TECHNICAL MANUAL

Human Enterovirus 71 (EV71) IgG ELISA Kit

- **SKU CODE:** HUFI08020
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Indirect ELISA Kit (Qualitative)
- **RUO:** Research-Use-Only

Revised: 12/25
For Reference Only

Human Enterovirus 71 (EV71) IgG ELISA Kit

Please read entire manual carefully before starting experiment. DO NOT mix reagents and use reagents from different kits or batches to prevent assay failure.

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1. Key features and Sample Types

Detection method:

Indirect (Qualitative ELISA)

Sample Type:

Serum, Plasma, Cell Culture Supernatant, cell or tissue lysate, Other liquid samples

Reactivity:

Human

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this unopened ELISA Kit at 4°C for 12 months. For opened kits, store individual components as described in the Kit Contents section. Date of expiration will be on the ELISA Box label.

3. Description and Principle

The Assay Genie Human Enterovirus 71 (EV71) IgG ELISA Kit enables qualitative detection of Human Enterovirus 71 (EV71) IgG in Human Serum, Plasma, Cell Culture Supernatant, cell or tissue lysate, Other liquid samples.

The kit uses Indirect ELISA technology: microplate wells pre-coated with Enterovirus 71 (EV71) IgG. Test samples are added to the wells and after incubation, unbound conjugates are washed with wash buffer. Then, an HRP-conjugate is added and binds specifically to target if present in the sample. Detection occurs via a colorimetric reaction where HRP oxidizes TMB, producing a blue signal that turns yellow upon addition of stop solution.

The resulting optical density (OD) is measured at 450 nm using a microplate reader. To improve accuracy and account for potential optical interference, a reference wavelength of 650 nm may also be applied.

This dual function kit includes validated Bradford Reagent to quantify total protein concentration for accurate sample normalization.

4. Kit Contents

The sealed kit can be stored at 2-8 °C. The storage condition for opened kit is specified in the table below. Each kit contains reagents for 96 assays including:

No	Component Name	Size	Storage
1	ELISA Microplate (Dismountable)	8×12	Place the test strips into a sealed foil bag with the desiccant. Store for 1 month at 2-8°C; Store for 12 months at -20°C.
2	EV71-IgG Positive Control	0.4 ml	2-8°C
3	EV71-IgG Negative Control	0.4 ml	2-8°C
4	Sample Dilution Buffer	12 ml	2-8°C
5	HRP-Conjugate	12 ml	2-8°C (Avoid direct light)
6	TMB Substrate A	6 ml	2-8°C (Avoid direct light)
7	TMB Substrate B	6 ml	2-8°C (Avoid direct light)
8	Stop Solution	6 ml	2-8°C
9	Wash Buffer(20X)	50 ml	2-8°C
10	Plate Sealer	5 pieces	-
11	Technical Manual	1 copy	-
12	Bradford Reagent	1 vial	4°C

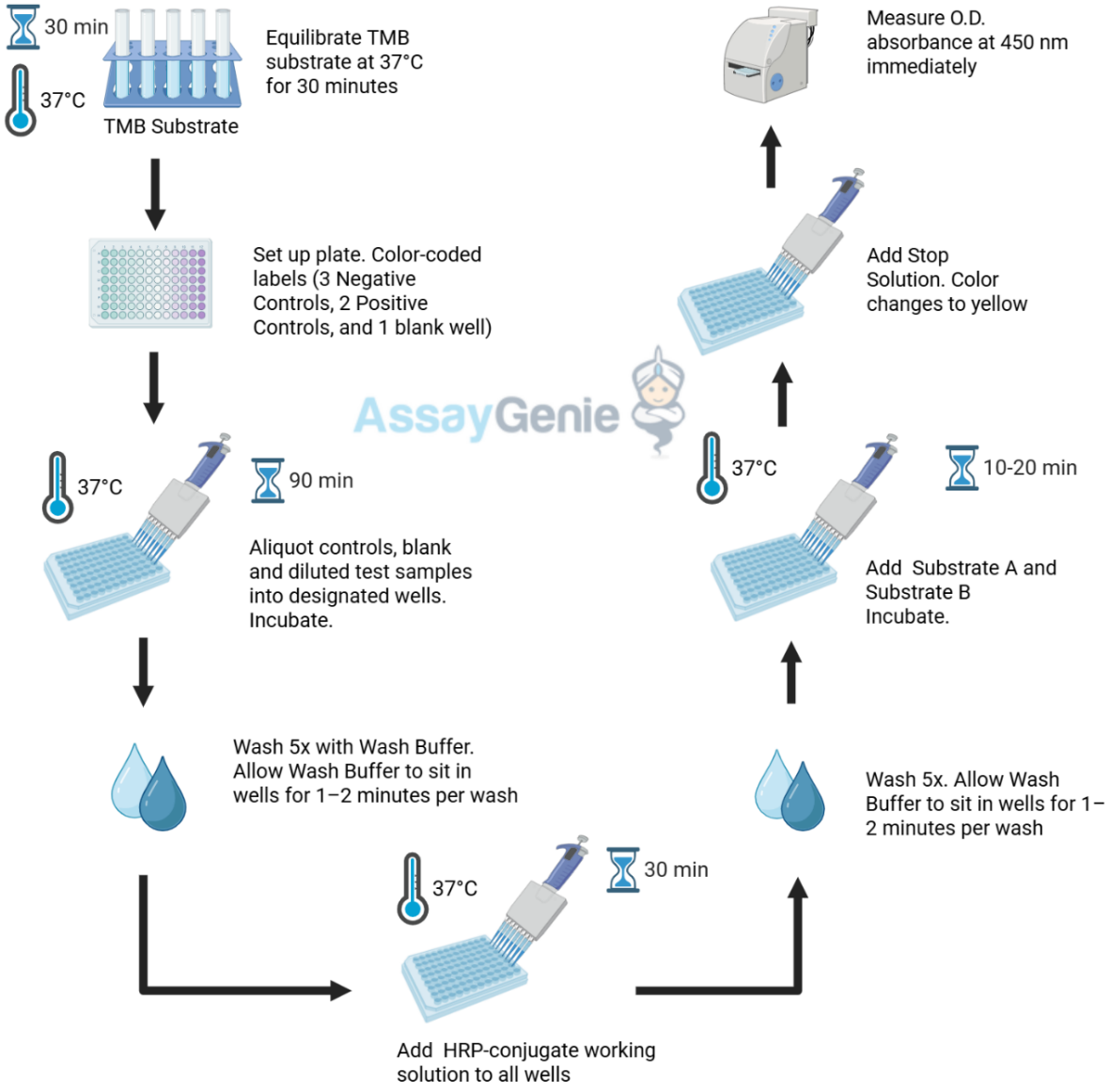
Additional materials required:

1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.

5. Precautions

1. This kit is for research purposes only and not for diagnostics or therapeutic uses.
2. Store all components as listed in this manual. Do not use the ELISA Kit after its expiration date.
3. Allow all reagents and samples to reach room temperature before use.
4. Ensure unopened and unused plates are kept dry to avoid contamination.
5. Before using the kit, centrifuge tubes to spin down standard and/or antibody.
6. Prepare all reagents, samples and standards as directed in this manual.
7. Duplicate wells are recommended for both standard and sample testing.
8. Do not let the microplate wells dry during the assay.
9. Maintain consistent incubation times and temperatures as variations can affect results.
10. Do not reuse tips and tubes to avoid cross contamination.
11. Avoid using the reagents from different batches together.

6. Assay Summary



7. Sample Preparation

The procedures outlined in this document are provided as general recommendations for sample preparation in ELISA assays. Due to the variability of biological samples and specific assay requirements, users are advised to optimize protocols based on their own experimental conditions.

***Note**: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

General Considerations

To prevent denaturation or degradation of target proteins, it is recommended to process samples promptly and store them under appropriate conditions.

- **Storage Conditions**:
    - **Short-term**: 2-8 °C for up to 5 days.
    - **Medium-term**: -20 °C for up to 6 months.
    - **Long-term**: -80 °C or cryopreservation in liquid nitrogen.
- **Thawing Protocol**: Frozen samples should be thawed rapidly in a 15-25 °C water bath to minimize ice crystal-induced damage. Thawed samples can be analyzed immediately or stored temporarily at 2-8 °C.
- **Freeze-Thaw Cycles**: Repeated freeze-thaw cycles should be strictly avoided due to their detrimental effect on protein stability.

A. Blood-Derived Samples

- **Serum:** Allow whole blood to coagulate at room temperature (2 h) or 2-8 °C overnight. Centrifuge at 1000 × g for 20 min and collect the supernatant. Store or use immediately.
- **Plasma:** Collect in anticoagulant tubes (EDTA, citrate, or heparin), mix gently, and centrifuge within 30 min at 1000 × g, 2-8 °C for 15 min. Store or assay as needed.

B. Tissue Homogenates

Tissue samples should be homogenized prior to use. Avoid buffers containing NP-40, Triton X-100, or DTT, as these strongly inhibit the assay. We recommend using 50 mM Tris + 0.9% NaCl + 0.1% SDS, pH 7.3.

The recommended protocol is as follows:

- **Sample Collection and Washing**
  - Place the target tissue on ice.
  - Rinse the tissue with pre-cooled PBS buffer (0.01 M, pH 7.4) to remove residual blood.
  - Weigh the tissue for further processing.
- **Homogenization**
  - Homogenize the tissue on ice using an appropriate lysis buffer.
  - The lysate volume should correspond to the tissue weight; typically, 9 mL PBS is used per 1 g of tissue. It is recommended to add protease inhibitors to the PBS (e.g., 1 mM PMSF). ***Note:*** *PBS buffer or mild RIPA lysis buffer can be used for homogenization. When using RIPA, adjust pH to 7.3.*
- **Cell Disruption**
  - Further disrupt the tissue using ultrasonic homogenization or freeze–thaw cycles.
    - Ultrasonic homogenization: Keep samples on an ice bath during sonication to avoid overheating.
    - Freeze–thaw cycles*:* Repeat twice for effective lysis.
- **Centrifugation and Storage**
  - Centrifuge the homogenate at 5000 × g for 5 minutes.
  - Collect the supernatant for immediate analysis, or aliquot and store at –20°C or -80°C for future assays.
- **Protein Concentration Measurement**
  - Determine total protein concentration using the Bradford Reagent included in this kit.
  - For ELISA assays, the total protein concentration should generally be 1–3 mg/mL.

- Tissues with high endogenous peroxidase levels (e.g., liver, kidney, pancreas) may react with TMB substrate, causing false positives. If this occurs, treat samples with 1% H_2O_2 for 15 minutes before repeating the assay.

Note: *Liver, kidney, and pancreas samples often contain high levels of endogenous peroxidase, which may react with the chromogenic substrate at elevated sample concentrations, potentially resulting in false positive signals.*
If analysis of these tissues is required, a gradient dilution assay is recommended. A proportional decrease in signal with increasing dilution typically indicates minimal interference and supports the accuracy of the results.
To further minimise potential interference, samples can be pre-treated with 1% hydrogen peroxide (H_2O_2) for 15 minutes prior to testing. To prepare the treatment solution, add 1 µl of pure H_2O_2 to 100 µl of sample (1% v/v).

C. Cell Culture Supernatant

Centrifuge the sample at 2500 rpm for 5 minutes at 2–8°C. Carefully collect the clarified cell culture supernatant for immediate analysis, or aliquot and store it at –80°C for future assays.

D. Cell Lysates

- **Suspension Cell Lysate:** Centrifuge the cell suspension at 2500 rpm for 5 minutes at 2–8°C and collect the cell pellet. Wash the pellet with pre-cooled PBS (0.01 M, pH 7.4) and mix gently. Repeat centrifugation and discard the supernatant. Add 0.5–1 mL of cell lysis buffer containing an appropriate protease inhibitor (e.g., PMSF, final concentration: 1 mM). Lyse the cells on ice for 30–60 minutes or disrupt them using ultrasonic homogenization.

- **Adherent Cell Lysate:** Remove the supernatant and wash the cells three times with pre-cooled PBS. Add 0.5–1 mL of cell lysis buffer supplemented with an appropriate protease inhibitor (e.g., PMSF at a final concentration of 1 mmol/L). Scrape the adherent cells using a cell scraper and transfer the cell suspension to a centrifuge tube. Lyse the cells on ice for 30–60 minutes, or disrupt the cells by ultrasonic treatment. Follow next steps for protein extraction and supernatant collection:
- **Protein Release and DNA Disruption**
    - During lysis, pipette gently or intermittently shake the tube to enhance protein extraction.
    - Mucilaginous material formed during lysis is DNA, which can be broken down by ultrasonic disruption (3–5 mm probe, 150–300 W, 3–5 seconds per cycle, with 30-second intervals for 1–2 minutes total).
- **Supernatant collection**
    - After lysis or ultrasonic treatment, centrifuge the lysate at 10,000 rpm for 10 minutes at 2–8°C. Collect the supernatant for immediate use or aliquot and store at –80°C for future assays.

Notes: *Refer to the "Tissue Sample Notes" for additional buffer and inhibitor recommendations*.

E. Other Sample Types

For more information about how to process other sample types, (e.g., body fluids, breast milk & more), please contact our Tech Support Team at techsupport@assaygenie.com.

7.1. Protein Quantification (Optional)

To quantify total protein levels, use the Bradford Reagent included in this kit. Visit Bradford Protein Assay Protocol to view the full protocol.

8. Reagent Preparation

Manual Washing

Discard the solution from the plate without touching the sides of the wells. Tap the plate firmly onto absorbent filter paper or another absorbent material. Completely fill each well with [WASH_BUFFER] µl of wash buffer and let it soak for 1 to 2 minutes. Then aspirate the contents from the plate and tap it again onto absorbent filter paper or other absorbent material. Repeat this process for the specified number of washes.

Automated Washing

Aspirate all wells, then wash the plate three times with [WASH_BUFFER] µl of wash buffer per well. After the final wash, invert the plate and tap it firmly on absorbent filter paper or another absorbent material. It is recommended to set the washer with a 1-minute soak time.

Note: *Set the height of the needles; be sure the fluid can be taken up completely.*

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

Note: *Dilution may be necessary to minimize matrix effects. However, if the target concentration in the sample is very low, the pre-treated sample can be added directly to the assay without dilution.*

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use (18 - 25°C). For repeated assays, please use only strips and standards required and store remaining reagents at the appropriate temperatures.

A. Wash Buffer:

Dilute 50mL of Concentrated Wash Buffer into 950 mL of Wash Buffer with deionized or distilled water. (Crystals that form in the concentrated wash buffer can be dissolved by placing the solution in a water bath at 40°C until fully dissolved (do not exceed 50°C). Mix well before proceeding to the next step. It is recommended to use the prepared wash buffer within one day. Any remaining buffer can be stored at 2-8°C for up to 48 hours.

9. Assay Procedure

1. **Plate Setup:** Label the wells for samples, 3 Negative Controls, 2 Positive Controls, and 1 blank well.
2. **Controls Loading:** Add [CONTROL] µL of Negative Control and Positive Control solutions to each respective well (except the blank well).
3. **Sample Loading:** Add [DILUTION_BUFFER] µL sample dilution buffer to sample wells and then add [SAMPLE] samples serum. Gently tap the plate to ensure thorough mixing.
4. **First Incubation:** Seal the plate with a cover and incubate at 37°Cfor 30 min.
5. **Washing:** Remove the cover and wash plate 5 times with [WASH_BUFFER] µl 1X Wash Buffer, allowing the buffer to sit in the wells for 1–2 minutes each time.
6. **HRP-Conjugate Addition:** Add [HRP_CONJUGATE] µL of HRP-conjugated antigen solution to each well containing controls and samples. Add the solution to the bottom of each well without touching the side walls.
7. **Second Incubation**: Cover the plate and incubate at 37°C for 30 minutes.
8. **Washing:** Remove the cover and wash plate 5 times with [WASH_BUFFER] µl 1X Wash Buffer, allowing the buffer to sit in the wells for 1–2 minutes each time.

9. **Color Development:** Add [SUBSA] µL of TMB Substrate A and [SUBSB] µL of TMB Substrate B to each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in the dark for 15 minutes. A blue color should develop in the Positive Control wells, while Negative Control wells should show no noticeable color.
10. **Stop Reaction:** Add [STOP] µL of Stop Solution to each well and mix thoroughly. The color should immediately change to yellow.
11. **OD Measurement:** Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the Stop Solution (using the blank well to set the zero reference.

10. Data Analysis

Equation

Cut-off Value = NCx+0.1

NCx: Mean Absorbance of Negative Control (A)

**If the mean absorbance (A) value for the negative control is less than [CUT_OFF], it should be recorded as [CUT_OFF]. If the negative mean A value is [CUT_OFF] or greater, record the actual value.*

**The absorbance of the blank well (containing only TMB and Stop Solution) should not exceed [CUT_OFF].*

Determination of results

Sample with absorbance values ≤ Cutoff Value are NON-REACTIVE and are considered NEGATIVE. Sample with absorbance values > Cutoff Value are considered POSITIVE.

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Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limit