# Human Echovirus IgG (ECHO IgG) ELISA Kit

**Catalog No.:** abx364936

**Size:** 96 tests

**Detection Range:** Qualitative

**Sensitivity:** Qualitative

**Storage:** Store all components at 4°C. Use any opened components within 1 month. Avoid freezing.

**Application:** For qualitative detection of Echovirus Antibody (ECHO IgG) in serum samples.

## Principle of the Assay

This kit is based on indirect enzyme-linked immuno-sorbent assay technology. The 96-well plate is pre-coated with the target antigen. Samples are added to the wells and incubated, then washed with wash buffer. Samples that contain ECHO IgG Antibody will bind to the pre-coated antigen to form an antigen-antibody complex. Unbound conjugates are washed away with wash buffer. Next, HRP-conjugated detection antibody is added to the wells and incubated. TMB substrate is added, which is catalyzed by HRP to produce a blue color product that changes into yellow after adding stop solution. The intensity of the yellow color is proportional to the amount of sample ECHO IgG Antibody bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the presence of ECHO IgG antibody can be determined.

### Kit components

1. 96-well microplate
2. Wash Buffer (20X): 50 ml
3. Sample Diluent: 12 ml
4. TMB Substrate A: 6 ml
5. TMB Substrate B: 6 ml
6. Stop Solution: 6 ml
7. Detection Reagent: 12 ml
8. Positive Control: 1 ml
9. Negative Control: 1 ml
10. Plate sealer: 3
11. Hermetic bag: 1

### Materials required but not provided

1. Microplate reader (450 nm)
2. Distilled water
3. Pipette and pipette tips
4. 1.5 ml microcentrifuge tubes
5. Centrifuge
6. Vortex mixer
7. Incubator

## Protocol

### A. Preparation of samples and reagents

#### 1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Prepare serum samples according to conventional methods. The tested serum should be clear, and free of any debris. Samples can be stored at 4°C for up to 1 week, or at -20°C for longer-term storage.

**Note:**

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

#### 2. Reagents

- **1X Wash Buffer:** Dilute the concentrated 20X Wash Buffer 20-fold with distilled water in a ratio of 1:19.

**Note:**

- Allow all reagents to equilibrate to room temperature before use.
- At low temperatures, a crystalline precipitate may form in the 20X Wash Buffer. Before diluting, bring the 20X Wash Buffer to room temperature and swirl gently until the precipitate is fully dissolved.

### B. Assay Procedure

1. Set 2 positive and 3 negative control, test sample and blank (zero) wells on the pre-coated plate respectively, and record their positions. *Add the solution to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles..*
2. Add 100 µl of Positive Control to each positive control well.
3. Add 100 µl of Negative Control to each negative control well.
4. Add 10 µl of sample and 100 µl of Sample Diluent to each sample well.
5. Tap the plate gently to mix, then cover with a plate sealer and incubate at 37°C for 30 minutes.
6. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash Buffer (approximately 300 µl) using a multi-channel pipette or auto-washer (a 30-60 second soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. After the final wash, invert the plate and blot it against clean absorbent paper towels.*
7. Add 100 µl of Detection Reagent to all wells.
8. Tap the plate gently to mix, then cover with a plate sealer and incubate at 37°C for 30 minutes.
9. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash Buffer (approximately 300 µl) using a multi-channel pipette or auto-washer (a 30-60 second soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. After the final wash, invert the plate and blot it against clean absorbent paper towels.*

10. Add 50 µl of TMB Substrate A and 50 µl of TMB Substrate B to each well.
11. Tap the plate gently to mix, then cover with a plate sealer and incubate at 37°C for 15 minutes in the dark.
12. Add 50 µl of Stop Solution to each well and mix thoroughly.
13. Measure the OD of each well with a microplate reader at 450 nm (or 450 nm/630 nm for dual wavelength). *If using dual wavelength method, blank well is not required. Read results within 10 minutes of adding Stop Solution.*

### C. Analysis

#### 1. Data Analysis:

- Mean OD of the Positive Control should be > 0.8.
- Mean OD of the Negative Control should be < 0.1.
- Mean OD of the Blank Well should be ≤ 0.08
- CUT OFF value = Negative Control + 0.1 (if the Mean OD of the Negative Control is < 0.05, calculate at 0.05; otherwise, calculate at the actual value)

If the Positive Control value is > 0.8, and the Negative Control value is < 0.1, the test is valid, otherwise, the test is invalid.
If OD of Samples < CUT OFF, the test samples are considered negative.
If OD of Samples ≥ CUT OFF, the test samples are considered positive.

**Precautions:**

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

## Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.