle. The results are given below:

SPECIMEN DATA	TOTAL	Qualisa™ Dengue IgG	Commercially available ELISA
Number of specimen tested	475	475	475
Number of positive specimens	25	25	25
Number of negative specimens	450	438	450

Sensitivity of **Qualisa™ Dengue IgG**: 100%
Specificity of **Qualisa™ Dengue IgG**: 97.3%

IMPORTANT NOTE

The wash procedure is critical, insufficient washing will result in poor precision and falsely elevated absorbance readings.

LIMITATIONS OF THE ASSAY

1. A single serum sample cannot be used to determine recent infection.
2. A serum specimen taken in an early stage during acute phase of infection may contain low levels of IgG antibody and render an IgG Index result negative.
3. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

BIBLIOGRAPHY

1. Halstead, SB. 1980. Immunological parameters of togavirus disease syndromes. p.107-173. In RW Schlesinger (ed.), The Toga Viruses. Academic Press, Inc., New York.
2. Gubler, DJ. 1989. Aedes aegypti and Aedes aegypti-borne disease control in the 1990's: top down or bottom up. Am. J. Trop. Med. Hyg. 40:571-578.
3. Henchal, EA and JR Putnak. 1990. The Dengue Viruses. Clin. Micro. Rev. #:376-396.
4. Halstead, SB. 1989. Antibody, Macrophages, Dengue Virus Infection, Shock, and Hemorrhage: A Pathogenic Cascade. Rev. of Inf. Dis. 11:S830-S839.
5. MMWR. 1991. 40:v9:145.
6. Gubler, DJ. 1989. Dengue, p.223-260. In T.P. Monath (ed.) The arboviruses: epidemiology and ecology. Vol.2 CRC Press, Inc., Boca Raton, Fl.
7. Halstead, SB and G Papaevangelou. 1980. Transmission of dengue 1 and 2 viruses in Greece in 1928. Am. J. Trop. Med. Hyg. 29: 625-637.
8. Kuno, G. and I Gomez and DJ Gubler. 1991. An ELISA procedure for the diagnosis of dengue infections. Journal of Virol. Meth. 33: 101-113.

9. Hayes Edward B. and Gubler Duane J. Dengue and Dengue Hemorrhagic Fever. Pediatric Infectious Disease Journal 11:311-317, 1992.
10. Kuno G., Gomez I. And Gubler D.J. An ELISA Procedure for the Diagnosis of Dengue Infections. Journal of Virological Methods 33:101-113, 1991.
11. Guzman Maria G. and Kouri Gustavo Advances in Dengue Diagnosis. Clinical and Diagnostic Laboratory Immunology 3:621-627, 1996.
12. Vaughn David W., Green Sharone, et al. Dengue in the Early Febrile Phase: Viremia and Antibody Responses. The Journal of Infectious Diseases 176:322-330, 1997.

Manufactured by:
Qualpro Diagnostics
A Division of Tulip Diagnostics (P) Ltd.
88/89, Phase II C, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.
Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

0820/VER-02