

Qualitative Human Coxsackievirus Group B Type 3 (CVB3) ELISA Kit

Cat.No: MBS109157

Store All Reagents At 2°C-8°C !

Package Size: 48T/Kit or 96 T/Kit

Valid Period: Six Months (2°C-8°C)

IN VITRO RESEARCH USE ONLY! NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

1. INTENDED USE

For the qualitative determination of Human Coxsackievirus Group B Type 3 (CVB3) in Human serum, plasma or other biological fluids.

2. PRINCIPLE OF THE ASSAY

The kit uses a sandwich enzyme-linked immunosorbent assay (ELISA) to analyze the existence or not of Human CVB3 in samples.

3. SAMPLE COLLECTION AND STORAGES

Serum - Centrifuge serum for approximately 20 minutes at $1000 \times g$ (or 3000 rpm) within 30 minutes after collection. Collect the supernatants carefully, assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for approximately 20 minutes at $1000 \times g$ (or 3000 rpm) within 30 minutes after collection. Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Other biological fluids - Remove particulates by centrifugation (approximately 20 minutes at $1000 \times g$ (or 3000 rpm) within 30 minutes after collection) and assay immediately or store samples at -20°C. Avoid repeated freeze-thaw cycles.

4. SAMPLE PREPARATION

Serum and plasma to be used within 7 days may be stored at 2-8°C, otherwise samples must be stored at -20 or -80°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature. The samples should be centrifuged adequately and no hemolysis or granule was allowed.

5. PRECAUTIONS

- 1) The operation should be carried out in strict according to the instructions.
- 2) Avoid cross-contamination when changing tips, and pipette reagents and samples into the center of each well.
- 3) The samples should be transferred into the assay wells within 15 minutes.
- 4) If the blue color too shallow after 15 minutes incubation with the substrates, it may be appropriate to extend the incubation time.
- 5) Do not mix the reagents from different batches
- 6) Chromogenic Substrate B is light-sensitive, please avoid prolonged exposure to light.

6. MATERIALS REQUIRED BUT NOT SUPPLIED

- 1) Distilled or deionized water.
- 2) Absorbent papers or paper towels.
- 3) Pipettes and disposable pipette tips.

- 4) An ELISA reader capable of measuring absorbance at 450 nm.
- 5) An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

7. REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

Items	Materials	96 Tests	48 Tests
1	Microelisa stripplate	12*8strips	12*4strips
2	Positive Control	0.5ml/vial	0.5ml/vial
3	Negative Control	0.5ml/vial	0.5ml/vial
4	Sample diluent	6.0ml	3.0ml
5	HRP-Conjugate reagent	10ml	5.0ml
6	20X Wash solution	25ml	15ml
7	Chromogen Solution A	6.0ml	3.0ml
8	Chromogen Solution B	6.0ml	3.0ml
9	Stop Solution	6.0ml	3.0ml
10	Closure plate membrane	2	2
11	User manual	1	1
12	Sealed bags	1	1

8. WASHING METHOD

Manual Washing - Dump the incubation mixtures of the wells into a sink or proper waste container. Using pipette or squirt bottle, fill each well completely with Wash Solution (1×), after about one minute's standing, invert and hit the plate onto absorbent papers or paper towels until no moisture appears. Repeat this procedure four times. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing - Aspirate all wells, then wash plates four times using Wash Buffer (1×). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µl/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

9. REAGENT PREPARATION AND STORAGE

The valid period of this kit is six months at 2°C-8°C. The kit should not be used beyond the expiration date. Wash Solution (1×) - Dilute one volume of Wash Solution (20×) with nineteen volumes of deionized or distilled water. Diluted Wash Solution is stable for one month at 2°C-8°C. Undiluted Wash Solution and other reagents are stable for six months at 2°C-8°C. When the kit is opened, please used up all Microelisa Stripplate as soon as possible after removed the plate from the foil pouch. The Microelisa Stripplate is detachable, so please return the unused wells to the foil pouch containing the desiccant pack, and reseal for preventing damp. The remaining reagents still need to be stored at 2°C-8°C.

10. ASSAY PROCEDURE

1) First, bring all reagents and samples to room temperature (18°C-25°C) naturally for 30min before starting assay procedures. DO NOT use hot water baths to thaw samples or reagents. If necessary, doing a low - speed centrifugation for one or two seconds to concentrate the Positive/Negative Controls to the bottom of the vials. The Microelisa Stripplate is detachable, detach unused part strips from the plate frame, return them to the foil pouch with the desiccant pack, and reseal for preventing damp.

2) Set Positive Control wells, Negative Control wells and Sample wells. Add Positive Control 50µl to each

Positive Control well, add Negative Control 50µl to each Negative Control well. Add sample 10µl and Sample Diluent 40µl to each Sample wells.

PC: Positive Control; NC: Negative Control; S01-S92: Sample

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	NC	NC	S01	S02	S03	S04	S05	S06	S07	S08
B	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
C	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32
D	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42	S43	S44
E	S45	S46	S47	S48	S49	S50	S51	S52	S53	S54	S55	S56
F	S57	S58	S59	S60	S61	S62	S63	S64	S65	S66	S67	S68
G	S69	S70	S71	S72	S73	S74	S75	S76	S77	S78	S79	S80
H	S81	S82	S83	S84	S85	S86	S87	S88	S89	S90	S91	S92

3) Add 100µl of HRP-conjugate reagent to Positive Control wells, Negative Control wells and Sample wells, cover with an adhesive strip and incubate for 60 minutes at 37°C.

4) Wash the Microtiter Plate 4 times.

5) Add Chromogen Solution A 50µl and Chromogen Solution B 50µl to each well successively. Gently mix and then protect from light to incubate for 15 minutes at 37°C.

6) Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7) Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

11. DETERMINE THE RESULT

1) Test validity: the average of Positive control well ≥ 1.00 ; the average of Negative control well ≤ 0.15 .

2) Calculate Critical (CUT OFF): Critical = the average of Negative control well + 0.15.

3) Negative Result: sample OD < Calculate Critical (CUT OFF) is Negative.

4) Positive Result: sample OD \geq Calculate Critical (CUT OFF) is Positive.

12. ASSAY PERFORMANCES

Intra-assay CV (%) and Inter-assay CV (%) are less than 15%.