Instructions for Use

# Zika Virus Total Antibody Detection Assay Development Kit

Reagents for the identification and qualitative measurement of Zika specific antibodies.

3 x 96 well plates


ELS61258

Read the documentation

For Research Use Only

# CONTENTS

## 1. INTRODUCTION

Zika virus (ZIKV) is a member of the virus family Flaviviridae and the genus *Flavivirus*, transmitted by Aedes mosquitoes, such as *A. aegypti* and *A. albopictus*. Its name comes from the Zika Forest of Uganda, where the virus was first isolated in 1947. Zika virus is related to Dengue, Yellow Fever, Japanese Encephalitis, and West Nile viruses, and many antibodies (e.g., those produced as a response to infection) cross-react between these viruses. This cross-reaction causes severe problems in identifying individuals seropostive for Zika as the much wider spread background of Dengue infections cause many false positives in most standard serological ELISAs.

The Zika virus antibody assay development kit uses an in-house manufactured Zika virus-specific antigen to virtually exclude cross reactions and is thus suitable for the accurate detection of serum antibodies to Zika virus, indicating exposure. The detection of Zika specific antibodies is relevant for epidemiological studies, particularly in populations with high prevalence of Dengue and Chikungunya virus.

## 2. INTENDED USE

This Zika virus total antibody assay development kit is designed to aid in the development of assays for the detection of Zika-specific antibodies in human serum. It is minimally cross-reactive with antibodies to Dengue virus (a closely-related flavivirus), and so can be used to develop assays which can distinguish human anti-Zika antibodies from other flavivirus and infectious disease antibodies, especially in epidemiological studies. Use of a Zika antibody assay is intended for detection of IgG in convalescent sera, although IgM/IgA are also detected earlier on in the infection cycle.

This product is FOR RESEARCH USE ONLY, and is not for use in diagnostic procedures.

## 3. PRINCIPLE OF THE ASSAY

The assay uses a Zika virus specific antigen coated microtitre plate. In the first reaction step, the diluted samples and controls are added in the coated wells, followed by addition of biotin labeled Zika virus specific antigen in step two. In case of a positive sample the antibodies are captured forming a double antigen sandwich. The third step of the assay requires addition of streptavidin-HRP to probe the biotin labeled antigen. Finally, an enzyme substrate is added to the wells to give a colored product. The reaction is stopped after specified time with an acid solution and the colorimetric change is determined for each well at 450/620nm or 450nm using an ELISA plate reader.

## 4. MATERIALS

### 4.1. Reagents supplied

This kit contains 5 reagents comprising a positive control, a negative control, a differential control, a recombinant Zika NS1 protein and a recombinant Zika NS1 protein conjugated to biotin.

**(1) Negative Control (ELS61258-NEG):** 1 vial containing 120µl foetal bovine serum non-reactive towards Zika NS1. Contains Proclin.

**(2) Positive Control (ELS61258-POS):** 1 vial containing 120µl foetal bovine serum, spiked with high level anti-Zika NS1 monoclonal antibody. Contains Proclin.

**(3) Differential Control (ELS61258-DIF):** 1 vial containing 120µl foetal bovine serum, spiked with low level anti-Zika NS1 monoclonal antibody. Contains Proclin.

**(4) Recombinant Zika virus NS1 protein (ZIKVSU-NS1-036):** 1 vial containing 36µg recombinant Zika NS1 protein – see label for antigen concentration. Total quantity/volume required per 96-well plate is 12µg in 12 ml (1µg/ml).

**(5) Biotin-labelled Zika NS1 Protein (ZIKVSU-NS1-BIO) 100X:** 1 vial containing 0.36ml recombinant biotin labelled Zika NS1 protein with BSA and Proclin. Total quantity/volume supplied sufficient for 36mls at working strength.

### 4.2. Materials and Equipment needed

- High-binding flat bottom microtitre plate – Greiner 762071 or equivalent
- BSA – Sigma A7906 or an equivalent grade
- Tween 20 – Sigma P1379 or equivalent grade
- DPBS pH 7.2 – GIBCO Catalogue no. 14190-136 or equivalent
- Pierce Poly Streptavidin HRP – Thermofisher Catalogue no. 21140 or equivalent
- TMB Substrate – Catalogue no. MO701A, Europa Bioproducts or equivalent
- Stop solution (1M HCl or 1N $H_2SO_4$).
- Microwell plate reader, equipped for the measurement of absorbance at 450nm

## 5. STABILITY AND STORAGE

Store the kit frozen at -20°C or below.
*Freeze in small aliquots to minimize damage due to freezing and thawing.*

## 6. ASSAY PROCEDURE

### 6.1. Reagent Preparation

Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. Perform all assay steps in the order given and without any delays. A clean, disposable tip should be used for dispensing each standard/control and sample.

Required buffers and diluents: The following buffers and diluents are not included and must be prepared fresh by the user (prepared buffers & diluents may be stored frozen -20°C for re-use):

- Coating buffer: Dulbecco's Phosphate Buffered Saline (DPBS)
- Blocking solution: DPBS + 3%BSA (w/v)
- Wash buffer: DPBS + 0.1% Tween20 (v/v)
- Dilution buffer: DPBS + 0.5%BSA (w/v) + 0.2% Tween20 (v/v)

Prior to use on day required, thaw the reagent(s) completely at ambient temperature (18-28°C). Mix liquid reagents by repeated gentle inversion or using a roller mixer prior to use.

*It is recommended to briefly centrifuge reagent tubes after mixing to collect material from the walls and cap.*

Biotin labelled Zika NS1 protein conjugate 100X concentrate:

Dilute the 100X conjugate to 1X working concentration using dilution buffer:

| **No. of strips** | **2** | **4** | **6** | **8** | **10** | **12** |
|---|---|---|---|---|---|---|
| 100X Zika NS1 biotin conjugate (µl) | 20 | 40 | 60 | 80 | 100 | 120 |
| Dilution buffer (µl) | 1980 | 3960 | 5940 | 7920 | 9900 | 11880 |

Mix thoroughly by roller mixing for 5-10 minutes.

Negative and positive controls: Dilute each control 1:101 (For example, 5µl control + 500µl dilution buffer, total volume equals 505µl) in dilution buffer. Mix thoroughly before adding it to the microwells.

Differential control: For diluting the differential control refer to the dilution factor indicated on the label. Use dilution buffer as diluent. Mix thoroughly before adding it to the microwells.

Samples: Samples that show a precipitate or are highly lipaemic should be centrifuged to clarify the sample as far as possible before use. Perform a 1:101 dilution of the samples (For example, 5µl sample + 500µl dilution buffer, total volume equals 505µl); ensure that the diluted samples are thoroughly mixed.

### 6.2. Assay Protocol

Prepare a plate plan to include duplicate wells for negative, positive, and differential controls, plus all samples required (see suggested plate layout, below).

Day 1:

1. Coat the required wells of a 96-well plate with 1µg/ml of Zika NS1 antigen using DPBS coating buffer. Add 100µl of the prepared coating solution in all the wells. Seal and incubate the plate overnight static at ambient temperature (18-28°C).

Day 2:

2. Aspirate the plate contents. Gently tap once on the paper towel to blot off any remainder of the coating solution. Add 3%BSA/PBS blocking solution, 300µl/well to block the plate. Incubate for 1-2 hours static at ambient temperature (18-28°C).
3. Aspirate the plate contents, add wash buffer 300µl per well and perform 3 wash/empty cycles, then invert the plate onto an absorbent paper and pat away any excess wash buffer.
4. Add 100µl of diluted controls/samples as per the plate plan. Seal and incubate the plate for 30 minutes at ambient temperature (18-28°C), shaken at 800rpm.
5. After 30 minutes of incubation, aspirate the plate contents, taking any safety precautions as required by sample type. Add wash buffer 300µl per well and perform 4 wash/empty cycles, then invert the plate onto an absorbent paper and pat away any excess wash buffer.
6. Add 100µl of diluted Zika NS1 biotin conjugate (1X) in every well, seal the plate and incubate for 30 minutes at ambient temperature (18-28°C) at 800rpm.
7. Aspirate the plate contents, add wash buffer 300µl per well and perform 5 wash/empty cycles, then invert the plate onto an absorbent paper and pat away any excess wash buffer.
8. Add 100µl of diluted poly streptavidin HRP (1:20,000) in all the wells, seal the plate and incubate for 30 minutes at ambient temperature (18-28°C) at 800rpm.
9. Aspirate the plate contents, add wash buffer 300µl per well and perform 5 wash/empty cycles, then invert the plate onto an absorbent paper and pat away any excess wash buffer.
10. Add 100µl of TMB substrate in all wells. Cover the plate with a new plate sealer and incubate the plate for 15 minutes at ambient temperature (18-28°C), 800rpm. The plate should be protected from direct sunlight.
11. After 15 minutes, add 100µl of stop solution to every used well. Read the plate at 450nm/620nm (or 450nm) using an ELISA plate reader within 10 minutes of stopping the reaction.

### 6.3. Plate Layout

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | **NC** | **NC** | S6 | S6 | S14 | S14 | S22 | S22 | S30 | S30 | S38 | S38 |
| B | **PC** | **PC** | S7 | S7 | S15 | S15 | S23 | S23 | S31 | S31 | S39 | S39 |
| C | **DC** | **DC** | S8 | S8 | S16 | S16 | S24 | S24 | S32 | S32 | S40 | S40 |
| D | S1 | S1 | S9 | S9 | S17 | S17 | S25 | S25 | S33 | S33 | S41 | S41 |
| E | S2 | S2 | S10 | S10 | S18 | S18 | S26 | S26 | S34 | S34 | S42 | S42 |
| F | S3 | S3 | S11 | S11 | S19 | S19 | S27 | S27 | S35 | S35 | S43 | S43 |
| G | S4 | S4 | S12 | S12 | S20 | S20 | S28 | S28 | S36 | S36 | S44 | S44 |
| H | S5 | S5 | S13 | S13 | S21 | S21 | S29 | S29 | S37 | S37 | S45 | S45 |

*NC – Negative control, PC – Positive control, DC – Differential control

## 7. RESULTS

### 7.1. Result Calculations

Calculate the Ratio Value (RV) for each sample by dividing the mean optical density (OD) value for each sample by the mean OD of the differential control:

$$\text{Ratio Value (RV)} = \frac{\text{Average optical density of sample or control}}{\text{Average optical density of differential control}}$$

### 7.2. Assay Performance Criteria

In order for the assay to be considered valid, the following criteria must be met:

- Negative control: The mean absorbance value of negative controls must be less than the mean differential control value
- Differential control: The mean absorbance value should be between 0.070 and 0.900 inclusive
- Positive control: The mean absorbance value of positive controls must be higher than the mean differential control value

*If these criteria are not met, the test is not valid and must be repeated*

### 7.3. Interpretation of Results

| Ratio Value (RV) | Result | Interpretation |
|---|---|---|
| < 0.25 | Negative | Negative for Zika Antibodies |
| ≥ 0.25 but ≤ 0.4 | Indeterminate | Positive for flavivirus antibodies |
| > 0.4 | Positive | Positive for Zika Antibodies |

Note: Indeterminate samples are to be considered positive for flavivirus antibodies, but may either be low levels of Zika antibodies, or very high levels of related flavivirus antibodies, most likely Dengue.

### 7.4. Example Data

This data is an example of the data typically produced with this assay procedure. However, the results obtained by users may not be identical to these. This data is intended as an example only and must not be used to calculate your sample values.

| Sample I.D/Control | O.D. 450/620nm | Ratio Value (RV) | Result |
|---|---|---|---|
| **Differential control** | 0.198 | - | - |
| **Negative control** | 0.012 | 0.06 | Negative |
| **Positive control** | 1.336 | 6.74 | Positive |
| **CDC Zika p23-L** | 1.349 | 6.81 | Positive |
| **CDC Zika p23-I** | 1.000 | 5.04 | Positive |
| **Zika sample nnnnnnnn 01** | 0.753 | 3.80 | Positive |
| **Zika sample nnnnnnnn 04** | 1.712 | 8.64 | Positive |
| **Zika sample nnnnnnnn 07** | 0.233 | 1.18 | Positive |
| **Dengue sample nnnnnnnn 20** | 0.023 | 0.11 | Negative |
| **Dengue sample nnnnnnnn 19** | 0.021 | 0.10 | Negative |
| **Dengue sample nnnnnnnn 16** | 0.015 | 0.07 | Negative |
| **Chikungunya 465 positive sample** | 0.016 | 0.08 | Negative |
| **WestNile positive sample** | 0.010 | 0.05 | Negative |

## 8. PRECAUTIONS AND WARNINGS

- Products are for Research Use or for Further Manufacturing Use only. Not for Diagnostic or Therapeutic Use.
- This kit is only designed for qualified personnel who are familiar with good laboratory practices.
- For potential hazardous substances, please check the safety data sheets.

## 9. ORDERING INFORMATION

Prod. No.: ELS61258 Zika Virus Total Antibody Detection Assay Development Kit