ROBOSCREEN DIAGNOSTICS

Instructions for Use

DENV IgG ELISA

Rev. 2/2020

CE

IVD

Order Number:

847-0108000106	96 reactions

Manufacturer:

Roboscreen GmbH
Hohmannstraße 7
04129 Leipzig
Germany

Phone: +49 341 989734 0
Fax: +49 341 989734 199

www.roboscreen.com
info@roboscreen.com

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Contents

1 Introduction

1.1 Intended use

The Dengue virus specific IgG ELISA is intended for the detection of IgG antibodies in response to a dengue virus infection in human serum and plasma samples. This ELISA is a confirmation test for DENV infection in patients. The Dengue virus specific IgG ELISA is not a screening test.

1.2 Warranty and technical support

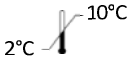
The manufacturer guarantees the correct functioning of the kit for the applications described in the instructions for use (IFU). During the warranty period, DENV specific IgG ELISA allows for precise and reproducible data collection in connection with excellent sensitivity. Any warranty claims shall only be valid if the general principles of Good Laboratory Practice (GLP) and the manufacturer's recommendations are observed.

To improve the application and design, Roboscreen GmbH reserves the right of product replacement or modification. The manufacturer may be contacted at any time for questions and problems or technical support concerning the detection of DENV IgG antibodies in human serum and plasma samples.

CONSULT INSTRUCTIONS FOR USE
This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.3 Notes on the use of this instruction for use

For easy reference and orientation, the IFU uses the following warning and information symbols as well as the shown methodology:

	REF Catalogue number
	Content Contains sufficient reagents for <N> tests
	Storage conditions
	Consult instructions for use
	Expiry date
	Manufactured by
	For single use only

The following abbreviations are used in the IFU:

DENV	Dengue virus
ELISA	Enzyme-linked immunosorbent assay
GLP	Good Laboratory Practice
HRP	Horseradish peroxidase
IFU	Instruction for use
OD	Optical density
RT	Room temperature (21.5 ± 3.5 °C)
TMB	Tetramethylbenzidine

2 Safety precautions

We recommend reading this chapter thoroughly before using this kit, to ensure the safety of the user and error-free utilization.
Any safety instructions and additional information of this IFU must be observed at all times.

Read and make sure you understand the operating instructions completely and thoroughly before carrying out the test. Use the currently valid version from the kit.

Notify the respective supplier in writing within one week from receiving the merchandise, should the test pack be substantially damaged. Damaged components must not be used to carry out the assay, however, they should be kept until the transport damages are finally settled.

Comply with Good Laboratory Practice and safety regulations. Wear laboratory coats, disposable Latex gloves and safety goggles whenever the need arises.

Reagents of this kit which contain hazardous substances may cause irritations to eyes and skin. See indications under *5 Kit components, storage and expiry date* and on the labels. Safety data sheets of this product are available upon request.

Chemicals and prepared or used reagents shall be disposed of as hazardous waste in compliance with the respective national regulations. The cleaning staff has to be instructed by experts with regard to any potential risks and the appropriate handling of such substances.

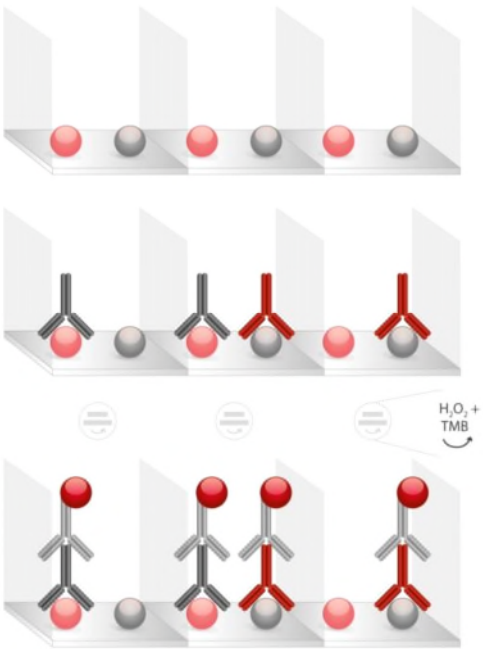
Avoid any contact with stop solution. This may cause irritations to the skin and chemical burns.

FOR SINGLE USE ONLY!
This kit is made for single use only!

ATTENTION!
Don’t eat or drink components of the kit!
The kit shall only be handled by educated personnel in a laboratory environment!

3 Test principle

The Dengue virus specific IgG ELISA is a sensitive immunoassay using specific antigen coated ELISA strips for binding of DENV serotype 1-4 (DENV 1-4) specific human IgG antibodies. Antibodies reacting to ZIKA virus are blocked by a specific blocking reagent (Rockstroh A, 2017). Bound human antibodies to DENV are detected using anti-human IgG HRP conjugate. Amount of bound conjugated HRP antibody is estimated using chromogenic substrate tetramethylbenzidine (TMB). Controls are included for the proof of reproducibility and evaluation of the assay within labs. This patented (WO2015/139784) serological assay was developed by the Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany. Due to DENV-specific antigen-coating of the immunostrips and use of specific inhibitors, unspecific cross-reactivity to heterologous arboviruses like ZIKA virus, West Nile virus (WNV), Yellow Fever virus (YFV), Chikungunya virus (CHIKV) or Tick-borne encephalitis virus (TBEV) is strongly reduced (Rockstroh A, 2015).



1. Ready for use: Immunostrips coated with DENV antigens of serotype 1-4.

2. Blocking of cross-reacting anti-ZIKA antibodies and specific binding of anti-DENV-IgG antibodies to the coated DENV-antigens.

3. Detection of bound IgG by HRP-conjugated anti-human IgG antibody + TMB/Peroxide.

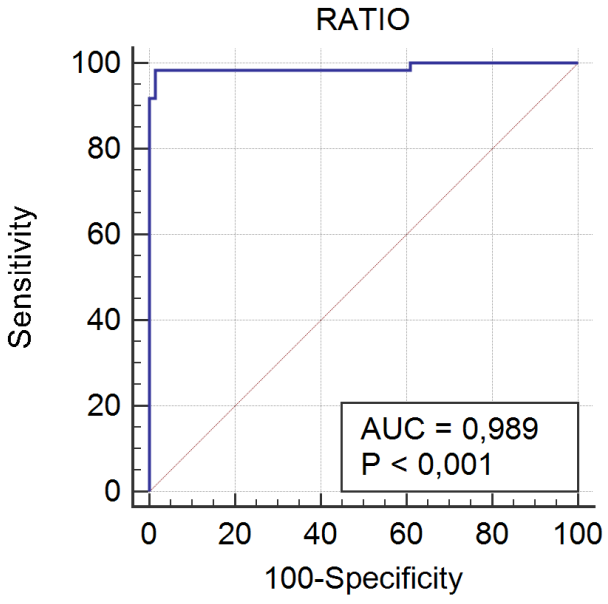
4 Performance assessment

Results of analytical validation

Reproducability of Dengue virus specific IgG ELISA was carried out on one DENV positive sample with 3 semi-logarithmic dilution stages and on two patient samples with a pre-dilution of 1:100 in Assay buffer with 6-fold repeat on 6 different immunostrips D1. The measurement at the optical density at 450-620 nm was evaluated. The ELISA showed a very small variation <10% over all measurements.

Results of clinical validation

130 clinical samples were included in the validation, of which 61 were samples from demonstrably DENV-infected patients and 69 samples from patients without DENV infection. The Dengue virus specific IgG ELISA showed a sensitivity of 98.4 % with a specificity of 98.6 %. The area under the ROC-curve (AUC) was 98.9 % (p<0.001).



5 Kit components, storage and expiry date

5.1 Kit components

Component	Σ 96	Description
D1 Immunostrips	12 x 8	Coated immunostrips containing DENV 1-4 antigen, blocked and stabilized. Ready to use.
D2 40X Wash buffer	1 x 50 ml	40X Wash buffer containing PBS, detergents and sodium merthiolate.
D3 Positive Control	3	Lyophilized positive control for test verification; contains Proclin 300
D4 Negative Control	3	Lyophilized negative control for test verification; contains Proclin 300
D5 10X HRP Anti-human IgG	1.5 ml	Horseradish peroxidase (HRP) conjugated anti-human IgG antibody; contains Kathon.
D6 Assay buffer	50 ml	Assay buffer containing PBS, detergent and Proclin 300.
D7 Calibrator	3	Lyophilized calibrator for OD-calibration of samples; contains Proclin 300.
D8 ZIKA Inhibitor	3	Lyophilized inhibitor for reduction of cross-reactivity to ZIKA antibodies; contains Proclin 300.
D9 Staining solution	20 ml	TMB/peroxide solution. Ready to use.
D10 Stop solution	25 ml	1 M sulphuric acid. Ready to use.

Component	Σ 96	Description
BR Blocking reagent	3	Protein-based blocking reagent.
Sealing tape	3	
Instruction for use	1	

5.2 Storage and expiry date

The kit is delivered at ambient temperature and should be stored at 6 ± 4 °C. Protect from heat and direct sunlight. Under these conditions, the kit has a shelf life as indicated on the kit box while retaining its endurance and stability.

Prepared kit components have the following expiry dates:

Component	Preparation step	Expiry date
D1	Coated immunostrips after opening of bag, taking out strips and closing of bag.	At 6 ± 4 °C up to 4 weeks.
D2	Ready-to-use 1X wash solution.	At 6 ± 4 °C up to 1 week.
D6+BR	Assaybuffer D6 containing blocking reagent.	At 6 ± 4 °C up to 1 week.
D6+BR+D8	Assaybuffer D6 containing blocking reagent and ZIKA inhibitor	At 6 ± 4 °C up to 4 h.
D3, D4, D7	Reconstituted controls and calibrator.	At 6 ± 4 °C up to 4 h.
D5	Ready-to-use 1X HRP conjugate.	At 6 ± 4 °C up to 4 h.

6 Components not included in the kit:

- Calibrated micropipettes with CV < 3 %
  Volume: 0.5-10 µl; 10-100 µL; 100-1000 µL
- Calibrated 8-channel micro-pipette with reagent reservoirs
- Vortex mixer
- Automated or semi-automated ELISA plate washing system
- Bidistilled or deionized water
- Paper towels, pipette tips and timer
- ELISA plate reader for reading absorbance at 450 and 620 nm
- Tubes or plates for sample dilution
- Tubes (10-50 ml) for preparation of components

7 Preparation of components

Ready-to-use Components

Immunostrips **D1**, staining solution **D9** and stop solution **D10** are ready-to-use components.

Components to be prepared

Dilution of 40X wash buffer **D2** with deionized/ bidistilled water in ratio 1:40
➢ **1X wash buffer**

⇩

Mixing of room tempered assay buffer **D6** with blocking reagent **BR**
➢ **D6+BR**

⇩

Dissolving of ZIKA inhibitor **D8** in **D6+BR**
➢ **D6+BR+D8**

⇩

Dissolving of controls **D3** and **D4** and calibrator **D7** in **D6+BR+D8**.
Dilution of **samples** with **D6+BR+D8**.
&
Incubation of samples, controls and calibrator for 60 min at RT.

⇩

Immunoassay

7.1 1X Wash solution

Mix 40X wash buffer **D2** by inversing 2-3 times before use. Dilute 40X wash buffer **D2** using bidistilled or deionized water before the first wash step of the immunoassay (see table below).

Number of immunostrips	Volume of 1X Wash solution	Volume of 40X Wash buffer D2	Volume of bidistilled or de-ionized water
1-4	400 ml	10 ml	390 ml
5-8	800 ml	20 ml	780 ml
9-12	1200 ml	30 ml	1170 ml

7.2 Assay buffer + Blocking reagent – D6+BR

For reduction of unspecific background signals assay buffer **D6** should be mixed with **blocking reagent**. For this purpose fill 15 ml of room tempered **D6** into the bottle containing **blocking reagent** and mix it intensively by vortexing up to 30 s.

For need of volumes > 15 ml pooling of several bottles is recommended as described below.

Number of immunostrips	Volume of D6+BR needed	Number of bottles with blocking reagent	Volume of assay buffer D6
1-4	1-15 ml	1	1 x 15 ml
5-8	15-30 ml	2	2 x 15 ml
9-12	30-45 ml	3	3 x 15 ml

7.3 Assay buffer with Blocking reagent (D6+BR) + ZIKA Inhibitor – D6+BR+D8

The use of the ZIKA inhibitor **D8** is recommended to completely suppress potential cross-reactions in human samples. The appropriate amount of **D8** tubes necessary for the planned number of immunostrips shell be prepared according to the table below.

At first, add 1 ml of **D6+BR** to a tube of **D8** and vortex for 2-3 s. Subsequently transfer the reconstituted **D8** in a 10 ml tube and add additional 7 ml **D6+BR** resulting finally in 8 ml **D6+BR** containing anti-Zika antibody blocking reagent (**D6+BR+D8**).

For need of volumes > 8 ml use more tubes of D8 and pool the prepared D6+BR+D8 solutions as described below.

Number of immunostrips	Volume of D6+BR+D8 needed	Number of D8 tubes	Volume of D6+BR
1-4	8 ml	1	1x (1 + 7 ml)
5-8	16 ml	2	2x (1 + 7 ml)
9-12	24 ml	3	3x (1 + 7 ml)

NOTE
Use **D6+BR+D8** for reconstitution of **D3**, **D4** and **D7** and for dilution of samples (see *8 Immunoassay, Sample preparation*)

7.4 Controls D3, D4 and Calibrator D7

Add **1.0 ml** of **D6+BR+D8** to each tube of **D3**, **D4** and **D7** and mix quickly, by vortexing 2-3 s.

7.5 1X HRP anti-human IgG antibody

NOTE
Use **D6+BR** for dilution of 10X HRP Anti-human IgG Antibody **D5**.

The required amount of 1X HRP anti-human IgG antibody is prepared according to the number of immunostrips used as described in the table below. Therefore, dilute 10X HRP Anti-human IgG **D5** with **D6+BR** in ratio 1:10 and mix by means of shaking the tube.

Number of immunostrips	Volume of 10X HRP D5	Volume of D6+BR
1 - 4	0.4 ml	3.6 ml
5 - 8	0.8 ml	7.2 ml
9 - 12	1.2 ml	10.8 ml

8 Immunoassay

8.1 Sample preparation

- Allow samples to reach 18 - 25 °C before use. For samples stored frozen keep them at 18 - 25 °C at least 30 min before pipetting onto plate.
- Mix samples before use by vortexing for 6-10 s.
- **Dilute samples 1:100** (v/v) using **D6+BR+D8** within separate tubes or a 96 well dilution plate.

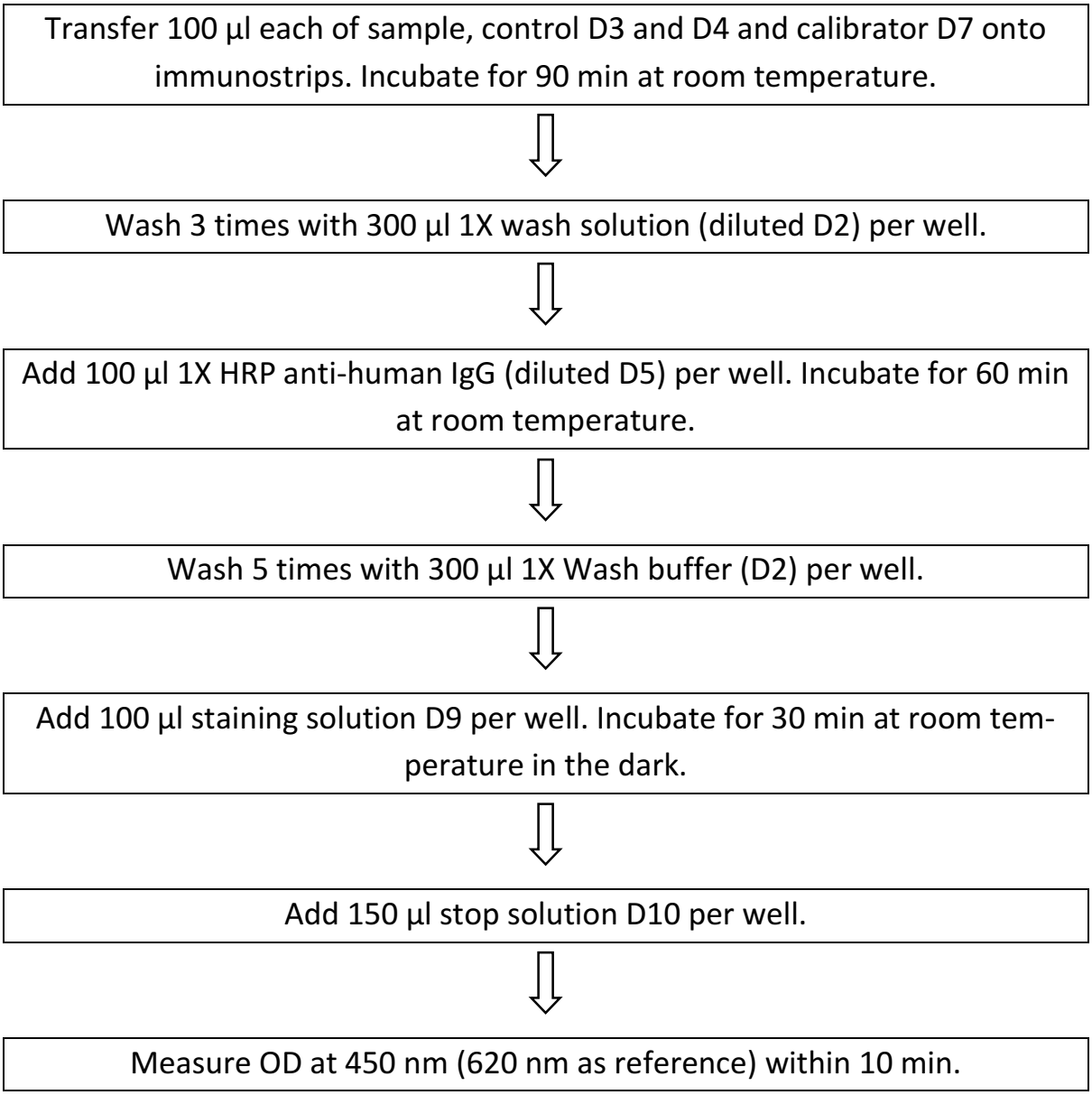
8.2 Reconstitution of reagents

- Reconstitute Controls **D3** and **D4** and calibrator **D7** before Blocking of anti-ZIKA antibodies in samples (see 8.3).

8.3 Blocking of anti-ZIKA antibodies in samples

- **Incubate** diluted **samples**, reconstituted controls **D3, D4** and calibrator **D7** for **60 min at RT** to block possibly contained anti-ZIKA antibodies.

9 Immunoassay protocol



Immunoassay procedure

1. Transfer 100 µl of each 1:100 diluted and for 60 min pre-incubated sample from dilution plate onto immunostrips. Pipetting of duplicates of each sample is recommended.

2. Transfer 100 µl of each reconstituted and pre-incubated control **D3** and **D4** and calibrator **D7** onto immunostrips. Pipetting of duplicates is recommended.

NOTE
Change the pipette tips between the different samples and between the controls and the calibrator.

3. Cover the strips with sealing tape and incubate at RT for 90 min.

4. Remove cover and wash **3** times with 300 µl Wash buffer manually or by use of a plate washer.

NOTE
Pull off the sealing tape carefully to avoid cross-contamination.

5. Transfer 100 µl of 1:10 diluted HRP conjugate **D5** into each well.

6. Cover the strips with sealing tape and incubate at RT for 60 min.

7. Remove cover and wash **5** times with 300 µl wash buffer manually or by use of a plate washer.

NOTE
Staining should be performed immediately after washing step 7 within 5 min.

8. Staining: Add 100 µl of staining solution **D9** into each well and incubate for 30 min at RT in the dark.

9. Terminate staining by pipetting of 150 µl stop solution **D10** into each well.

10. Measurement of absorbance: Mix plate with shaker of the reader for 3-5 s and let it settle down for 5 s. Measure the OD at 450 nm using 620 nm as reference wave length within 10 minutes after termination of the reaction.

NOTE
In high concentrated controls or samples staining components may be precipitated some time after termination. In this case additional mixing before reading is recommended.

10 Data analysis

The OD of the measured values is determined as the difference of the measured OD at 450 nm minus the OD at reference wavelength 620 nm ($OD_{450/620\ nm}$).

Quality criteria of the assay

- $OD_{450/620\ nm}$ value of negative control **D4** (blank) should be **< 0.1**.
- $OD_{450/620\ nm}$ value of positive control **D3** should be **> 0.5**.
- $OD_{450/620\ nm}$ of **D7** is used as **Calibrator** for calculation of results of sample measurement.

Calculation of results

We recommend calibration of $OD_{450/620}$ of each measured sample using $OD_{450/620}$ of calibrator **D7**.

The resulting ratio allows an accurate discrimination between non-infected persons and DENV 1-4 infected patients.

$$\text{Ratio} = \frac{\text{OD sample - OD D4}}{\text{OD D7 - OD D4}}$$

For discrimination, a **cut-off of 3.0** should be used for the ratio. A ratio **≤ 3.0** indicates no IgG antibodies against DENV 1-4. A ratio **> 3.0** indicates IgG antibodies against DENV 1-4 and clearly confirms a dengue virus infection.

Ratio	Interpretation
< 3.0	No evidence of DENV specific IgG antibodies in the sample.
≤ 3.0	Evidence of DENV specific IgG antibodies in the sample.

11 References

Rockstroh A, et al. 2017. Specific detection of dengue and Zika virus antibodies using envelope proteins with mutations in the conserved fusion loop. *Emerg Microbes Infect.* 8. Nov 2017, S. 6(11):e99. doi: 10.1038/emi.2017.87.

Rockstroh A, et al. 2015. Recombinant Envelope-Proteins with Mutations in the Conserved Fusion Loop Allow Specific Serological Diagnosis of Dengue-Infections. *PLoS Negl Trop Dis.* 13. Nov 2015, S. 9(11):e0004218. doi: 10.1371/journal.pntd.0004218. eCollection 2015 Nov.