Influenza A ELISA
Influenza B ELISA
IgG / IgM Testkit
IgA Testkit

Order No.:
Influenza A: EC118.00 (IgG/IgM Testkit) / EC118A00 (IgA Testkit)
Influenza B: EC119.00 (IgG/IgM Testkit) / EC119A00 (IgA Testkit)

Color Coding:
Influenza A:  IgG/IgM:  light-blue
              IgA:  light-blue / black
Influenza B:  IgG/IgM:  light-blue / transparent
              IgA:  light-blue / red

FOR IN VITRO DIAGNOSIS ONLY

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REV 27 / Influenza A / Influenza B ELISA IgG/IgM/IgA GB
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1. **Intended Use**

   The influenza ELISA tests are used to detect human influenza A or influenza B virus antibodies in serum. The antigens used are influenza antigens which are, in accordance with the vaccine recommendations of the WHO for the Northern Hemisphere, updated annually with recombinant haemagglutinins (HA) as follows:

   - **Season 2013/2014**
     - Influenza A:  
       1. HA A/Christchurch/16/2010 ((H1N1) A/California/7/2009-like virus)
       2. HA1 A/Texas/5/2012 (A (H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/2011)
     - Influenza B:  
       1. HA B/Massachusetts/2/2012

2. **Diagnostic Relevance**

   The cause of influenza A/B (viral flu) is one of the orthomyxoviruses. Extremely high genetic variability is characteristic of influenza viruses, based on an above-average mutation frequency and the capacity for gene exchange. These features lead to the epidemics and endemics typical of influenza.

   Influenza virus infections are distributed worldwide, and besides humans, birds and mammals represent another natural reservoir for Influenza A.

   Flu (influenza) is an acute respiratory tract infection. Spread is airborne and it is highly contagious. The incubation period is 1-3 days. In the northern hemisphere, flu occurs mainly between December and April, but sporadic cases are observed throughout the year.

   The early diagnosis of an influenza infection is best made by isolating the virus (culture/ PCR/ EIA/ IFT) from nasal and throat swabs (2). Culturing the virus followed by immunohistochemical detection using monoclonal antibodies is achieved as gold standard for direct detection of Antigens.

   In serological diagnosis, ELISA and CBR are used to find type-specific antibodies (2) and to differentiate the individual immunoglobulin classes. The measurement of subtype-specific antibodies is unimportant in clinical diagnosis because of the high variability and an infection rate of 50 % (3). An IgG antibody titre can only indicate contact with the antigen, but this does not necessarily mean immunity. This also applies for proven vaccination titres (1). Serology is only of limited use to diagnose an acute event, as the antibodies generally occur only 2-3 weeks after the onset of the disease (2). They are of increased significance in epidemiological surveys.

3. **Test Principle**

   The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. **Package Contents**

   4.1 **IgG/IgM Testkit**

   1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
   2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7.2, with preservative and Tween 20
   3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7.2, with preservative and Tween 20
   4. **IgG negative Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
   5. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
   6. **IgG positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
   7. **IgM negative Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
   8. **IgM cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
   9. **IgM positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
   10. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
   11. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
12. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
13. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

### 4.2 IgA Testkit
14. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
15. **PBS-Dilution Buffer** (blue, ready to use) 2x50ml, pH 7.2, with preservative and Tween 20
16. **PBS-Washing Solution** (20x concentrated) 50ml, pH 7.2, with preservative and Tween 20
17. **IgA negative Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
18. **IgA cut-off Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
19. **IgA-positive Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
20. **IgA-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
21. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
22. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

### 5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is notusable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

<table>
<thead>
<tr>
<th>Material</th>
<th>Status</th>
<th>Storage</th>
<th>Shelflife</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Samples</td>
<td>Diluted</td>
<td>+2 to +8°C</td>
<td>max. 6h</td>
</tr>
<tr>
<td></td>
<td>Undiluted</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
<tr>
<td>Controls</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Microtitreplate</td>
<td>After Opening</td>
<td>+2 to +8°C (storage in the provided bag with desiccant bag)</td>
<td>3 months</td>
</tr>
<tr>
<td>Rheumatoid factor -</td>
<td>Undiluted, After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Absorbent</td>
<td>Diluted</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
<tr>
<td>Conjugate</td>
<td>After Opening</td>
<td>+2 to +8°C (protect from light)</td>
<td>3 months</td>
</tr>
<tr>
<td>Tetramethylbenzidine</td>
<td>After Opening</td>
<td>+2 to +8°C (protect from light)</td>
<td>3 months</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Washing Solution</td>
<td>Final Dilution (ready-to-use)</td>
<td>+2 to +25°C</td>
<td>4 weeks</td>
</tr>
</tbody>
</table>

### 6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

### 7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the Sekisui Virotech user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma. Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not be used (false positive/negative results).

8.2 Preparation of Reagents

The Sekisui Virotech System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are parameter specific and only to use with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

8.3 Virotech ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG- IgM- and IgA-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!
8.4 Usage of ELISA processors

All Sekisui Virotech ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

Sekisui Virotech recommends the following procedure:

1. Sekisui Virotech recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.

2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.

3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG-, IgA- and IgM-antibodies. Their concentration can be expressed in Virotech units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) Virotech Units (VE)

The Virotech Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the Virotech Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

\[
VE_{(positive\ control)} = \frac{OD_{(positive\ control)}}{OD_{(cut-off\ control)}} \times 10
\]

\[
VE_{(patient\ serum)} = \frac{OD_{(patient\ serum)}}{OD_{(cut-off\ control)}} \times 10
\]

9.3 Interpretation of Results

With influenza IgG antibodies occur about 2-3 weeks after infection (2). Therefore a positive result may give notice of an acute or recent infection (please note vaccination management). However these results should always be considered in the context of all available medical findings. A final diagnosis is only possible in consideration with anamnesis, clinic and laboratory data. The appearance of increased IgA or IgM titers may give additional notice of an acute infection. They might also occur in reinfections and may persist up to one year.

It is advantageous to recognize titer changes in the Ig-classes to make influenza diagnostic more secure. The examination of titer courses (first serum shortly after infection, second serum after 14 days) may serve as help when interpreting unclear diagnosis.
9.4 Interpretation Scheme IgG, IgM and IgA

<table>
<thead>
<tr>
<th>Result (VE)</th>
<th>Evaluation</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 9.0</td>
<td>Negative</td>
<td>Antibodies without significant concentration</td>
</tr>
<tr>
<td>9.0 - 11.0</td>
<td>Borderline</td>
<td>No significantly increased antibody concentration repeat test, if necessary obtain 2nd sample</td>
</tr>
<tr>
<td>&gt; 11.0</td>
<td>Positive</td>
<td>Significantly increased antibody concentration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Notice of acute infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Notice of past infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Vaccination antibodies</td>
</tr>
</tbody>
</table>

9.5 Limits of the Test
1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. No sera from patients with acute influenza infection have been available for evaluation. Therefore the performance data are based upon tests of vaccination sera and blood bank sera.
3. Crossreactivities between influenza A and influenza B may occur.
4. The RKI recommends that the diagnostic laboratory test should be repeated within a short interval if there are still clinical signs of the disease.

10. Performance Data

10.1 Prevalence (Expected Values)
The following tables show the results of the examination of blood bank sera:

### Influenza A ELISA

<table>
<thead>
<tr>
<th></th>
<th>IgG n=78</th>
<th>IgM n=78</th>
<th>IgA n=78</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Negative</td>
<td>35</td>
<td>45</td>
<td>74</td>
</tr>
<tr>
<td>Borderline</td>
<td>11</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
<td>41</td>
<td>3</td>
</tr>
</tbody>
</table>

### Influenza B ELISA

<table>
<thead>
<tr>
<th></th>
<th>IgG n=78</th>
<th>IgM n=78</th>
<th>IgA n=78</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Negative</td>
<td>49</td>
<td>63</td>
<td>74</td>
</tr>
<tr>
<td>Borderline</td>
<td>6</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
<td>29</td>
<td>3</td>
</tr>
</tbody>
</table>

10.2 Intra-assay-Coefficient of Variation (Repeatability)
In one assay, strips of different plates of one batch have been tested with the same serum sample. The obtained coefficient of variation for Influenza A IgG is < 13%, for Influenza A IgM and IgA as well as for Influenza B IgG, IgM, IgA < 9%.

10.3 Inter-assay-Coefficient of Variation (Reproducibility)
Three sera were tested in 10 independent test runs by different persons in different laboratories. The obtained variation coefficient values for Influenza A and Influenza B are <15%.
11. Literature

12. Test Procedure Scheme

Preparation of Patient Samples and Washing Solution

▼ Washing Solution: Fill up concentrate to 1 liter with aqua dest./demin.

▼ IgG-/IgA-samples – Dilution 1:101

  e.g.:  
  10 µl serum/plasma + 1000 µl Dilution Buffer  
  (Serum Dilution Buffer is ready to use)

▼ IgM-samples - Dilution 1:101

  Rheumafactor-absorption with RF-SorboTech  
  e.g.:  
  5 µl serum/plasma + 450 µl Dilution Buffer +  
  1 drop RF-SorboTech, incubate for 15 min. at room temperature.

Testprocedure

Samples Incubation  30 minutes at 37°C  
100 µl Patient Samples  
blank value (Dilution Buffer) and controls

Wash 4times

Conjugate Incubation  30 minutes at 37°C  
100 µl Conjugate  
IgG, IgM, IgA

Wash 4times

Substrate Incubation  30 minutes at 37°C  
400 µl Washing Solution  
Remove Residues on a Cellulose Pad

100 µl Substrate

Stopping  50 µl Stopping Solution  
shake carefully

Measure Extinctions

Photometer at 450/620nm  
(Reference Wavelength 620-690nm)