ASTAM DIAGNOSTICS

PRODUCT INSERT

REF DAGE

PRATHAM®
DENGUE NS1 ANTIGEN ELISA

INTENDED USE

PRATHAM® Dengue NS1 Antigen ELISA Test is a solid phase, enzyme linked immunosorbent assay for the detection of dengue NS1antigen in human serum/plasma. For professional use only.

INTRODUCTION

Dengue is caused by any of the four distinct but anti-genically related serotypes of flavivirus (DEN-V1, DEN-V2, DEN-V3 & DEN-V4) found largely in tropic and subtropic areas. The viruses are transmitted to human by mosquito Aedes aegypti and Aedes albopictus causing dengue fever with severe flu like symptoms. WHO estimates that 55-60 million cases of dengue fever occur worldwide each year, including a more severe form called the dengue hemmorraghic fever(DHF) and dengue shock syndrome (DSS). During primary infection the classical symptoms are sudden onset of fever, intense headache, myalgia, arthralgia and rash. Secondary infection is most common in many parts of South East Asia and South America. During Primary infection a Non Structural Protein (NS-1) levels are raised and can be detected in early clinical phase of the disease from day 1-9. NS 1 Ag appears in serum / plasma before the onset of IgM & IgG antibodies and its detection in suspected dengue patient can be used as an early marker for the disease.

TEST PRINCIPAL

PRATHAM® Dengue NS1 Antigen ELISA Test an antibody specific for DENV NS1 has been pre-coated into a micro well strips. Standards or samples are pipetted into the wells and any DENV NS1 present is bound on the plate. After washing away any unbound substances, a Horseradish Peroxides (HRP) conjugated primary antibody binds to DENV NS1 is added to each well and incubates. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and colour develops in proportion to the amount of total DENV NS1 bound in the initial step. The colour development is stopped by the addition of acid and the intensity of the colour is measured at a wavelength of 450nm ±2nm. The concentration of total DENV NS1 in the sample is then determined by comparing the O.D of samples to the standard curve.

KIT COMPONENTS

Component	Description of Reagent	Presentation
Coated Microwells	96 Microwell (12x8) coated with monoclonal anti-Dengue NS1 Antibodies	1
Positive Control	Dengue NS1 Positive and HIV/HCV/HBsAg non-reactive Serum dilution in stabilizer solution with preservatives.	1x1.5 ml
Negative Control	Inactivated and stabilized human serum non reactive for Dengue NS1.	1x1.5 ml
Conjugate	Monoclonal anti-Dengue NS1 conjugated to HRP. 21X	1x0.8 ml
Conjugate dilution buffer	Buffered solution containing proteins and preservatives.	1x12 ml
Sample dilution buffer	Buffered solution containing stabilizing proteins and preservatives.	1x6 ml
Substrate	Solution containing Tetramethibenzidine (TMB) and hydrogen peroxide. Ready to use.	1x10 ml
Wash buffer	Buffer containing surfactants (40X). To be diluted 40 times with distilled or deionized water.	1x25 ml
Stop solution	Diluted sulphuric acid.	1x10 ml
Plate sealer	Adhesive paper sheet for covering plate on incubation time	2 Nos.
Pack insert	Instruction for use	1 Nos.

MATERIAL REQUIRED BUT NOT PROVIDED

Microplate reader capable of measuring absorbance at 450nm, Pipettes and Pipettes tips, Deionized or distilled water, 1X PBS, Automated microplate washer, ELISA Reader, Disinfectant, Timer, Gloves, Biohazard Waste Container etc.

STORAGE AND STABILITY

PRATHAM® Dengue NS1 Antigen ELISA test components are stable up to expiry date indicated on the component label/box label. Dengue NS1 Antigen ELISA kit needs to be stored at 2-8ºC.

SPECIMEN COLLECTION AND STORAGE

1. No prior preparation of the patient is required.
2. Collect blood specimen by venipuncture according to the standard procedure.
3. Specimen should be free of particulate matter and microbial contamination.
4. Preferably use fresh sample. However, specimen can be stored refrigerated for 24 hours. For long storage, freeze at - 20ºC or below. Specimen should not be frozen and thawed repeatedly. Maximum of two freeze/thaw cycles are allowed.
5. Do not use heat inactivated specimen.
6. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
7. No not use turbid, lipaemic, haemolysed, clotted or contaminated specimen.

WARNING AND PRECAUTIONS

- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Before use, allow reagents to reach room temperature (+18-30ºC.
- Carefully reconstitute or dilute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugate.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the micro plate to dry between the end of the washings operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- Use a new pipette tip for each sample.
- Washing the micro plate is a critical step in the procedure: Follow the recommended number of washings cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washings may lead to inaccurate results.
- Inadequate removal of residual wash buffer can cause inconsistent color development. Microwell strips should be tapped and blotted on absorbent paper or towels to minimize residual wash buffer.
- Never use the same container to distribute conjugate and development solution.
- Check the pipettes and other equipment for accuracy and correct operations.
- Do not change the essay procedure.

Preparation of Reagents prior to use:
Wash Buffer Preparation: Dilute wash buffer 40 times for example add 5 ml concentrated buffer to 195 ml distilled or deionized water.
Conjugate Preparation: Dilute conjugate 21 times as shown below:

Reagent (Strip)	1	2	3	4	5	6	7	8	9	10	11	12
Conjugate (µl)	50	100	150	200	250	300	350	400	450	500	550	600
Conjugate dilution buffer (ml)	1	2	3	4	5	6	7	8	9	10	11	12

TEST PROCEDURE

1. Bring all the reagents and specimen to room temperature before use.
2. Take out required number of strips and immediately close the pouch.
3. Prepare data sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Leave well A1 as substrate control.
6. Add 50µl sample dilution buffer in each well except A1.

7. Add 100µl sample or controls in separate well except well A1.
8. Apply plate sealer and incubate for 60 minutes at 37ºC.
9. Wash each well by filling approximately 350µl diluted wash buffer and aspirating/flicking off six times Blot dry.
10. Add 100µl diluted conjugate in each well except A1 and incubate for 30 minutes at 37ºC.
11. Wash six times as in step 9 and blot dry.
12. Add 100µl substrate in each well including A1 and incubate at room temperature away from light for 15 minutes.
13. Stop reaction by adding 100µl stop solution. The stop solution should be added in the same sequence as substrate addition.
14. Blank ELISA reader with well "A-1".
15. Read the absorbance at 450nm with 630nm or above as reference within 30 minutes of stopping the reaction.

Quality Control

Negative Control: The individual absorbance value of negative controls should be less than 0.1.
Positive Control: The individual absorbance value of positive controls should be more than 1.0.
Calculation of Cut of Vlue
The cut off value = 0.2 + NC(Avg)
Set up the cut-off value
Example

NC	**Absorbance**
A1	**0.028**
B1	**0.033**

Avg. of NC= (0.028+0.033)/2= 0.030
cov = 0.2+0.030=0.230

INTERPRETATION OF RESULTS

Negative: Specimen with absorbance value less than or equal to the cut-off value are considered Negative for Dengue.
Positive: Specimen with absorbance value more than to the cut-off value are considered positive for Dengue. These specimens should be retested in duplicate. On retesting if the absorbance value of the duplicates is less than cut of value, the specimen is considered as negative. If reactive sample that reacts in either or both duplicates are considered repeatedly reactive.
Repeatedly reactive: If a sample is repeatedly reactive the probability of Dengue NS1 antigen are high, especially with patients at high risk or high absorbance values. Such samples should be retested with other tests and correlated with clinical symptoms.

INTERNAL QUALITY CONTROL

1. Positive and negative controls should be included in each test batch.
2. Acceptable performance is indicated when a uniform milky suspension with no agglutination in observed with the Dengue NS1 Negative Control and agglutination with large aggregates is observed with the Dengue NS1 Positive Control.

PERFORMANCE CHARACTERISTICS

The kit has been evaluated with the 30 known panel of Dengue NS1 Ag positive and 100 negative samples. Following is the in-house evaluation.

Description	Negative	Positive	Total
Dengue known Negative Specimen	100	0	100
Dengue known Positive Specimen	0	30	30

Assay Sensitivity : 100%
Assay Specificity : 100%

LIMITATION OF THE TEST

1. **PRATHAM® Dengue NS1 Antigen ELISA** is a screening test. All reactive samples should be further confirmed by supplemental assays like PCR or Viral isolation.
2. Absence of Dengue NS1 does not indicate that an individual is absolutely free of Dengue infection.
3. The clinical diagnosis must be interpreted with clinical signs and symptoms of the patient. The results from this kit are not be themselves diagnostic and should be considered in association with other clinical data and patient symptoms.

4. As with all diagnostic tests, a definitive clinical diagnostic should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
5. In case the kit is not stored properly or the test is not performed as per the recommended instructions, it may lead to erroneous results.

DISPOSAL

Consider all test run with human specimen as potentially infectious and discard using standard biosafety practices.

DISCLAIMER:

Whilst every precaution has been taken to ensure the diagnostic ability and accuracy of this product the product is used outside of the control of the Manufacturer and Distributor and the result may accordingly be affected by environmental factors and / or user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.

The manufacturer and distributors of this product shall not be liable for any losses, liability, claims, coasts or damages whether direct or indirect or consequential arising out of or related to an incorrect diagnosis, whether positive or negative in the use of this product.

REFERENCES

1. Shu PY, Huang JH. (2004) Current advances in dengue diagnosis. Clinical and Diagnostic Laboratory Immunology; 1:642-50.
2. Halstead SB, Selective primary health care: strategies for control of disease in the developing world: XI, Dengue, Rev. Infect. Dis. 1984; 6:251-264.
3. Ruechusatsaeat K, et al. Daily observation of antibody levels among dengue patients detecte by enzyme-linked immunosorbent assay (ELISA). Japanese J. Trop. Med. Hygiene 1984; 22:9-12.
4. Ampaiwan C, Wathanee C, Viroj P, Kanchan T, Sarapee L, Sutee Y. (2008). The use of Nonstructural protein 1 antigen for the early diagnosis during the febrile stage in patients with infection. Indian J Med Microbiol 2010;28:107-10.
5. Sekaran SD, Lan EC, Mahesawarappa KB, Appanna R, Subramanium G. (2007). Evaluation of a dengue NS1 capture ELISA assay for the rapid detection of dengue. J Infect.

SYMBOLS

Read instructions for use	Name of Manufacturer	For single use only
No. of test	Expiry Date of Kit.	Date of manufacturing of IVD Kit
IVD In-vitro diagnostic use	Keep away from Sunlight	REF Reference Catalogue Number
Storage Condition		

ASTAM DIAGNOSTICS

Manufactured By:
ASTAM DIAGNOSTICS PVT. LTD.
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