ab108746

Influenza Virus B IgG Human ELISA Kit

Instructions for Use

For the qualitative determination of IgG class antibodies against Influenza Virus B in Human serum or plasma (citrate).

This product is for research use only and is not intended for *in vitro* diagnostic use.

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ab108746 Influenza Virus B IgG Human ELISA Kit
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1. Introduction

ab108746 Influenza Virus B IgG Human ELISA Kit is intended for the qualitative determination of IgG class antibodies to Influenza Virus B in human serum or plasma (citrate).

Influenza are RNA viruses of the family Orthomyxoviridae. Influenza viruses are divided into three types, designated A, B, and C which are differentiated by the specificity of a soluble antigen associated with the internal ribonucleoprotein component of the virion. The virions are spherical particles of 80-120 nm in diameter consisting of the ribonucleoprotein component and enveloped by matrix protein and a lipid bilayer which contains two spike line structures: viral hemagglutinin (H) and viral neuraminidase (N). Influenza viruses are respiratory tract pathogens which are transmitted by direct contact, large-droplet infection, or by contaminated surfaces. Influenza types A and B are responsible for epidemics of respiratory illness that occur almost every winter and are often associated with increased rates of hospitalization and death. Type C infections usually cause either a very mild respiratory illness or no symptoms at all; it does not cause epidemics and does not have the severe public health impact that influenza types A and B do. Normally influenza is a self-limiting disease lasting for 3 to 7 days, but some people develop serious and potentially life-threatening medical complications.
2. Assay Summary

*ab108746 is for the qualitative immunoenzymatic determination of IgG-class antibodies to Influenza Virus B and is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.*

Microtiter strip wells are precoated with Influenza Virus B antigens to bind corresponding antibodies of the specimen. Incubated for 1 hour.

After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgG conjugate is added. This conjugate binds to the captured Influenza Virus B specific antibodies. Incubated for 30 mins.

The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. Incubated for 15 mins. The intensity of this product is proportional to the amount of Influenza Virus B specific IgG antibodies in the specimen.

Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorption at 450 nm is read using an ELISA microwell plate reader.
3. Kit Contents

- **Influenza Virus B Coated Wells (IgG):** 12 breakapart 8-well snap-off strips coated with Influenza Virus B antigen; in resealable aluminium foil.
- **IgG Sample Diluent ***:** 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; colored yellow; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Solution (20x conc.)*:** 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
- **Influenza Virus B anti-IgG Conjugate**: 1 bottle containing 20 ml of peroxidase labeled rabbit antibody to human IgG; colored blue, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3’,5,5’-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **Influenza Virus B IgG Positive Control***: 1 bottle containing 2 ml; colored yellow; ready to use; red cap.
- **Influenza Virus B IgG Cut-off Control***: 1 bottle containing 3 ml; colored yellow; ready to use; green cap.
- **Influenza Virus B IgG Negative Control***: 1 bottle containing 2 ml; colored yellow; ready to use; blue cap.
• Strip holder: 1
• Cover foil: 1

* contains 0.1 % Bronidox L after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

4. Storage and Handling

The reagents are stable up to the expiry date stated on the label when stored at 2-8 °C.

5. Additional Materials Required

• ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
• Incubator 37 °C
• Manual or automatic equipment for rinsing wells
• Pipettes to deliver volumes between 10 and 1000 µl
• Vortex tube mixer
• Deionised or (freshly) distilled water
• Disposable tubes
• Timer
6. Preparation of Reagents

It is very important to bring all reagents, samples and standards to room temperature (20-25°C) before starting the test run.

1. **Coated snap-off Strips**: The ready to use break apart snap-off strips are coated with Influenza Virus B antigen. Store at 2-8°C. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2-8 °C; stability until expiry date.*

2. **Influenza Virus B anti-IgG Conjugate**: The bottle contains 20 ml of a solution with anti-human-IgG horseradish peroxidase, buffer, stabilizers, preservatives and an inert blue dye. The solution is ready to use. Store at 2-8°C. *After first opening stability until expiry date when stored at 2-8 °C.*

3. **Controls**: The bottles labeled with Positive, Cut-off and Negative Control contain a ready to use control solution. It contains 0.1% Kathon and has to be stored at 2-8°C. *After first opening stability until expiry date when stored at 2-8 °C.*

4. **IgG Sample Diluent**: The bottle contains 100 ml phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the specimen. This ready to use solution has to be stored at 2-8°C. *After first opening stability until expiry date when stored at 2-8 °C.*

5. **Washing Solution (20x conc.)**: The bottle contains 50 ml of a concentrated buffer, detergents and preservatives. Dilute
Washing Solution 1+19; e.g. 10 ml Washing Solution + 190 ml fresh and germ free redistilled water. The diluted buffer will keep for 5 days if stored at room temperature. *Crystals in the solution disappear by warming up to 37 °C in a water bath.* After first opening the concentrate is stable until the expiry date.

6. **TMB Substrate Solution:** The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2-8 °C away from the light. *The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first opening stability until expiry date when stored at 2-8 °C.*

7. **Stop Solution:** The bottle contains 15 ml 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2-8 °C. *After first opening stability until expiry date.*

7. **Preparation and Collection of Specimen**

Use human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2-8 °C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70 °C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.*
Heat inactivation of samples is not recommended.

**Sample Dilution:**

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10µl sample and 1ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

**8. Assay Method**

**Test Preparation**

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:

- 1 well (e.g. A1) for the blank
- 1 well (e.g. B1) for the negative control
- 2 wells (e.g. C1+D1) for the cut-off control and
- 1 well (e.g. E1) for the positive control
It is recommended to determine controls and samples in duplicate, if necessary. Perform all assay steps in the order given and without any appreciable delays between the steps. A clean, disposable tip should be used for dispensing each standard and each sample. Adjust the incubator to 37° ± 1°C.

Assay Procedure:

1. Dispense 100 µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   **Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.**
5. Dispense 100µl Influenza Virus B anti-IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature. Do not expose to direct sunlight.**
7. Repeat step 4.
8. Dispense 100µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature in the dark.**
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. *Any blue colour developed during the incubation turns into yellow.*
    **Note:** Highly positive samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2.
11. Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the Stop Solution.

**Measurement:**

Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

*If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!*
Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard and sample.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. Data Analysis

A. Run Validation Criteria

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

Substrate blank in A1: Absorbance value < 0.100.
Negative control in B1: Absorbance value < 0.200 and < cut-off.
Cut-off control in C1 and D1: Absorbance value 0.150 – 1.300.
Positive control in E1: Absorbance value > cut-off.

If these criteria are not met, the test is not valid and must be repeated.
B. Calculation of Results

The cut-off is the mean absorbance value of the Cut-off control determinations.

*Example:* Absorbance value Cut-off control 0.39 + absorbance value Cut-off control 0.37 = 0.76 / 2 = 0.38

Cut-off = 0.38

C. Interpretation of Results

Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off.

Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative → grey zone.

It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.

Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.
D. Results in Abcam Units

Sample (mean) absorbance value \( \times 10 = \) [Abcam Units = NTU]  
Cut off

Example: \( \frac{1.786 \times 10}{0.38} = 47 \) NTU [Abcam Units = NTU]

| Cut-off: | 10 | NTU |
| Grey zone: | 9-11 | NTU |
| Negative: | <9 | NTU |
| Positive: | >11 | NTU |

E. Precision

| Inter-assay | n | Mean | Cv (%) |
| Pos. Serum | 20 | 2.61 | 3.2 |

| Intra-assay | n | Mean | Cv (%) |
| Pos. Serum | 8  | 2.57 | 3.4 |

F. Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 86.7 %.
G. Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 95.8 %.

H. Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

10. Limitations

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.
11. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>concentration of target is too high</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th></th>
<th>Wells are insufficiently washed</th>
<th>Wash wells as per protocol recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contaminated wash buffer</td>
<td></td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td>Waiting too long to read plate after adding STOP solution</td>
<td></td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Low sensitivity</th>
<th>Improper storage of ELISA kit</th>
<th>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</th>
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<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
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For further technical questions please do not hesitate to contact us by email ([technical@abcam.com](mailto:technical@abcam.com)) or phone (select “contact us” on [www.abcam.com](http://www.abcam.com) for the phone number for your region).
ab108746 Influenza Virus B IgG Human ELISA Kit
Abcam in the USA
Abcam Inc
1 Kendall Square, Ste B2304
Cambridge,
MA 02139-1517
USA

Toll free: 888-77-ABCAM (22226)
Fax: 866-739-9884

Abcam in the USA
Abcam Inc
1 Kendall Square, Ste B2304
Cambridge,
MA 02139-1517
USA

Toll free: 888-77-ABCAM (22226)
Fax: 866-739-9884

Abcam in Japan
Abcam KK
1-16-8 Nihonbashi
Kakigaracho,
Chuo-ku, Tokyo
103-0014
Japan

Tel: +81-(0)3-6231-094
Fax: +81-(0)3-6231-0941

Abcam in Europe
Abcam plc
330 Cambridge Science Park
Cambridge
CB4 0FL
UK

Tel: +44 (0)1223 696000
Fax: +44 (0)1223 771600

Abcam in Europe
Abcam plc
330 Cambridge Science Park
Cambridge
CB4 0FL
UK

Tel: +44 (0)1223 696000
Fax: +44 (0)1223 771600

Abcam in Hong Kong
Abcam (Hong Kong) Ltd
Unit 225A & 225B, 2/F
Core Building 2
1 Science Park West Avenue
Hong Kong Science Park
Hong Kong

Tel: (852) 2603-682
Fax: (852) 3016-1888

Abcam in Hong Kong
Abcam (Hong Kong) Ltd
Unit 225A & 225B, 2/F
Core Building 2
1 Science Park West Avenue
Hong Kong Science Park
Hong Kong

Tel: (852) 2603-682
Fax: (852) 3016-1888

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