ab108724 Anti-Cytomegalovirus (CMV) IgG Human ELISA Kit

For the qualitative measurement of IgG class antibodies against Cytomegalovirus (CMV) in Human serum and plasma (citrate).

This product is for research use only and is not intended for diagnostic use

Storage and Stability:
Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Amount	Storage Condition (Before Preparation)
Cytomegalovirus antigens Coated Microplate (12 x 8 wells)	96 Wells	4°C
IgG Sample Diluent***	100 mL	4°C
Stop Solution	15 mL	4°C
20X Washing Solution*	50 mL	4°C
Cytomegalovirus anti-IgG HRP Conjugate**	20 mL	4°C
TMB Substrate Solution	15 mL	4°C
Cytomegalovirus IgG Positive Control***	2 mL	4°C
Cytomegalovirus IgG Cut-off Control***	3 mL	4°C
Cytomegalovirus IgG Negative Control***	2 mL	4°C
Cover foil	1 unit	4°C
Strip holder	1 unit	4°C

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

Materials Required, Not Supplied
These materials are not included in the kit, but will be required to perform this assay:
- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi and single channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

Reagent Preparation
- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

1x Washing Solution: Prepare 1X Washing Solution by diluting 20X Washing Solution with deionized water. To make 200 mL 1X Washing Solution combine 10 mL 20X Washing Solution with 190 mL deionized water. Mix thoroughly and gently.

Sample Collection and Storage
- Use Human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days of sample collection, the specimen should be kept at 4°C; otherwise it should be aliquoted and stored deep-frozen (-20 to -80°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 Preparation
Before assaying, all samples should be diluted 1:100 with IgG Sample Diluent. Add 10 µL sample to 1 mL IgG Sample Diluent to obtain a 1:100 dilution. Mix gently and thoroughly.

Plate Preparation
- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition.
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).

Assay Procedure
- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Please read the test protocol carefully before performing the assay. Reliability of results depends on strict adherence to the test protocol as described.
- If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.
- All controls (Cytomegalovirus IgG Positive, Cytomegalovirus IgG Negative and Cytomegalovirus IgG Cut-off) must be included with each assay performed to determine test results
- Assay all standards, controls and samples in duplicate.

1. Prepare all reagents, standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 100 µL of controls and diluted samples into appropriate wells. Leave one well for substrate blank.
4. Cover wells with the foil supplied in the kit and incubate for 1 hour at 37°C.
5. Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining 1X Washing Solution by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.
   Note: Complete removal of liquid at each step is essential for good assay performance.
6. Add 100 µL Cytomegalovirus anti-IgG HRP Conjugate into all wells except for the blank well. Cover with foil.
7. Incubate for 30 minutes at room temperature. Do not expose to direct sunlight.
8. Repeat step 5.
9. Add 100 µL TMB Substrate Solution into all wells
10. Incubate for exactly 15 minutes at room temperature in the dark.
11. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
    Note: Any blue color developed during the incubation turns into yellow.
12. 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 PBS for example 1:1 is recommended. Then dilute the sample 1:100 with IgG Sample Diluent and multiply the results in Standard Units by 2 (See Section 14. Calculations.)

13. Measure the absorbance of the specimen at 450 nm within 30 minutes of addition of the Stop Solution.
Dual wavelength reading using 620 nm as reference wavelength is recommended.

Calculations

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

- **Substrate blank:** Absorbance value < 0.100
- **Negative control:** Absorbance value < 0.200 and < cut-off
- **Cut-off control:** Absorbance value 0.150 – 1.300
- **Positive control:** Absorbance value > cut-off

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

Calculation of Results

Calculate the mean background subtracted absorbances for each sample and compare to mean Cut-off control value.
The Cut-off control value is the mean absorbance value of the Cut-off control wells.
Example: Absorbance value Cut-off control Well 1 = 0.156
Absorbance value Cut-off control Well 2 = 0.168
Mean Cut Off value: (0.156 + 0.168)/2 = 0.162

Interpretation of Results

Samples are considered to give a positive signal if the absorbance value is greater than 10% over the cut-off value.
Samples with an absorbance value of less than 10% above or below the Cut-off control value should be considered as inconclusive (grey zone) i.e. neither positive or negative. It is recommended to repeat the assay using fresh samples. If results of the second test are again less than 10% above or below the Cut-off control value the sample has to be considered negative.
Samples are considered negative if the absorbance value is lower than 10% below the cut-off.

Results in Standard Units

$$\frac{\text{Patient (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{Standard Units}$$

$$\text{Example: } \frac{1.786 \times 10}{0.38} = 47 \text{ Standard Units}$$

Cut-off: 10 Standard Units
Grey zone: 9-11 Standard Units
Negative: <9 Standard Units
Positive: >11 Standard Units

Typical Sample Values

Sensitivity

The sensitivity is 98 % and is defined as the probability of the assay scoring positive in the presence of the specific analyte.

Precision

Positive Serum	Intra-Assay	Inter-Assay
n=	12	4
Mean	2.86	77.2
%CV	1.9	6.2

Specificity

The specificity is 97.5 % and is defined as the probability of the assay scoring negative in the absence of the specific analyte.

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.

Troubleshooting

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

Technical Support

Copyright © 2020 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print
For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)