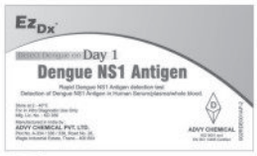
EzDx™ Dengue NS1 Test Procedure

1. FIRST, read carefully the Product Insert on how to use the **EzDx™ Dengue NS1 Antigen** kit.
2. Now open the kit and look for the following.

I) Test device individually foil pouched with a desiccant

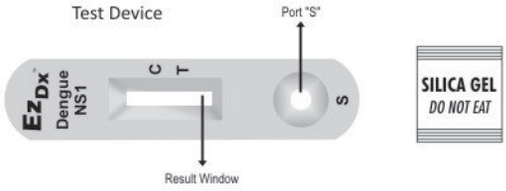


II) Sample Dropper

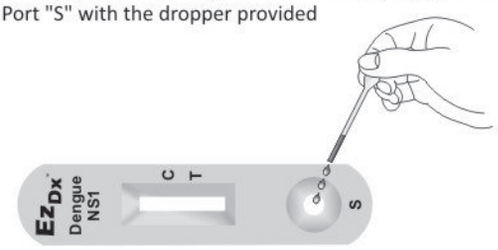
II) Product Insert

3. Next, look at the expiry date at the back of the pouch. Use another kit, if expiry date has passed.

Open the pouch and look for the following.



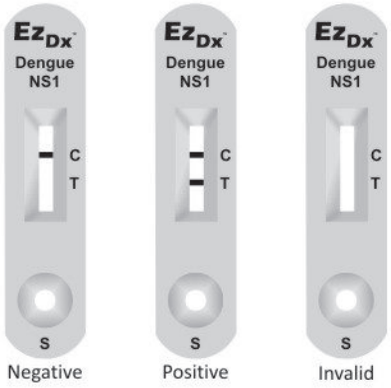
4. Add 3 drops(60µl) of serum/plasma or 4 drops(80µl) of whole blood into Port "S" with the dropper provided



5. **INTERPRET TEST RESULTS AT THE END OF 20 MINUTES.**

CAUTION : Do not read test after 20 minutes, since it may give incorrect results.

6. **Interpretation of Test Results.**



Ez Dx™

Dengue NS1 Antigen

Rapid Dengue NS1 Antigen Detection Test
Detection of Dengue NS1 Antigen in Human Serum/Plasma/Whole Blood

Ref: 002RDE010KI-2 **Product Code: RK DEN 002**

INTRODUCTION

Dengue is a major health concern in many tropical and subtropical countries in the world. The accurate and efficient diagnosis of primary dengue infection is important for clinical care and surveillance. A specific test is needed to confirm infection during the acute phase primary infections. Dengue is an enveloped flavivirus with 3 structural (C, prM and E) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS, NS4A, NS4B and NS5). NS1 antigen recognized as a marker of acute phase of dengue infection, a period for which traditional serological antibodies based methods are of limited value. NS1 antigen was found circulating in sample of infected patients from the first day and up to 9 days after onset of fever in primary dengue cases.

INTENDED USE

EzDx™ Dengue NS1 Antigen is a qualitative rapid test for the detection of Dengue NS1 Antigen in human Serum / Plasma/ Whole blood. This test is for diagnostic use only in acute dengue infection.

PRINCIPLE

EzDx™ Dengue NS1 Antigen test device consist of two lines; "C" (Control Line) & "T" (Dengue NS1 Antigen Test Line). Test line is coated with antibodies, anti-dengue NS1 Ag. When a sample is added to the device, Dengue NS1 antigen if present in the sample will bind to the anti-dengue NS1 conjugate making antigen antibodies complex. This complex migrates along the membrane to the test region and forms the visible Color line at "T" as antibody-antigen-antibody complex. The intensity of the test bands in the device will vary depending upon the amount of antigen present in the sample. The appearance of colour in a specific test region should be considered as positive for that particular antigen. A procedural control line "C"should always develop in the test device window to indicate that the test has been performed properly. This test is intended for professional use and must be used by trained personnel.

MATERIALS PROVIDED

EzDx™ Dengue NS1 Antigen test kit contains the following items to perform the assay:

1. **EzDx™ Dengue NS1** Ag devices individually pouched with desiccant.
2. Sample Dropper
3. Product Insert

WARNING AND PRECAUTION

1. For in- vitro diagnostic use only.
2. For best results, strict adherence to these instructions is required. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.
3. The **EzDx™ Dengue NS1 Antigen** test device should remain in its sealed pouch until ready for use since the test devices are sensitive to humidity as well as heat.
4. Use separate syringe or clean pipette tips/ sample Droppers for different samples to avoid cross-contamination of samples which could cause erroneous results. Do not pipette by mouth.
5. Do not smoke, eat or drink in areas where specimens or kits are handled.
6. Wear disposable gloves while handling specimens and performing the tests, and thoroughly wash hands afterwards.
7. As known relevant interference, haemolytic samples, rheumatoid factors contained samples and lipaemic, icteric samples can lead to impair the test results.
8. All patients samples should be handled as if they are capable of transmitting diseases. Follow established GLP for proper disposal of samples, used dropper/pipette tips or syringes and used test devices.
9. Do not reuse test devices.

10. All reagents must be at room temperature before performing the test.
11. Do not use reagents beyond the stated expiration date marked on the package label.
12. The components in this kit have been quality control tested as standard batch unit. Do not mix components from different lots.
13. Do not use the test kit if the pouch is damaged or the seal is broken.

KIT STORAGE AND STABILITY

(1) The test device should be stored at 2 - 40°C (36°-104°F). **DO NOT FREEZE**
(2) The test device is sensitive to humidity as well as to heat.
(3) Do not use the test device beyond the expiration date. Expiration date of this Kit is as indicated on the kit cartons as well as on individual pouches.
(4) Do not use the test kit if the pouch is damaged or the seal is broken.
(5) Do not re-use the test device

SPECIMEN COLLECTION, STORAGE & PRECAUTION

1) Whole blood

(1) Collect the whole blood into the collection tube (containing anticoagulants such as EDTA, Heparin or Oxalate) by venipuncture. Guidelines as recommended by the National Committee for Clinical Laboratory Standard (NCCLS) should be followed when collecting, transporting and processing patient samples.
(2) Optimal results were obtained when patient samples were tested immediately after collection. If immediate testing is not possible then the samples may be stored at 2-8°C for up to 3 days. For storage more than 3 days, freezing is recommended. The samples should be brought to room temperature prior to use. Using the samples in the long term keeping more than 3 days can cause non-specific reactions. Ideally samples when stored at 2-8°C, the whole blood samples should be used within 3 days.

2) Plasma or Serum

(1) [Plasma] Collect the whole blood into the collection tube (containing anticoagulants such as EDTA or Heparin or Oxalate) by venipuncture and then centrifuge blood to get plasma specimen.
(2) [Serum] Collect the whole blood into the collection tube (NOT containing anticoagulants) by venipuncture, leave to settle for 30 minutes for blood coagulation and then centrifuge blood to get serum.

Note:

- If plasma or serum specimens are not tested immediately, they should be refrigerated at 2-8°C. (For storage period longer than 2 weeks, freezing is recommended. The Samples should be brought to room temperature (15 - 30°C) prior to use.

- Plasma or serum specimens containing a precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

TEST PROCEDURE

1. Collect specimen according to instruction in specimen collection and storage.
2. Allow the **EzDx™ Dengue NS1 Antigen** test kit components and specimens to attain room temperature prior to testing. Open the pouch just before performing the assay.
3. Remove the test devices from the sealed pouch.
4. Add 3 drops (60µl) of serum/plasma or 4 drops (80µl) of whole blood using the sample dropper provided into the port "S"
5. Read the result at the end of 20 minutes.
6. Interpret the result. Refer figure for interpretation of the test results.
   Caution: Do not read the test after 20 minutes, since it may give incorrect results.

INTERPRETATION OF THE RESULTS

Whole blood samples may cause red background to appear in the result window. If this is not masking the test line the result remains valid.

A) **EzDx™ Dengue NS 1 Antigen device:**

Positive:

Appearance of coloured line, one each in test region "T" and control region "C" indicates that the sample is Positive for Dengue NS1 Antigen.

Negative :

Appearance of one distinct coloured line in the control region "C" only, indicates that the sample is "Negative" for Dengue NS1 Antigen.

Invalid:

If Coloured Control line "C" does not appear, the test is invalid. In this case, please repeat the test using a fresh device, and follow the test procedure exactly.

LIMITATION OF THE TEST

1. **EzDx™ Dengue NS1 Antigen** test is designed for primary screening of acute dengue infection and recent infection can only be established with the clinical and laboratory findings. It's a qualitative test and does not indicate the amount of NS1 antigen in sample.
2. The test is for In Vitro Diagnostic Use Only. Do not read results after 20 minutes

PERFORMANCE CHARACTERISTICS

The **EzDx™ Dengue NS1 Antigen** rapid detection test kit has been tested with NS1 positive and negative clinical samples confirmed by ELISA. The results obtained are as follows:

Sr. No.	Sample Type	Total No. of Sample Confirmed by ELISA	Test Results of EzDx™ Dengue NS1 Ag Rapid test Detection kit		Sensitivity (%)	Specificity (%)
			NS1 Positive	NS1 Negative		
1	NS1 Positive	52	51	1	98.08	-
2	NS1 Negative	190	0	190	-	100

REFERENCES

1) Alcon S., Talarmin A., Debruyne M., Falconar A., Deubel V., Flamand M. 2002.Enzyme-linked immunosorbent assay specific to dengue virus type 1 non structural protein NS1 reveals circulation of the antigen in the blood during acute phase of disease in patients experiencing primary or secondary
2) Kumarasamy V, Wahab AH, Chua SK, Hassan Z, Chem YK, et al.: Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. J Virol Methods 2007, 140:75-79.
3) Hang VT, Nguyet NM, Trung DT, Tricou V, Yoksan S, et al.: Diagnostic Accuracy of NS1 ELISA and Lateral Flow Rapid Tests for Dengue Sensitivity, Specificity and Relationship to Viraemia and Antibody Responses. PLoS Negl Trop Dis 2009, 3:e360.

DISCLAIMER:

While every precaution has been taken to ensure the diagnostic ability and accuracy of this product, this product is used outside of the control of the manufacturer and the 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.

WARNING:

The Manufacturers and Distributors of this product shall not be liable for any loses, liability, claims, costs 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.

For any complaint/query and suggestions:

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SYMBOL LEGENDS

Symbol	Explanation of symbol	Symbol	Explanation of symbol
	Consult instruction for use		Keep Dry
	Do not use if package is damaged	LOT	Batch code No.
IVD	In vitro diagnostic device		Manufacturer
	Store at 2°c - 40°c		Date of Manufacturer
	Keep away from sunlight		Use by (date or month of expiry)
2	Do not reuse		