Varicella-Zoster-Virus IgG ELISA

Enzyme immunoassays for the quantitative determination of IgG antibodies against Varicella-Zoster-Virus in human serum and plasma.

REF RE58271

12x8

2-8 °C

EU: For research use only. U.S.: For research use only. Not for use in diagnostic procedures.
2. INTENDED USE
The Varicella Zoster Virus IgG EIA kit is an enzyme immunoassay for the detection of Varicella Zoster Virus specific IgG antibodies in human serum and is used as an aid in the diagnosis of Varicella Zoster Virus infections and for the detection of immunity. The assay must be performed strictly in accordance with the instructions set out in this instructions for use. No responsibility can be held for any loss or damage (except as required by statute) how so ever caused by or arising out of non-compliance with the instructions provided.

3. INTRODUCTION
Varicella Zoster Virus (VZV) belongs to the Herpes virus group, which also includes Herpes Simplex Virus (HSV) types 1 and 2, Cytomegalovirus (CMV) and Epstein-Barr virus (EBV). A common characteristic of these viruses is that they can cause latent infections. VZV is the etiological agent of two diseases in man: varicella (chicken pox) and herpes zoster (shingles). Varicella is an ubiquitous and common communicable disease. It follows after primary exposure of VZV in susceptible persons, most often in a child, herpes zoster is an endemic sporadic disease appearing most frequent in the elderly people. As varicella is the manifestation of primary infection, herpes zoster is the manifestation of virus reactivation in an infected host after a period of latency usually lasting several decades. Infection with VZV is of particular concern in immunocompromised hosts and in neonates, who are at risk of serious complications. Both primary and reactivated VZV infections elicit antibody responses of different immunoglobulin classes. Crossreactivity between VZV and HSV may exist and interpretation of VZV serological results should be related with clinical data. Heterologous rises, such as a rise in HSV titer in a patient, occur only when the subject has been infected with the heterologous agent. In such cases, titer-rises to the current agent greatly exceed that of the heterologous agent. Serological diagnosis of VZV infections can be performed by testing for the presence of VZV-specific IgG seroconversion or significant titer-rise measured preferably in paired sera drawn in an interval of 10 to 14 days and testing for the present VZV-specific IgA or significant VZV-IgM antibodies. Screening for seropositivity can be performed by testing for the presence of VZV-IgG antibodies in serum.

4. PRINCIPLE OF THE ASSAY
The method for quantitative determination of specific IgG to Varicella Zoster Virus is illustrated below.

1. Well coated with Varicella Zoster Virus antigen is incubated with diluted human serum for 60 minutes at 37°Celsius.
2. After washing with washbuffer antibody conjugated with horseradish peroxidase (conjugate) is added. Incubation for 60 minutes at 37°Celsius.
3. After washing with washbuffer TMB is added. TMB acts as a substrate to peroxidase.
4. By incubating the wells in the dark for 30 minutes at room-temperature the colour of the substrate will turn to blue.
5. The enzymatic conversion of TMB is stopped by adding sulphuric acid, 0.5 M. The optical density is measured with a photometric reading instrument at 450 nanometer.
4.1 Assay principle
The Varicella Zoster Virus IgG EIA is an indirect immunosorbent assay for the detection of Varicella Zoster Virus (VZV) specific IgG in human serum. The VZV antigen (a clinical isolate) is propagated in vitro in human epithelial fibroblasts, purified and inactivated. This VZV antigen is coated to the solid phase of the microtiterstrip wells. This antigen will specifically bind human IgG present in each serum. Anti-IgG labelled to peroxidase (the conjugate) will complex to captured VZV-specific IgG. The conjugate will act as the indicator for the immunological reaction between the human IgG in serum and the VZV specific antigen coated on the wells of the microtiterplate. TMB that acts as a chromogen will induce colour proportionally to the amount of VZV specific IgG bound. By making use of a set of 4 calibrators, the results obtained with each sample can be expressed quantitatively in Arbitrary Units per mL (AU/mL).

5. REAGENTS AND ACCESSORIES

5.1 Reagents provided in the kit
The kit contains the following reagents. A distinction can be made between reagents that are specific for the assay and universal reagents.

<table>
<thead>
<tr>
<th>SYMBOL</th>
<th>CAT. CODE</th>
<th>DESCRIPTION</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL 200 AU</td>
<td>4460-19</td>
<td>Calibrator 200 AU/mL (RED, ready-to-use)</td>
<td>1.3 mL</td>
</tr>
<tr>
<td>CAL 50 AU</td>
<td>4460-15</td>
<td>Calibrator 50 AU/mL (ROSE, ready-to-use)</td>
<td>1.3 mL</td>
</tr>
<tr>
<td>CAL 10 AU</td>
<td>4460-13</td>
<td>Calibrator 10 AU/mL (GREEN, ready-to-use)</td>
<td>1.3 mL</td>
</tr>
<tr>
<td>CAL 0 AU</td>
<td>4460-11</td>
<td>Calibrator 0 AU/mL (YELLOW, ready-to-use)</td>
<td>1.3 mL</td>
</tr>
<tr>
<td>CONJ Ab</td>
<td>9000-46</td>
<td>PO-labelled anti-IgG conjugate (100 x concentrated)</td>
<td>0.25 mL</td>
</tr>
<tr>
<td>MTP Ag</td>
<td>4460-08</td>
<td>Microtiterplate coated with VZV antigen (96 wells)</td>
<td>1 plate</td>
</tr>
<tr>
<td>SOLN TMB</td>
<td>9000-19</td>
<td>TMB Substrate Solution, ready-to-use.</td>
<td>15 mL</td>
</tr>
<tr>
<td>DILAS</td>
<td>9000-03</td>
<td>Dilution buffer (BLUE, ready-to-use)</td>
<td>120 mL</td>
</tr>
<tr>
<td>WASHBUF</td>
<td>9000-06</td>
<td>Wash buffer (10 x concentrated)</td>
<td>60 mL</td>
</tr>
<tr>
<td>H2SO4</td>
<td>9000-08</td>
<td>Stop solution (ready-to-use)</td>
<td>15 mL</td>
</tr>
</tbody>
</table>

5.2 Materials provided with the kit
- Resealable bag, 2 x
- Instructions for use, 1 x
- Certificate of Analysis, 1 x

5.3 Equipment and materials required, but not supplied
- Pipettes to deliver volumes between 10 µL and 1000 µL
- Volumetric laboratory glassware
- Deionised (or distilled) water
- Incubator 37°C
- Clean disposable tubes for diluting patients sera (capacity appr. 3 mL)
- Clean disposable tubes for diluting conjugate and TMB (capacity 12 mL)
- Automatic plate washer (optional)
- Microtiter plate reader, equipped for measuring absorbances at 450 nm (optionally equipped for dual wavelength measurement at 450 and 620 nm)
- Vortex tube mixer
- Timer
6. COMPOSITION AND HANDLING OF REAGENTS

6.1 Specific kit reagents

6.1.1 Microtiterplate
Microtiterplate (96 wells) with 8-well breakable strips coated with Varicella Zoster Virus specific antigen. The strips are ready to use and should be stored at 2-8 °C. After opening replace any unused wells in the resealable plastic bag and store in the kitbox between 2-8 °C. Resealed strips expire after one month.

6.1.2 Calibrator 200 AU/mL
A vial containing 1.5 mL of human serum, highly reactive for IgG against VZV prediluted in PBS buffer, BSA, preservatives and an inert red dye. The VZV-IgG reactivity is set such that this calibrator contains 200 AU/mL. The reagent is ready to use. After use, close cap, replace in the kitbox and store between 2-8 °C. Handled in this way, the calibrator will expire as indicated on the vial label.

6.1.3 Calibrator 50 AU/mL
A vial containing 1.5 mL of buffered solution, reactive for IgG antibodies against VZV prediluted PBS buffer, BSA, preservatives and an inert light-red dye. The VZV-IgG reactivity is set such that this calibrator contains 50 AU/mL. The reagent is ready to use. After use, close cap, replace in the kitbox and store between 2-8 °C. Handled in this way, the calibrator will expire as indicated on the vial label.

6.1.4 Calibrator 10 AU/mL
A vial containing 1.5 mL human serum, with a low reactivity for IgG to VZV prediluted in PBS buffer, preservatives and an inert green dye. The VZV-IgG reactivity is set such that this calibrator contains 10 AU/mL. The reagent is ready to use. After use, close cap, replace in the kitbox and store between 2-8 °C. Handled in this way, the calibrator will expire as indicated on the vial label.

6.1.5 Calibrator 0 AU/mL
A vial containing 1.5 mL human serum, without reactivity for IgG to VZV prediluted in PBS buffer, preservatives and an inert yellow dye. After use, close cap, replace in the kitbox and store between 2-8 °C. Handled in this way, the calibrator will expire as indicated on the vial label.

6.2 Universal reagents

6.2.1 Conjugate
A vial containing 0.25 mL antibody specific for human IgG labelled to peroxidase, PBS buffer, BSA and preservatives. Use only the amount of working strength conjugate needed for the assay-run and keep the concentrated conjugate at 2-8 °C. Handled in this way, the conjugate will expire as indicated on the vial label.

Note: The working strength conjugate cannot be stored and should be used immediately after preparation.

6.2.2 Dilution buffer
The bottle contains 120 mL PBS buffer, proteins, preservatives and an inert blue dye. The reagent is ready to use. After use, close lid, replace in the kitbox and store between 2-8 °C. Handled in this way, the dilution buffer will expire as indicated on the bottle label.

6.2.3 Wash buffer
The bottle contains 60 mL PBS buffer, Tween® 20 and preservatives. The wash buffer is 20 times concentrated. The working concentration must be prepared according to protocol. After use, close lid, replace in the kitbox and store between 2-8 °C. Handled in this way, the wash buffer will expire as indicated on the bottle label. Stability at working concentration is one week at room temperature or one month at 2-8 °C.

6.2.4 TMB substrate (chromogen)
The bottle contains 15 mL TetraMethylBenzidine (TMB) chromogen/substrate solution and is presented in an amber bottle. The TMB solution is at working strength and is ready to use. Take out only the amount of TMB needed for the assay-run. After opening take out the volume needed and immediately re-close the bottle.
The TMB should at all times kept away from direct light, as this can induce the auto-coloration. Always check the colour of the TMB prior to use. The TMB should be clear, colourless or with a very faint blue tinge. The TMB solution should be stored at 2-8°C. Handled in this way, the TMB will expire as indicated on the bottle label.

6.2.5 TMB diluent
The bottle contains 15 mL citrate buffered solution without preservatives. The reagent is ready to use. After use, close lid, replace in the kitbox and store between 2-8 °C. Handled in this way, the diluent buffer will expire as indicated on the bottle label.

6.2.6 Stop Solution
The bottle contains 20 mL 0.5 M sulphuric acid solution. The reagent is ready to use. After use, close cap and replace in the kitbox. Handled in this way, the Stop Solution will expire as indicated on the vial label.

7. COLLECTION, HANDLING AND STORAGE OF SERUM SPECIMENS
Either human serum or plasma may be used. Samples must not be haemolized, nor contain particulate material. To obtain sera for the detection of VZV IgG antibodies, patient blood should be drawn and allowed to clot at room temperature. Centrifuge within one day, transfer the serum into a vial. Sera may be stored at 4°C for up to 7 days. If storage time exceeds 7 days, store at -20°C to -70°C. Avoid repeated freeze-thaw cycles.

8. PROCEDURE

8.1 Washing procedure
Efficient washing is a fundamental requirement of EIA's. It is essential that each washing procedure is carried out with care to obtain reproducible inter- and intra- assay results.

Prepare the washbuffer: mix per 8-well strip 1.5 mL wash buffer (20x) with 28.5 mL distilled water. Alternatively, mix the total volume (60 mL) of the wash buffer (20x) with 1140 mL distilled water.

Both manual washing and washing with an automatic plate washer can be done:

8.1.1 Manual washing
1. Empty the contents of each well by turning the strips in the holder upside down followed by a firm short vertical movement. Keep the strips tightened by pressing the sides of the strip holder.
2. Fill all the wells to the rims (300-350 µL) with rinsing buffer, for instance with a 8-channel pipet. Be aware of carry-over.
3. Turn the strips upside down and empty the wells by a firm short vertical movement.
4. This washing cycle (2 and 3) should be carried out 5 (five) times.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove residual washing solution in the wells.
6. Take care that none of the wells dries out before the next reagent is dispensed. Therefore, proceed immediately with the next step.

8.1.2 Washing with automatic microtiterplate wash equipment
When using automatic plate wash equipment, check that all wells can be aspirated completely, that the washbuffer is accurately dispensed reaching the rim of each well during each washing cycle. The washer should be programmed to execute 5 (five) washing cycles. After the last cycle, remove the washing buffer from the wells by tapping firmly the plate on absorbant towels.

8.2 Assay and reagent preparation procedure

Note: Bring all reagents to room temperature (18-23°C) before assaying. Perform all assay steps in the order given and without any appreciable delays between the steps.
1. **Dilute** patient sera (1+100): mix 1.0 mL dilution buffer (BLUE) with 10 µL patient serum. After each dilution step thoroughly mix with a vortex to ensure adequate mixing. The calibrators are ready to use and need no further dilution.

2. Leave as many wells as needed in the holder. Label appropriately.

   **Note:** *Bring the coated strips to room temperature before opening the pouch to avoid development of condensed water in the wells. Place unused strips in the pouch, securely reseal and store at 2-8°C in the kit-box.*

3. **Dispense** per well 100 µL of the calibrators in duplicate (see scheme). Use 8 wells for calibrators: 200 AU/mL (RED), 50 AU/mL (ROSE), 10 AU/mL (GREEN) and 0 AU/mL (YELLOW). **Dispense** 100 µL of each diluted patient sample (BLUE) into a well.

4. **Incubate** the wells in a second resealable bag or in 100% moist atmosphere for 1 hour at 37°C.

5. **Prepare** working strength anti-IgG-PO conjugate: mix per 8-well strip 1.0 mL dilution buffer (BLUE) with 10 µL anti-IgG-PO conjugate (100x). See scheme below.

<table>
<thead>
<tr>
<th>Number of 8-well strips in use</th>
<th>Dilution buffer (BLUE)</th>
<th>anti-IgG PO Conjugate (100x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 mL</td>
<td>10 µL</td>
</tr>
<tr>
<td>2</td>
<td>2 mL</td>
<td>20 µL</td>
</tr>
<tr>
<td>6</td>
<td>6 mL</td>
<td>60 µL</td>
</tr>
<tr>
<td>12</td>
<td>12 mL</td>
<td>120 µL</td>
</tr>
</tbody>
</table>

6. When incubation has completed, **aspirate** the liquid and **wash** the 8-well strips 5 (five) times with wash buffer according to the washing protocol (see 8.1).

7. **Dispense** 100 µL per well working strength anti-IgG-PO conjugate (BLUE).

8. **Incubate** the strips in the resealable bag or in a 100% moist atmosphere for 1 hour at 37°C.
Note: In case the incubations can not be performed in a 100% moist atmosphere, the background OD levels may rise. In that case it is advised to incubate with 150 µL conjugate per well.

Note: Use only clean disposable containers.

| Dilution scheme for preparation of TMB |

9. **Wash** the strips 5 (five) times with washbuffer according to the washing protocol (see 8.1).

10. **Dispense** 100 µL TMB solution ready-to-use per well.

11. **Incubate** for 30 min at room temperature (18-23 °C), away from direct or intense light.

12. **Add** 100 µL stop solution per well (colour shift: blue ⇒ yellow) in the same order and the same rate as for TMB-substrate

13. **Measure the absorbance** of specimens with a spectrophotometer at 450 nm (optionally with a 620 nm reference filter) within 10 minutes of adding the stop solution.

9. **CALCULATION OF RESULTS**

9.1 Calculations

Calculate the mean absorbance value of the calibrators. The concentration of VZV specific IgG in a patient sample is determined by comparing the absorbance values of patient samples with that of the calibrators. The calibrators have fixed values expressed in AU/mL which represent the reactivity of the sera. Plot the validated mean absorbances of the standards against the fixed AU/mL values into a curve as indicated in the example calibration curve (figure below). The concentration expressed in Arbitrary Units per mL of individual serum sample can now be read by interpolation from this calibration curve. Similarly, a curve fitting program such as spline analysis can also be used to calculate the AU/ml values.

![Example VZV-IgG Calibration Curve](image)

**Note:** Do not use this example calibration curve to read your absorbance results. In each run and each microtiterplate a calibration curve should be produced!
9.2 Validation of test
The following criteria must be met to validate each run. Validation should be based on mean values.

1. Calibrator 0 AU/mL: $OD < 0.6 \times OD$ of the 10 AU/mL calibrator
2. Calibrator 10 AU/mL: $0.150 < OD < 0.500$
3. Calibrator 50 AU/mL: $0.500 < OD < 1.400$
4. Calibrator 200 AU/mL: $OD \geq 1.400$

**Note:** If these criteria are not met, the run should be considered invalid and must be repeated. The ranges for the OD values can be seen as a guide. When OD values of a run are out of the indicated range, the validity ranges given for the calibrators should be considered as the ultimate criteria against which a run is considered valid.

9.3 Interpretation of results

Detection of seropositivity for VZV
To determine seropositivity of VZV by serology interpretation of the concentration in AU/mL of VZV antibodies in a serum is as follows:

<table>
<thead>
<tr>
<th>RESULTS</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>A serum should be considered positive for VZV specific IgG antibodies when the concentration is $\geq 15$ AU/mL. Interpretation needs to be done with care as indicated in section 13, Limitations of the assay.</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>A serum should be considered negative for VZV specific IgG antibodies when the concentration is $&lt;10$ AU/mL. Interpretation needs to be done with care as indicated in section 13, Limitations of the assay.</td>
</tr>
<tr>
<td>EQUIVOCAL</td>
<td>A serum may be considered equivocal, if the VZV IgG concentrations between 10 and 15 AU/mL. In such case it is advised to confirm the results by testing that serum again in duplicate. In the case the repeated result is again equivocal, a second serum should be tested and judged for a change in result.</td>
</tr>
</tbody>
</table>

Detection of infections with VZV
To estimate a VZV infection by serology it is advised to test serum pairs. Also, it is advised to perform a combination of IgG, IgM and IgA testing. The second serum of a pair can be drawn 14-21 days after the first serum is obtained.

**Note:** Each serum pair should be tested at the same day in the same assay to allow interpretation of significant antibody level rises.

If absorbance values of one or both sera in a serum pair exceed that of the 200 AU/mL calibrator, retest both sera at a 1:1000 dilution. To obtain the concentration in AU/mL of a serum with 1:100 dilution, multiply by 10.

$$\text{Concentration (1:100) = Concentration (1:1000) x 10 AU/mL}$$

A significant difference in antibody level between two sera is found when,
1. the first serum is negative and the second serum has a concentration of $> 11$ AU/mL (i.e. seroconversion), or,
2. there is a threefold difference in concentration between the two sera.

**Note:** If the results of a serum pair do not meet the criteria for a significant antibody level difference, it can be concluded that no indication is found for a seroconversion or a significant IgG antibody level rise, although an active or recent VZV infection can NOT be excluded.
10. CHANGES IN PROCEDURE AND PERFORMANCE
IFU 4460 V08: Instructions for Use are changed as follows: Front page: address and Logo are updated. All pages: reference to Meddens is made.

11. SPECIFIC PERFORMANCE CHARACTERISTICS
When the kit is employed according to the instructions given, and the appropriate equipment is used in optimal conditions, the following performances could be reached. The performance of the kit can be expressed by different performance parameters namely assay precision, analytical specificity, diagnostic specificity and diagnostic sensitivity. This section will be completed according to EC-directive 98/79/EC.

12. TRACEABILITY OF CALIBRATORS
The level of the calibrators as presented in this kit, represents the level as used in the clinical trials as shown above. This is organised such that a manufacturer’s working reference is maintained to which manufacturer’s product reference is calibrated. This manufacturer’s product reference is used for validating kit performance. In this way the sensitivity and specificity of each lot represents that as shown above.

13. LIMITATIONS OF THE ASSAY
- Bacterial contamination or repeated freeze-thaw cycles of the specimens may affect the absorbance values of the samples with consequent alterations of antibody to VZV levels.
- Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis, in fact, should take into consideration clinical history, symptomatology, as well as serological data. Serological data, however, have restricted value in immunosuppressed patients.
- The performance characteristics mentioned in section 11 are acquired with the utmost care. However, a negative result does not totally exclude a recent VZV infection. Therefore results need to be interpreted with caution.
- For the estimation of a primary or recurrent VZV infection by serology please refer to section 9.3.

14. WARNINGS AND PRECAUTIONS
- All reagents supplied are for in vitro use only.
- All calibrators contain serum or plasma from human origin, provided in this VZV-IgG testkit, have been assayed for Hepatitis B antigen, anti-HCV and anti-HIV antibodies and found negative. However, these sera must be considered as potentially infectious, and sera should be handled by appropriate procedures.
- The reagents included in the kit have been formulated with materials of animal origin. These materials are sourced where possible from countries that have no current status of outbreaks of TSE’s or other transmittable infectious agents within cattle, or are treated during the manufacturing process in such a way as to protect personnel and preserve the performance of the device. However, the reagents must be considered as potentially infectious, and should be handled by appropriate procedures.
- Avoid contact of substrate, sulfuric acid, washing and dilution buffer with skin and mucous membranes. If these reagents come into contact with skin or mucous membranes, wash with abundant tap water.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the undersurface of the strips, prevent damage and dirt.
- Use only components that are provided in this kit: intermixing between kits may cause interpretation problems.
- The reagents supplied should be used only as indicated in this instruction manual.
- Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
- Do not pipette solutions by mouth.
- Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands thoroughly at the end of each assay.
- Avoid splashing or forming an aerosol. Any reagent spill should be washed with 3% sodium hypochlorite solution and disposed of as though potentially infectious.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Disposal should be according to local, state or national legislation. Dispose of through authority facilities or pass to chemical disposal company. Disposable ignitable materials must be incinerated; liquid waste and non-ignitable materials must be decontaminated with sodium hypochlorite at a final concentration of 3% for at least half an hour. Liquid waste containing acid must be neutralized before treatment. Any materials to be reused must be autoclaved using an overkill approach. A minimum of one hour at 121 °C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.

- TMB substrate solution is a stabilized chromogenic substrate for use with horse radish peroxidase immunoassays. It contains both 3,3', 5,5' tetramethylbenzidine and hydrogen peroxide (H2O2) in low concentrations. This formulation contains no DMF or DMSO. TMB is known to be sensitizing to the skin when exposed to high concentrations. TMB substrate is very light sensitive and direct exposure to sunlight should be avoided. To avoid contamination of the entire bottle of substrate, never pour back unused substrate solution. Always pour necessary volume of substrate into a separate container for use.

- Stop Solution (stopping reagent, H2SO4, 0.5 M):
R36/38 : irritating to eyes and skin. S26 : In case of contact with eyes, rinse immediately with plenty of water, and seek medical advice.

15. LITERATURE

Examples of results obtained with Meddens Diagnostics Varicella Zoster Virus ELISA's (IgG, IgM and IgA), Evaluation of laboratory results and clinical data (December 1994) Scientific Product Support, Meddens Diagnostics, V001
16. QUICK REFERENCE PROTOCOL

<table>
<thead>
<tr>
<th>QUICK REFERENCE PROTOCOL FOR VZV IgG EIA*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PREPARATION OF REAGENTS</strong></td>
</tr>
<tr>
<td><strong>A. Dilute</strong> patient test serum</td>
</tr>
<tr>
<td>mix 1.0 mL dilution buffer (BLUE) + 10 µL patient test serum.</td>
</tr>
<tr>
<td><strong>B. Prepare</strong> diluted conjugate:</td>
</tr>
<tr>
<td>mix per 8-well strip: 1.0 mL dilution buffer (BLUE) +10 µL anti-IgG-PO conjugate (100x).</td>
</tr>
<tr>
<td><strong>C. Prepare</strong> wash buffer: mix per 8-well strip 27.0 mL distilled water + 3.0 mL Wash Buffer (10x).</td>
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<td></td>
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</tbody>
</table>

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Read the entire protocol before starting the assay

INSTRUCTIONS FOR USE: IFU4460-V08, 2 April 2007
<table>
<thead>
<tr>
<th>Symbols / Symbole / Symbôles / Símbolos / Σύµβολα</th>
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<tbody>
<tr>
<td><strong>REF</strong></td>
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<tr>
<td><strong>LOT</strong></td>
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</tbody>
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Symbols of the kit components see MATERIALS SUPPLIED.

Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.

Voir MATERIEL FOURNI pour les symbôles des composants du kit.

Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.

Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.

Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.

Για τα σύµβολα των συστατικών του κιτ συµβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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**IBL AFFILIATES WORLDWIDE**

<table>
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<tr>
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<th>Address</th>
<th>Telephone</th>
<th>Fax</th>
<th>E-Mail</th>
<th>Web</th>
</tr>
</thead>
<tbody>
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<td><a href="http://www.IBL-International.com">http://www.IBL-International.com</a></td>
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<td>Fax: -60 73 86</td>
<td><a href="mailto:IBL@IBL-International.com">IBL@IBL-International.com</a></td>
<td><a href="http://www.IBL-International.com">http://www.IBL-International.com</a></td>
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<td><a href="http://www.IBL-Transatlantic.com">http://www.IBL-Transatlantic.com</a></td>
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**LIABILITY:** Complaints will only be accepted in written and if all details of the test performance and results are included (complaint form available from IBL or supplier). Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

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