Human CMV-IgG (cytomegalovirus-Immunoglobulin G) ELISA Kit

(Do not mix reagents of different batches and different product numbers in the kit, otherwise t he kit will not work properly)

Catalogue No.: EH5341

Size: 96T/48T

Reactivity: Human

Range: 1.563-100ng/ml

Sensitivity: 0.938ng/ml

Application: This immunoassay kit allows for the quantitative determination of CMV-IgG in human serum or plasma.

Storage: 2-8°C.

Expiry Date: see kit label

Principle: Indirect

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T/48T)	Storage
Micro ELISA Plate(Dismountable)	8 ×12/8×6	2-8°C /-20°C
Standard	1ml×1/0.5ml×1	2-8°C
Sample dilution buffer	30ml×1/30ml×1	2-8°C
Assay buffer	12ml×1/6ml×1	2-8°C
HRP- Anti-Human IgG Antibody	12ml×1/6ml×1	2-8°C (Avoid Direct Light)
TMB substrate A	6ml×1/6ml×1	2-8°C (Avoid Direct Light)
TMB substrate B	6ml×1/6ml×1	2-8°C (Avoid Direct Light)
Stop solution	6ml×1/6ml×1	2-8°C
Wash buffer (25X)	30ml×1/15ml×1	2-8°C
Plate Sealer	5pieces/3pieces	
Product Description	1 copy	

Wuhan Fine Biotech Co., Ltd.

1-2 Floor, BLD 22, Optics Valley Biopharmaceutical Accelerator, No.388 Gaoxin 2nd Road,

East Lake High Tech Development Zone, Wuhan, 430074, Hubei, China

Tel: (0086)027-87384275 Fax: (0086)027-87800889 www.fn-test.com

Background

CMV (Cytomegalovirus) is a double-stranded DNA virus belonging to the herpesviridae family that is widely present in human populations worldwide. It is capable of infecting humans and incubating in the host for a long time, usually presenting as an asymptomatic infection in people with healthy immune systems, but in individuals with immunosuppressed or immature immune systems (such as newborns, organ transplant recipients, or HIV patients), CMV infection can lead to serious complications such as pneumonia, retinitis, hepatitis, and encephalitis. CMV is transmitted through body fluids, such as saliva, urine, blood, milk and semen, and the virus can remain dormant in the host for life after infection. After virus infection, serum CMV IgM antibodies can be detected for 4-6 months, and in some cases can last for several years. Serum CMV IgG antibodies are relatively constant throughout life. The positive rate of CMV-IgG varies significantly across regions and populations, and generally increases with age. In developed countries, the positive rate of CMV-IgG in the adult population is about 30% to 70%, while in developing countries or resource-limited areas, the positive rate can be as high as 90% or more.

Principle of the Assay

This kit was based on indirect enzyme-linked immune-sorbent assay technology. CMV-Ag was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP- Anti-Human IgG Antibody, if there were any CMV-IgG in the samples, it would form a CMV -Ag - CMV -IgG - HRP- Anti-Human IgG complex. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

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Precautions for Use

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manuf acturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required But Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of THREE washes.

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Automated Washing

Aspirate all wells, wash plate THREE times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- Serum: Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.
- Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20°C.

Note: Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

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Recommended Sample Dilution Ratio

Please refer to the following table of recommended dilution ratio for limited samples for reference. (ND: Not Detected)

Sample Type	Recommended Dilution Ratio	Content
Serum from healthy adults who have not been infected with varicella-herpes virus	undiluted	ND
Serum from a adult infected with varicella-herpes virus(n=5)	1/2-1/5	26.4-87.8ng/ml

If other dilution ratio for your sample model is required, please refer to the universal dilution ratio below. (The ratio is suitable for single-well assay. For duplicate assay, please follow the calculation: volume of sample and diluent x number of duplicate well)

For 2 fold dilution (1/2): Add 20ul sample into 20ul sample diluent and mix gently.

For 5 fold dilution (1/5): Add 10ul sample into 40ul sample diluent and mix gently.

For 10 fold dilution (1/10): Add 5ul sample into 90ul sample diluent and mix gently.

For 20 fold dilution (1/20): Add 3ul sample into 57ul sample diluent and mix gently.

For 50 fold dilution (1/50): Add 3ul sample and 147ul sample diluent and mix gently.

For 100 fold dilution (1/100): Add 3ul sample and 197ul normal saline into 120ul sample diluent and mix gently.

For 1000 fold dilution (1/1000): Two step dilution. Create a 50-fold dilution first, then, create a 20-fold dilution and mix gently.

Notes: The volume in each dilution is not less than 3ul. Dilution factor should be within 100 fold. Mixing during dilution is required to avoid foaming.

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Reagent Preparation and Storage

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25℃). For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

1. Wash Buffer

Dilute 30ml (15ml for 48T) concentrated wash buffer to 750ml (375ml for 48T) wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is 18MΩ.) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 25-fold dilution and mix well. Store the rest solution at 2-8℃.

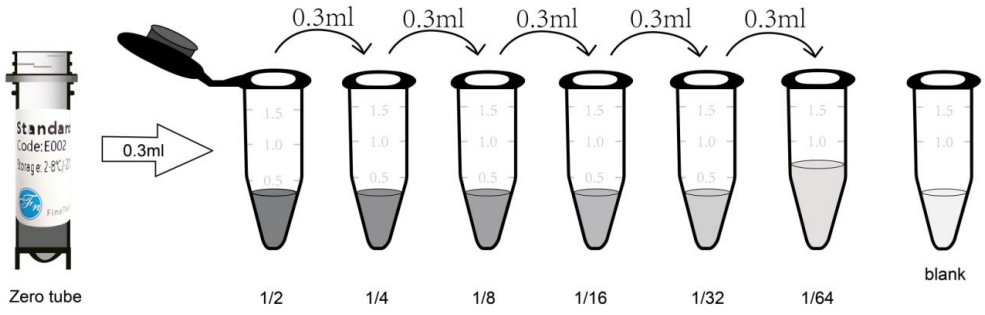
Crystals formed in the concentrated wash buffer can be heated by water bath at 40℃ till complete dissolution. (Heating temperature should be below 50℃.) Mix well for the next step. It's better to use up the prepared wash buffer in one day. Store the rest buffer at 2-8℃ within 48h.

2. Standards

2.1. **Concentrated standard（400ng/ml）**: Centrifuge standards tube for 1min at 2000xg. Label it as Zero tube.

2.2 **Zero tube(100ng/ml)**: Add 200ul of the above **concentrated standard** solution into 600ul of Sample dilution buffer and mix thoroughly.

2.3. Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Sample dilution buffer into each tube. Add 0.3ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contain 0.3ml Sample dilution buffer.



Prepare standard solutions

Notes: Store the **Concentrated standard** tube at 2-8℃. Other diluted working solutions containing standards should be used in 2h.

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Assay Procedure

1. **Label** the standard wells, sample wells and 1 blank well, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors.
2. Standards loading: Aliquot 100ul of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube into each standard well. Also add 100ul Sample dilution buffer into the control (blank) well.
3. Assay buffer loading: Add 100ul Assay buffer into each sample well.
4. Samples loading: Add 10ul samples into each sample well. Gently tap the plate for 10s to ensure thorough mixing then static incubate for 30 minutes at 37°C.
5. Remove the cover, wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for one minute each time.
6. Add 100 µL HRP- Anti-Human IgG Antibody to each well.
7. Seal the plate with a cover and incubate at 37°C for 30 min.
8. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for one minute each time.
9. Add 50 µl of TMB substrate A and 50 µl of TMB substrate B into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark for 15 min. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.
10. Add 50 µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
11. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. (Use the blank well to set zero)

Calculation of Results

1. Calculate the mean OD450 value (using the original OD450 value or the corrected OD450 value) of the duplicate readings for each standard, control, and sample. Then, obtain the value of calculation by subtracting the OD450 blank.

2. Create a four parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. (Remove the OD450 blank during plotting.) Alternatively, you can use the curve fitting software offered by the microplate reader (e.g. Thermo SkanIt RE software, Curve Expert 1.3 or 1.4).

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3. Calculate the sample concentration by substituting OD450 value into the standard curve.

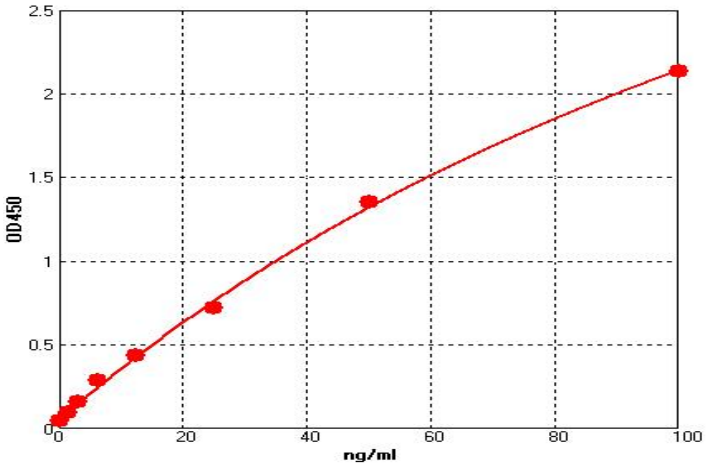
Note: If the sample is added undiluted, the sample dilution caused by incubation of both the sample and Assay buffer should be considered. In this case, the final concentration should be multiplied by 11 from the calculated value. If the sample is 1/2 diluted before adding the plate wells, the final concentration should be multiplied by 22 from the calculated value. And so on.

Typical Data & Standard Curve

This product has been tested by Quality Control Department and meets performance specifications mentioned in the manual. (The humidity in the laboratory is 20%-60%, and the temperature is 18°C - 25°C. TMB was balanced to 37°C before color development, and incubated at 37°C for 15 minutes in the dark after adding the enzyme label plate holes.)

The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.

STD.(ng/ml)	OD-1	OD-2	Average	Corrected
0	0.048	0.049	0.049	0.000
1.56	0.092	0.093	0.093	0.044
3.13	0.159	0.161	0.160	0.112
6.25	0.281	0.299	0.290	0.242
12.5	0.434	0.435	0.435	0.386
25	0.720	0.719	0.720	0.671
50	1.354	1.356	1.355	1.307
100	2.134	2.141	2.138	2.089



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Precision

Intra-assay Precision: samples with low, medium and high concentration are tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration are tested 20 times on three different plates.

Item	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	3.16	13.02	49.64	3.15	13.66	50.95
Standard deviation	0.16	0.61	2.75	0.16	0.85	2.95
CV(%)	4.95	4.68	5.54	4.95	6.2	5.79

Recovery

Add a certain amount of CMV-IgG into the sample. Calculate the recovery by comparing the measured value with the expected amount of CMV-IgG in the sample.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	87-102	98
EDTA Plasma(n=5)	95-101	99
Heparin Plasma(n=5)	93-101	96

Linearity

Dilute the sample with a certain amount of CMV-IgG at 1:2, 1:4 and 1:8 to get the recovery range.

Sample	1:2	1:4	1:8
Serum(n=5)	83-100%	80-95%	87-95%
EDTA Plasma(n=5)	89-94%	81-100%	85-102%
Heparin Plasma(n=5)	86-101%	86-101%	83-95%

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Stability

Perform the stability test for the sealed kit at 37°C and 2-8°C and get relevant data.

Elisa kit(n=5)	37°C for 1 month	2-8°C for 6 months	2-8°C for 12 months
Average (%)	80	95-100	85-98

ELISA Troubleshooting

If the ELISA result is unsatisfied, please take a screenshot for the staining result and store the OD data. Keep used strips as well the rest reagents. Contact us to solve your problem with the kit's catalogue number and batch number. You can also refer to the following table to check the reason.

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect order for adding reagents	Confirm the required reagent added in each step. Also repeat the assay and verify.
	Use components from different kits	Use the component included in the same kit. Also repeat the assay and verify.
	Forget to add some reagents	Verify whether the required reagent is added.
Overflow OD	Use components from different kits, or prepare the working solution with higher concentration	Use the component included in the same kit. Also repeat the assay and verify.
Poor standard curve	Inappropriate curve fitting model	Try to plot the curve by different fitting models.
Samples without signal	The amount of pilot sample is lower than the detection range.	Decrease dilution ratio or concentrate the sample.
	The detection target is incompatible with the buffer.	Verify the compatibility of sample storage buffer with the pilot sample.
	Incorrect preparation of sample	Please refer to sample preparation guideline and regularly store.
	Longer storage of sample or freeze-thaw cycle	Aliquot and store samples according to the assay requirement.
High CV%	Precipitate is formed in the well during staining.	Increase the dilution ratio of the sample.

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	Unclean plate	Don't touch the bottom of the plate during the assay.
	Foam is found in the well.	Avoid foaming during reading in a microplate reader.
	Each well is washed unevenly.	Check whether the tube of the washer is smooth.
	Reagents are not completely mixed.	Mix all reagents completely.
	Inconsistent pipetting	Use calibrated pipette and correct pipetting method.
Standard curve with low signal	Standards are improperly reconstituted.	Before opening, shortly centrifuge the lyophilized standard tube till complete dissolution.
	Standards have been degraded.	Follow suggested storage conditions for standards.
	When pipetting, the required volume is incorrect or inaccurate.	Use calibrated pipette and correct pipetting method.
	Expired kit	Don't use expired products.
	Improper storage	Follow suggested storage conditions for all components.
	The well is over dried.	The assay and sample loading process can't be terminated. Especially after washing the plate, add reagents immediately. Seal the plate during incubation.
	Slow colorimetric reaction	Before use, equilibrate the whole bottle of TMB substrate for 30min at 37°C. Extend the incubation time.
	The wavelength of the microplate reader is incorrect.	Check the wavelength and read the OD450 value again.
	The well is washed excessively.	Follow suggested washing times in this manual.
High Background	Insufficient washing	Follow suggested washing times in this manual.
	Wash buffer is contaminated.	Use the prepared wash buffer immediately. During manual washing, add wash buffer

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		without touching the well.
	Too many detection reagents or higher concentration.	Use calibrated pipette and correct pipetting method.
	Reading of assay result is not in time.	Read the assay result immediately after adding the stop solution.
	TMB substrate is incubated in strong light.	During colorimetry, incubate in the dark.

Declaration

1. Limited to current conditions and scientific techniques, all raw materials are not completely identified and analyzed. This product may have a technology-related quality risk.

2. During the Elisa kit development, some endogenous interferons (not all) in the biological sample have been removed or decreased.

3. The final assay result is related to the validity of reagents, experimental operation and environment. Our company is only responsible for this kit, excluding sample consumption during using this kit. Before use, please consider and prepare enough samples required by the assay.

4. To get a satisfied assay result, please use all reagents offered by this kit. Don't use any product from other vendors. Strictly follow instructions of this manual.

5. During assay procedure, incorrect reagents preparation and parameter setting of the microplate reader may result in the abnormal result. Before assay, please read this manual carefully and adjust instruments.

6. Even if the assay is performed by the same person, results in two independent assays may be different. Thus, each step in the assay should be controlled to ensure the reproducibility.

7. Before delivery, this kit is subject to the strict QC. Influenced by transportation conditions and experimental devices, the assay result got by the customer may be different from original data. Inter-assay CV between different batches may be caused by reasons before.

8. This kit is not compared to similar kits from other vendors or methods for testing the same detection target. Thus, assay results may be inconsistent.

9. This kit allows for research use only. For IVD or other purposes, our company is not responsible for relevant consequences and doesn't bear any legal liability.

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