Size : 274 x 218 mm

# QUALISA™ Dengue NS1

**Enzyme Linked Immunosorbent Assay (ELISA) for detection of Dengue NS1 Antigen in human serum or plasma.**
**FOR IN VITRO DIAGNOSTIC USE ONLY**
**Store at 2°C to 8°C**

## INTENDED USE

**QUALISA™ Dengue NS1** is intended to be used for qualitative detection of Dengue NS1 ( Serotypes 1-4) antigen in human serum or plasma. For In Vitro Diagnostic use only.

## SUMMARY AND EXPLANATION

**QUALISA™ Dengue NS1** is a solid phase Enzyme Linked Immunosorbent Assay (ELISA) that employs highly purified Agglutinating sera to the Dengue NS1 antigen.

## PRINCIPLE OF THE ASSAY

Microwell strips are coated with Agglutinating sera for Dengue NS1. Another Agglutinating sera for dengue NS1 antigen is conjugated to horseradish peroxidase (HRPO). Samples along with positive and negative controls are added to the coated wells and incubated. The wells are washed to remove unbound components and the bound enzyme is detected by adding substrate. The reaction is stopped after specified time with acid and absorbance is determined for each well at 450 nm with an ELISA reader. The cutoff value is calculated by given formula and absorbances of all wells are compared with the cutoff value. Any sample having absorbance more than cutoff value is considered reactive.

## KIT COMPONENTS

**QUALISA™ Dengue NS1** has following components:

1. Coated microwells: Microwells are coated with the Agglutinating sera for Dengue NS1 antibody.
2. Positive control: Agglutinating sera for mouse globulin with stabilizer. Produces a positive reaction.
3. Negative control: Bovine Serum Albumin with stabilizer. Produces a negative reaction and is used for cut-off calculation.
4. Conjugate: Agglutinating sera for Dengue NS1-HRP conjugate.
5. Conjugate diluent: Buffered solution containing stabilizing proteins and preservatives. Ready to use.
6. Sample diluent: Buffered solution containing stabilizing proteins and preservatives. Ready to use.
7. Substrate: Solution containing Tetramethyl benzidine (TMB) and hydrogen peroxide. Ready to use.
8. Wash buffer: Buffer containing surfactants.
9. Stop solution: Diluted sulfuric acid. Ready to use.

## STORAGE AND STABILITY

1. **QUALISA™ Dengue NS1** kit is stable at 2-8°C upto the expiry date printed on the label.
2. Coated microwells should be used within one month of opening the pouch provided that once opened, the pouch must be resealed properly to protect from moisture. If the color of the desiccant has changed from blue to white at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted conjugate must be used immediately.
4. Diluted wash buffer is stable upto one week when stored at 2-8° C.

## MATERIAL REQUIRED BUT NOT PROVIDED

1. Manual or automatic pipette.
2. Pipette tips.
3. Incubator.
4. Absorbent sheets.
5. ELISA washer.
6. ELISA reader.
7. Pipetting troughs or boats.
8. Disinfectant.
9. Reagent grade water.
10. Disposable gloves.
11. Timer.
12. Biohazard waste container.
13. Serological pipettes.

## SAMPLE COLLECTION

1. Collect blood specimen by venipuncture according to the standard procedure.
2. Serum or plasma can be used.
3. Specimen should be free of particulate matter and microbial contamination.
4. Preferably use fresh sample. However, specimen can be stored refrigerated for short duration. For long storage, freeze at -20° C or below. Do not freeze samples in frost-free freezer.
5. Specimen should not be frozen and thawed repeatedly.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.

## PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. Dispose off all the reagents and material used as they contain infectious agent.
8. Neutralize acid containing waste before adding hypochlorite.

9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.
14. Do not let the dispensing tip of ELISA washer touch liquid in the wells.
15. Incubation times and temperatures other than those specified may give erroneous results.
16. Samples must remain confined to microwells during testing. Cross contamination of reagents or samples may give erroneous results.
17. Microwells strips must be tapped vigorously and blotted on absorbent paper or towels to minimize residual wash buffer. Inadequate removal of residual wash buffer can cause inconsistent color development.
18. Adequate washing of wells is extremely important. Inadequately washed wells will exhibit high background values and may show false positive values. For manual washing, aspirate the contents of the wells and then fill each well with the diluted wash buffer solution. Drain off all the wash buffer solution from the wells by inverting and then shaking residual wash buffer from the wells with a sharp 'flicking' of the wrist. Repeat these steps for six washes. The wells should then be firmly tapped on a absorbent paper or towel to remove all traces of residual wash buffer.

## REAGENT PREPARATION

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

| No. of Strips | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 50 X Enzyme Conjugate | 20 µl | 40 µl | 60µl | 80 µl | 100 µl | 120µl | 140 µl | 160µl | 180 µl | 200 µl | 220µl | 240µl |
| Conjugate Diluent | 980 µl | 1960 µl | 2940µl | 3920 µl | 4900 µl | 5800µl | 6860 µl | 7840µl | 8820 µl | 9800 µl | 10780µl | 11760µl |

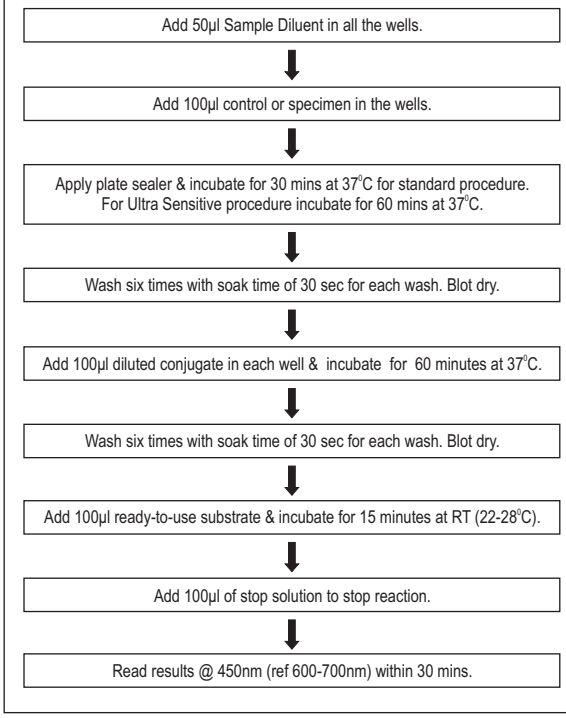
## TEST PROCEDURE

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 ELISA protocol sheet indicating the location of controls and specimen.
4. Use controls in duplicate.
5. Add 50µl Sample diluent in each well.
6. Add 100µl control or specimen in separate wells.
7. Apply plate sealer and incubate for 30 minutes at 37º C for Standard Procedure & 60 minutes for Ultra-sensitive procedure.
8. Wash each well Six times by filling approximately 350 µl diluted wash buffer, giving 30 seconds soak time for each wash and blot dry.
9. Add diluted 100µl Agglutinating sera for Dengue NS1- HRP conjugate in each well and incubate for 1 hour at 37º C.
10. Wash six times as in step 8. Blot dry.
11. Add 100µl substrate in each well and incubate for 15 minutes at room temperature (22-28°C) away from direct light.
12. Stop reaction by adding 100µl stop solution. The stop solution should be added in the same sequence as substrate addition.
13. Read the absorbance at 450 nm with 600-700 nm as reference within 30 minutes of stopping the reaction.

## RUN CRITERIA

1. The individual absorbance value of negative controls should be less than 0.1.
2. The individual absorbance value of positive controls should be more than 1.0.
3. If the test run does not meet above criteria, test run is invalid and should be repeated.

**QUALISA ™ Dengue NS1 Procedural flow chart**



## CALCULATIONS

The cut-off value (COV) is calculated by adding 0.3 to average absorbance value of negative control.
Example: **COV = Avg.NC + 0.3**

| Absorbance of Negative Control (NC) | |
|---|---|
| Reading - 1 | 0.038 |
| Reading - 2 | 0.040 |
| Average NC reading | 0.039 |

| Absorbance of Positive Control (PC) | |
|---|---|
| Reading - 1 | 2.440 |
| Reading - 2 | 2.585 |
| Average PC reading | 2.512 |

Cutoff value = Avg NC + 0.3 = 0.039 + 0.3 = 0.339

## SAMPLE DATA

| Well | Absorbance | Mean | Cutoff | Result |
|---|---|---|---|---|
| NC | 0.038 | 0.039 | 0.339 | |
| NC | 0.040 | | | |
| PC | 2.440 | 2.512 | | |
| PC | 2.585 | | | |
| Sample 1 | 2.236 | | | Reactive |
| Sample 2 | 0.902 | | | Reactive |
| Sample 3 | 0.22 | | | Non-Reactive |

## INTERPRETATION OF RESULTS

1. Samples with absorbance values less than the cutoff value are considered non reactive by **QUALISA™ Dengue NS1** and are considered negative for Dengue NS1 antigen.
2. Samples with absorbance value equal to or greater than cutoff value are considered reactive.
3. In case of samples with high OD, there are possibilities of black precipitate formation after the addition of stop solution. This will not interfere with the interpretation of results.

## NOTE

All ELISA readers have a linear measuring range (approx 2.5Å). Beyond this range the OD values are non linear. That is why some instruments can be pre-programmed to show 'out of range' values if the OD exceeds 2.5 or 3. **QUALISA ™ Dengue NS1** positive control may show 'out of range' values in such instruments even after dilution. Please note that this indicates a valid run (provided the negative control value is < 0.1) and does not interfere with the assay results.

## PERFORMANCE CHARACTERISTICS

| Specimen Data | Total | QUALISA™ Dengue NS1 | Commercially available ELISA |
|---|---|---|---|
| Number of specimen tested | 122 | 122 | 122 |
| Number of Suspected Dengue Specimens | 19 | 19 | 19 |
| Number of negative specimen | 100 | 99 | 99 |

Sensitivity of **QUALISA™ Dengue NS1** : 100%
Specificity of **QUALISA™ Dengue NS1** : 99%

## LIMITATION OF THE PROCEDURE

- **QUALISA™ Dengue NS1** kit alone cannot be used to diagnose infection by Dengue virus, clinical correlation is required.
- NS1 antigen is an in-vitro diagnostic marker for Dengue diagnosis in early phase only. Clinical diagnosis can be established only by physician. A negative result does not preclude the possibility of exposure or infection with the Dengue virus.
- Patient should be re-tested after 2-4 days with other diagnostic markers in case of clinically non-correlated results.
- Serological cross reactivity across the other flavi-virus group may occur in certain cases.

## BIBLIOGRAPHY

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