AB Diagnostic Systems

REF **DK.059.01.8** Σ **96** IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia CMV IgG
Enzyme immunoassay for the qualitative and quantitative determination of IgG antibodies to *Cytomegalovirus* (CMV) in human serum or plasma

This Package Insert provides information for Professional Use of the kit.
The kit contains sufficient reagents for 96 (one breakable plate) assays including controls; the kit is intended for manual testing with a possibility of fractional (one strip) use of the kit or for use of the kit on open type automated analyzer for enzyme immunoassay.

I. INTENDED USE

The abia CMV IgG kit is intended for the qualitative and quantitative detection of IgG antibodies to *Cytomegalovirus* (CMV) in human serum (plasma) by a microplate immunoenzymometric assay.

This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public. All the reagents are for professional *in vitro* diagnostic use only.

The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture.

II. INTRODUCTION

Cytomegalovirus (CMV) is a member of the human herpesvirus group. Transmission occurs by contact to body fluids (saliva, genital secretions, urine, breast milk) and vertically in utero or during delivery. CMV can also be transmitted by blood transfusion, transplantation of organs and stem cells. Once established, CMV results in a lifelong latent infection that can reactivate later. In addition, reinfection may occur by a new viral strain [1].

Anti-CMV IgG is used for screening, to assess serological status, to determine immunity and to evaluate the risk of CMV disease. Seroconversion to anti-CMV IgG is evidence for recent primary infection. Anti-CMV IgG in combination with IgM indicates primary or recurrent infection, and without IgM indicates past infection. A titer increase in sequential samples may indicate active infection [1, 2]. An increase in IgG level does not always confirm a secondary infection, as this may be due to non-specific polyclonal stimulation of the immune system. CMV is one of the main causes of intrauterine infections. The diagnosis of primary CMV infection during pregnancy can be made by one of the following results: 1) the appearance of CMV-specific IgG in a woman who was previously seronegative; 2) detection of CMV antibody IgM with low IgG levels [3].

CMV infection has either asymptomatic or nonspecific symptoms. The greatest severity of the disease is observed in persons with immunosuppression. In this case, anti-CMV IgG control is used [4].

III. PRINCIPLE OF THE TEST

Scheme of the test procedure is an indirect two-stage immunoassay. Microtiter strip wells precoated with the recombinant analogs of CMV antigens to bind corresponding antibodies. The antigen-antibody complex reacted with HRP analysed anti-human-IgG antibodies. The presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a color change in the TMB-Substrate.

IV. CONTENT OF THE KIT abia CMV IgG

4.1 Contents of the reagent kit.

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
CMV-Ag Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with a mix of recombinant proteins, which represent the recombinant analogs of CMV antigens. Store at 2-8 °C until expiration date.	1 plate
Conjugate	Concentrate of the polyclonal antibodies against human IgG, conjugated with HRP enzyme. Transparent or slightly opalescent yellow liquid. Preserving agent: 0.1% ProClin 300, 0.04% gentamicin sulfate, 0.1% phenol, 0.01% thimerosal. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Positive Control, Inactivated	Inactivated human serum (plasma), containing anti-CMV IgG. Transparent or slightly opalescent red colored liquid. Preserving agent: 0.2% sodium azide, 0.04% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 1.5 ml
Negative Control, Inactivated	Inactivated human serum (plasma), not containing anti-CMV IgG. Transparent or slightly opalescent green colored liquid. Preserving agent: 0.2% sodium azide, 0.04% ProClin 300. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.5 ml
Calibrator, Inactivated	Inactivated human serum (plasma) containing anti-CMV IgG. Approximate* concentration 1.6 U/ml, negative for HBsAg, antigen p24 HIV-1, HIV-1,2 and anti-HCV. * Exact level is given on the label on a lot specific basis and certificate of analysis. The Calibrator was calibrated against Reference Preparation CMV Immunoglobulin 12/1996, Paul-Ehrlich-Institute, Germany. Transparent or opalescent colorless liquid. Preserving agent: 0.1% sodium azide, 0.1% phenol. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 2.5 ml

Preliminary Sample Diluent	Sample buffer that is used for preliminary dilution of samples. Transparent or slightly opalescent violet-blue colored liquid. Preserving agent: < 0.1% sodium azide. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Sample Diluent	Sample buffer that is used to dilute samples. Transparent or slightly opalescent pink colored liquid. Preserving agent: 0.1% sodium azide, 0.006% thimerosal. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 11.0 ml
Washing Solution (concentrated 25-fold)	Phosphate saline buffer (pH 7.4–7.7). Transparent or slightly opalescent colorless or light yellow liquid, sediment may form that dissolves completely at 35-39 °C and shaking. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 ml
Stopping Reagent	0.2 M sulphuric acid solution. Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml
TMB-Substrate	Tetramethylbenzidine in citric acid buffer, containing H_2O_2.Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 14.0 ml
Plate for preliminary dilution of sera	Polystyrene plate with transparent wells.	1 plate
Protective films for EIA plates		2
Polyethylene bag with a Zip-Lock		1
Disposable plastic dishes for liquid reagents		2
Disposable tips		16

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following requirements:

- The temperature in the lab should be 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. If labels are lost or labels/vials damage, vials should be disposed, and **kit cannot be used**.
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.

- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugates.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- Use a new distribution tip for each sample.
- Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate and color development solution.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay's procedure.
- Use high quality water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- Human origin material used in the preparation of Positive Control, Negative Control and Calibrator has been tested and found negative for HBsAg, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2), antigen p24 HIV-1.
- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patients samples as if capable of transmitting infectious disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces.

- Avoid any contact of the TMB-Substrate and the Stopping Reagent with the skin and mucosa.
- Provide adequate ventilation.
- All materials contacted with specimens or reagents, including liquid and solid wastes, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations.

Warning!

Conjugate contains ProClin 300.
H317: May cause an allergic skin reaction.
P261: Avoid breathing vapours.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352: IF ON SKIN: Wash with plenty of water.
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.

Danger!

Positive Control, Negative Control, Calibrator and Sample Diluent contain sodium azide.
H312: Harmful in contact with skin.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 IF ON SKIN: Wash with plenty of soap and water.
P312: Call a POISON CENTER or doctor/physician if you feel unwell.

Danger!

Stopping Reagent contains 0.2 M/l sulphuric acid.
H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/ eye protection/face protection.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/ physician.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT:

- Distilled or deionized water.
- Automatic or semiautomatic, adjustable or preset single-channel and multi-channel pipettes with a changeable volume for a set of liquids.
- Disposable pipette tips.
- Microplate incubator at (37.0 ± 1.0) °C.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm or with 405 nm and 620-680 nm filters.
- Open type automated analyzer with 450 nm or with 405 and 620-680 nm filters (for automated procedure).
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Collection of blood samples should be implemented according to the current practices. Serum, plasma (citrate, heparin, EDTA) may be used. Separate serum or plasma from blood cells as soon as possible to avoid any haemolysis. Extensive haemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results. The samples after heat inactivation at 56 °C for 30 minutes may be analyzed.

Samples can be stored at 2-8 °C not more than for 48 hours; they may be deep-frozen at -20 °C. Samples that have been frozen and defrosted more than 1 time cannot be used.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **CMV-Ag Coated Strips.** Strips are wrapped in a sealed foil-lined bag. Open the bag and remove the tray. Select the number of Coated Strips required for the assay. Return unused strips in the bag. After the bag has been opened the Coated Strips are stable during the shelf life of the kit at 2-8 °C, provided that the foil-lined bag is resealed in Zip-Locked plastic bag. The silica gel bag should not be removed from the foil packaging.
- **Conjugate;**
- **Positive Control;**
- **Negative Control;**
- **Calibrator;**
- **Preliminary Sample Diluent;**
- **Sample Diluent;**
- **Stopping Reagent;**
- **TMB-Substrate.**

2. Reagents to prepare:

- **Working Washing Solution**. Thoroughly shake Washing Solution concentrate. To make Working Washing Solution take required amount of concentrate and mix with distilled or deionized water (1:24 ratio) in a separate vial. Thoroughly mix the solution. The prepared Working Washing Solution is stable for 14 days at 18-24 °C or for 28 days at 2-8 °C.

The required volumes of Working Washing Solution for the certain number of strips or plate are tabulated in Table 2.

Table 2

Number of strips to be used		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Working Washing Solution	Washing Solution (×25), ml	4.0	8.0	12.0	16.0	20.0	24.0	28.0	32.0	36.0	40.0	44.0	50.0
	High quality water, ml	96.0	192.0	288.0	384.0	480.0	576.0	672.0	768.0	864.0	960.0	1056.0	1200.0

3. Storage of unused reagents

After opening the vials the unused components of the kit: Positive Control, Negative Control, Calibrator, Preliminary Sample Diluent, Sample Diluent, Washing Solution (concentrated 25-fold), Stopping Reagent can be stored in tightly sealed vials until the kit expiration date at 2-8 °C. Coated Strips can be stored until the kit expiration date at 2-8 °C.

Conjugate, TMB-Substrate can be stored in tightly sealed vials within 3 months at 2-8 °C.

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature (18-24 °C) for 30 min.

Step	The assay procedure
1	Add 90 µl of Preliminary Sample Diluent into the wells of the plate for preliminary samples dilution and 10 µl of the tested serum (plasma) samples. Carefully mix by pipetting. Violet-blue color should change to blue-green.
2	Add 100 µl of Positive, Negative Controls and Calibrator into the wells. 1 strip – Positive Control to 1 well, Negative Control to 1 well and Calibrator to 2 wells; 2 strip and more – Positive Control to 1 well, Negative Control to 2 wells and Calibrator in 3 wells.
3	Add 90 µl of Sample Diluent and 10 µl of the preliminary diluted samples to the rest of the wells (the final serum dilution ratio is 1:100). Carefully mix fluid in wells by gentle pipetting. Cover the strips with a protective film.
4	Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C.
5	Aspirate the contents of the wells and wash the plate **4 times** with the Working Washing Solution. Add into each well not less than 380 µl of Working Washing Solution and remove Washing Solution into the container with disinfecting solution. Do not leave any fluid in the wells. Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect the assay precision.
6	Add 100 µl of Conjugate to all the wells of the plate. Cover the plate with a protective film.
7	Incubate for **30 min** in a microplate incubator at (37.0 ± 1.0) °C. Remove fluid from wells, wash the plate **4 times** as described in step 5.

8	Add 100 µl of TMB-Substrate into all the wells.
9	Incubate at 18-24 °C for a **20 min** in a dark place.
10	Add 150 µl of Stopping Reagent into wells to stop the reaction results are read by microplate plate reader at wavelength of 450 nm, with reference filter at 620-680 nm. In case of overflow absorbance values, read at 405 nm, with reference filter at 620-680 nm.

Scheme of the assay is represented in Annex.

Automated analyzer

Validated test protocols and dilution tables of reagent working solutions for different EIA-analyzers can be obtained from the manufacturer upon request (see section XIV). For the instrumentation without established validated protocol follow the section "TEST PROCEDURE" and ensure all requirements described in the section "PRECAUTIONS" are fulfilled. All protocols for automated analyzers must be fully validated before use.

When preparing working reagent solutions for automated EIA procedure, it is necessary to consider "dead" volume of vials and containers used for loading working solutions in the EIA analyzer.

XI. RESULTS

Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. **Positive Control:** the absorbance value should not be less than 1.000 at 450/620-680 nm.
2. **Negative Control:** the absorbance value should not be more than 0.200 at 450/620-680 nm.
3. **Calibrator:** the absorbance value should not be less than 0.250 at 450/620-680 nm.

Qualitative results:

The presence or absence of anti-CMV IgG is determined by the ratio of the OD of each sample to the calculated Cut-Off value.

Calculate Cut-Off value as:

Cut-Off = average OD value of Calibrator / A, (A=4) (1),

where **A** – is a coefficient defined by manufacturer during statistical processing for each lot.

Quantitative results:

Concentration [U/ml] = OD sample x B / average OD value of Calibrator, (B=1.6) (2),

where **B** – anti-CMV IgG concentration of the calibrator (U/ml). Exact level is given on the label on a lot specific basis and certificate of analysis.

If the OD of tested sample exceeds 2.900, the sample should be measured at 405/620-680 nm.

Interpretation of results:
Sample is positive, if the OD value is ≥ Cut-Off (≥ 0.4 U/ml).
Sample is negative, if the OD value is < Cut-Off (< 0.4 U/ml).

XII. PERFORMANCE CHARACTERISTICS

1. Analytical sensitivity

The analytical sensitivity was calculated by adding 2 standard deviations from the mean of 20 replicate analyses of Negative Control and was found to be 0.023 U/ml.

2. Interferences

Hemoglobin (up to 2 mg/ml), bilirubin (up to 0.3 mg/ml) and lipids (up to 5 mg/ml) have no influence on the assay results.

3. Cross reactivity

No cross reactivity was found for samples with anti-HSV-1,2, anti-VZV and anti-EBV.

4. Diagnostic sensitivity

Diagnostic sensitivity of abia CMV IgG with 72 anti-CMV IgG positive samples is 100% (95%CI: 94.9-100%).

5. Diagnostic specificity

Diagnostic specificity of abia CMV IgG with 141 anti-CMV IgG negative samples is 98.5 % (95% CI: 94.8-99.6).

6. Trueness. Agreement with certified reference measurement procedure

The abia CMV IgG was compared with the “SERION ELISA classic Cytomegalovirus IgG”, Virion/Serion. 204 serum and plasma samples are tested.

	SERION ELISA classic Cytomegalovirus IgG			
abia CMV IgG		Positive	Negative	Indeterminate
	Positive	86	2	0
	Negative	0	114	2

The abia CMV IgG has not a “gray area”, so indeterminate results were not included in the calculation. The agreement to comparative assays is 99% (95%CI: 96.5-99.7%).

7. Precision

The precision of the abia CMV IgG was determined by 20 days × 3 samples × 2 replicates covering the measuring range.

Intra-assay (within run) precision

Data	Serum sample #1	Serum sample #2	Serum sample #3
Mean (U/ml)	0.64	1.51	2.08
S_r	0.03	0.07	0.03
CV (%)	5.3	4.5	1.3

Inter-assay (between-run) precision

Data	Serum sample #1	Serum sample #2	Serum sample #3
Mean (U/ml)	0.64	1.51	2.08
S_{rr}	0.03	0.06	0.12
CV (%)	4.9	4.3	5.6

8. Recovery

The samples were prepared by adding defined amount of anti-CMV IgG (Calibrator) to Control serum.

Sample	Measured concentration, U/ml	Expected concentration, U/ml	Recovery, %
Control serum	2.17	-	-
Calibrator	1.6	-	-
Control serum + Calibrator	1.737	1.885	102.9

9. Linearity

The linearity of the test was investigated using serial dilutions of patient sera with high anti-CMV IgG concentration. The abia CMV IgG is linear in the measurement range 0.437-3.047 U/ml by protocol 450/620-680 nm and 0.458-3.099 U/ml by protocol 405/620-680 nm.

10. Hook effect

Up to anti-CMV IgG concentration of 24.5 U/ml no hook effect was observed.

XIII. LIMITS OF THE TEST

- A single study for the definition of anti-CMV IgG is poorly informative, it only indicates infection with cytomegalovirus and does not allow to establish the stage of infection. The concentration anti-CMV IgG depends on the state of the patient's immune system. The active replication of the virus is usually indicated by an increase in the concentration of anti-CMV IgG in paired samples of serum taken at an interval of 10-15 days.
- The diagnosis of acute CMV infection is possible only in the presence of clinical manifestations and a complex of laboratory studies (detection of an increase in the level of anti-CMV IgG, the detection of high levels of anti-CMV IgM, the isolation of the virus in the urine or positive PCR result).
- Attention should be paid to persons with a negative anti-CMV IgG result. This especially applies to recipients of organs and blood components that can become infected during transplantation, as well as pregnant. In case of infection with CMV during pregnancy, the risk of intrauterine infection of the fetus is significantly increased. Therefore, periodic testing of seronegative pregnant is recommended to identify of primary CMV infection. The presence of primary CMV infection can also be detected in the definition of anti-CMV IgG avidity.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

Expiry date is indicated on the packaging.

Keep in dark dry place at 2-8 °C. Freezing is prohibited.

Transportation should be done at 2-8 °C. Transportation at 9-25 °C is allowed not more than during ten (10) days.

AB Diagnostic Systems GmbH
Sportfliegerstraße 4, Berlin, 12487, Germany
Tel. +49 30 208987160, Fax: +49 30 208987199
E-Mail: info@ab-ds.de, www.ab-ds.de

XV. REFERENCES

1. Revello M.G., Gerna G. State of the art and trends in Cytomegalovirus diagnostics. Chapter II. 18, in: Cytomegaloviruses: from molecular pathogenesis to intervention, M. J. Reddehase, N. Lemmermann (eds.), Caister Academic Press, Norfolk UK, 2013: 380-399.

2. Dollard S.C., Staras S.A., Amin M.M. et al. National prevalence estimates for cytomegalovirus IgM and IgG avidity and association between high IgM antibody titer and low IgG avidity. Clin Vaccine Immunol. 2011; 18(11):1895-1899.

3. Khalil A., Heath P., Jones C., Soe A., Ville Y.G. Congenital Cytomegalovirus Infection: Update on Treatment. Scientific Impact Paper No. 56.BJOG 2018; 125:1 – 11.

4. Drago F., Aragone M.G., Lugani C., Rebora A. Cytomegalovirus infection in normal and immunocompromised humans. Dermatology 2000; 200:189-195.

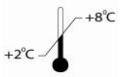
5. Li T.D., Li J.J., Huang X. et al. Baseline antibody level may help predict the risk of active human cytomegalovirus infection in a HCMV seropositive population. Eur J Clin Microbiol Infect Dis. 2017; 36(5):863-868.

6. Xi H., Jinjie L., Shengxiang G. et al. Establishment and validation of an enzyme-linked immunosorbent assay for IgG antibody against cytomegalovirus based on pp150 antigen. J Virol Methods. 2017; 240:21-25.

7. Wu D., Wu Y., Wang L. et al. Evaluation of a Novel Array-Based Toxoplasma, Rubella, Cytomegalovirus, and Herpes Simplex Virus IgG Enzyme Linked Immunosorbent Assay and Its Comparison with Virion/Serion Enzyme Linked Immunosorbent Assays. Annals of Laboratory Medicine 2014; 34(1):38-42.

8. Carlier P., Harika N., Bailly R., Vranken G. Laboratory evaluation of the new Access ® cytomegalovirus immunoglobulin IgM and IgG assays. J Clin Virol. 2010; 49(3):192-7.

XVI. EXPLANATION OF SYMBOLS

IVD	For *in vitro* diagnostic use		
	Manufacturer		Consult Instruction for use
	Date of manufacture CCYY-MM		Symbol “exclamation mark”
	Expiry date CCYY-MM-DD		Symbol “corrosion”
REF	Catalog number		Symbol “health hazard”
	Sufficient for	**Danger! Warning!**	Signal words
LOT	Batch code		Keep away from sunlight
	Storage temperature limitation		Keep dry
	Fragile, handle with care		Top

Annex

Scheme of the assay

1	**Add**	90 µl of Preliminary Sample Diluent and 10 µl of the samples (conduct on the plate for preliminary dilution of samples)
2	**Add**	100 µl of Positive Control, Negative Control, Calibrator
3	**Add**	90 µl of Sample Diluent
4	**Add**	10 µl of preliminary diluted samples
5	**Incubate**	30 min, (37.0 ± 1.0) °C, microplate incubator
6	**Wash the plate**	Working Washing Solution, not less than 380 µl, 4 times
7	**Add**	100 µl of Conjugate
8	**Incubate**	30 min, (37.0 ± 1.0) °C, microplate incubator
9	**Wash the plate**	Working Washing Solution, not less than 380 µl, 4 times
10	**Add**	100 µl of TMB-Substrate
11	**Incubate**	20 min, 18-24 °C in a dark place
12	**Add**	150 µl of Stopping Reagent
13	**Read the optical density**	450 nm/620-680 nm or 405 nm/620-680 nm