AB Diagnostic Systems

REF **DK.060.01.8** Σ **96** IVD **In vitro diagnostic medical device**

INSTRUCTIONS FOR USE
abia CMV IgM
Enzyme immunoassay for the detection of IgM 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 IgM kit is intended for the detection of IgM antibodies specific 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 [1].

The presence of anti-CMV IgM may indicate one of the following: primary infection, re-infection, reactivation. The highest risk of infection of the fetus is observed in the primary infection in the mother during pregnancy (30-40%) [2]. Of all pregnancies with confirmed vertical transmission, only 10% to 20% of the fetuses will have evidence of clinical infection at birth [3]. For the diagnosis of primary CMV infection during pregnancy the detection of CMV antibody IgM with low IgG levels is shown.

For CMV infection is characteristic: 1) IgM may persist for many months after the primary CMV infection; 2) IgM can be detected during a secondary infection; 3) there may be cross reactivity with IgM due to another viral infection, for example, Epstein-Barr virus; 4) IgM can be detected as a result of nonspecific polyclonal stimulation of the immune system [4].

III. PRINCIPLE OF THE TEST

Scheme of the test procedure is an indirect two-stage immunoassay. Microtiter strip wells precoated with the recombinant antigens of CMV to bind corresponding antibodies. The antigen-antibody complex reacted with HRP-labeled anti-human-IgM 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. The color development is stopped with the addition of Stopping Reagent, changing the color to yellow. The color intensity of the sample is directly proportional to the concentration of anti-CMV IgM.

IV. CONTENT OF THE 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.	1 plate
Conjugate	Anti-human IgM polyclonal antibodies, conjugated with HRP enzyme. Transparent or slightly opalescent yellow colored liquid. Preserving agents: 0.096% ProClin 300, 0.004% gentamicin sulfate.	1 vial 11.0 ml
Positive Control	Control sample, containing Anti-GST antibodies, conjugated with HRP enzyme. Transparent or slightly opalescent red colored liquid. Preserving agents: 0.10% ProClin 300, 0.01% phenol.	1 vial 1.2 ml
Negative Control	Control sample with heat inactivated human plasma, not containing IgM antibodies to CMV. Transparent or slightly opalescent green colored liquid. Preserving agents: 0.04% ProClin 300, 0.2% sodium azide, 0.001% gentamicin sulfate.	1 vial 2.5 ml
Preliminary Sample Diluent	Saline buffer that is used for preliminary dilution of samples. Transparent or slightly opalescent violet-blue colored liquid. Preserving agent: 0.09% sodium azide.	1 vial 11.0 ml
Sample Diluent	Saline buffer that is used to dilute samples. Transparent or slightly opalescent pink colored liquid. Preserving agent: 0.10% ProClin 300.	1 vial 11.0 ml
Washing Solution (concentrated 25-fold)	Phosphate-saline solution (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.	1 vial 50.0 ml
Stopping Reagent	Sulfuric acid solution (H_2SO_4) 0.2M. Transparent colorless liquid.	1 vial 25.0 ml
TMB-Substrate	Tetramethylbenzidine in citric acid buffer, containing H_2O_2. Transparent colorless liquid.	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 laboratory 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 the kit reagents if the appearance of reagents is non-compliant (see section IV Content of the Kit).
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- 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 purified 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.
- Do not reuse the coated plates.
- Do not reuse the removed protective film.
- Use purified water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.
- Reagents are non-flammable. External packaging is not self-igniting, explosive.
- Do not misuse. Do not use for cosmetic and domestic purposes, do not eat.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- ★ Negative Control was prepared using inactivated human origin material not containing HBsAg, HIV-1 p24 antigen, antibodies to HIV-1,2 and hepatitis C virus.
- 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, Positive Control and Sample Diluent contains ProClin 300.★
H317: May cause an allergic skin reaction.
H412: Harmful to aquatic life with long lasting effects.
P261: Avoid breathing vapours.
P272: Contaminated work clothing should not be allowed out of the workplace.
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.
P273: Avoid release to the environment.

Danger!

Negative Control contains sodium azide. ★
H312: Harmful in contact with skin.
H412: Harmful to aquatic life with long lasting effects.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352: IF ON SKIN: Wash with plenty of water.
P312: Call a POISON CENTER/doctor if you feel unwell or Get medical advice/attention if you feel unwell.
P273: Avoid release to the environment.

Danger!

Stopping Reagent contains 0.2M sulfuric acid. ★
H314: Causes severe skin burns and eye damage.
P260: Do not breathe vapours.
P264: Wash hands/face thoroughly after handling
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P301+P330+P331: IF SHALLOWED: Rinse mouth. DO NOT induce vomiting!
P303+P361+P353: IF ON SKIN: 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.

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

- Purified 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 450 and 620-680 nm filters.
- Laboratory clock.
- Open type automated analyzer with 450 nm and 620-680 nm filters (for automated procedure).

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 cannot be analyzed.

Samples can be stored at 2-8 °C not more than for 48 hours. For long-term storage separated serum/plasma should be frozen at or below -20 °C. Samples that have been frozen and defrosted more than 1 time cannot be used.

Samples with expressed bacterial growing, hemolysis, hyperlipidemia must not be analyzed.

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. Place the unused strips back into the foil-lined bag; reseal the foil-lined bag in Zip-Lock polyethylene bag. The silica gel bag should not be removed from the foil packaging.
- **Conjugate;**
- **Positive Control;**
- **Negative Control;**
- **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 purified water (1:24 ratio) in a separate vial. Thoroughly mix the solution.

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**	**1 well**
Working Washing Solution	Washing Solution (×25), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0	0.2
	Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0	4.8

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 samples. Carefully mix by pipetting. Violet-blue color should change to blue-green.
2	Add 100 µl of Positive and Negative Controls into the wells. 1 strip – Positive Control to 1 well and Negative Control to 2 wells; 2 strips and more – Positive Control to 1 well and Negative Control to 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.
8	Remove fluid from wells, wash the plate **4 times** as described in step 5.
9	Add 100 µl of TMB-Substrate into all the wells.
10	Incubate at 18-24 °C for a **20 min** in a dark place.
11	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. Reading of the absorbance at 450 nm only is possible.

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 XV). 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

1. 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. If obtained repeat results do not meet the criteria, please contact the manufacturer.

1. **Positive Control**: the absorbance value should not be less than 1.0;
2. **Negative Control**: the absorbance value should not be more than 0.2.

Calculate Cut-Off value as:

Cut-Off = average OD value of Negative Control + A, (A=0.200)

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

2. Interpretation of Results

Sample is positive, if the OD value is ≥ Cut-Off.
Sample is negative, if the OD value is < Cut-Off.

XII. PERFORMANCE CHARACTERISTICS

1. Interferences

Hemoglobin (up to 28.32 mg/ml), bilirubin (up to 0.3 mg/ml), lipids (up to 11.4 mg/ml), rheumatoid factor (up to 221 IU/ml) have no influence on the assay results.

2. Cross reactivity

No cross reactivity was found for samples with antibody to *Rubella virus*, *Epstein-Barr* virus (EBV).

3. Diagnostic sensitivity

Diagnostic sensitivity of abia CMV IgM with 60 anti-CMV IgM positive samples is 93.3% (95%CI: 84.1-97.4).

4. Diagnostic specificity

Diagnostic specificity of abia CMV IgM with 1527 anti-CMV IgM negative samples is 97.3 % (95% CI: 96.3-98.0).

5. Trueness. Agreement with certified reference measurement procedure

The abia CMV IgM was compared with the "SERION ELISA classic Cytomegalovirus IgM", Virion/Serion. 153 serum and plasma samples are tested.

	"SERION ELISA classic Cytomegalovirus IgM"			
abia CMV IgM		Positive	Negative	Indeterminate
	Positive	74	18	6
	Negative	15	37	3

The abia CMV IgM has not a "gray area", so indeterminate results were not included in the calculation. The agreement to comparative assays is 77.1% (111/144) (95%CI: 69.6-83.2%).

6. Precision

The precision of the abia CMV IgM 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)	12.5	3.8	1.9
S_r	0.4	0.1	0.1
CV (%)	3.5	3.5	5.0

Inter-assay (between-run) precision

Data	Serum sample #1	Serum sample #2	Serum sample #3
Mean (U/ml)	12.5	3.8	1.9
S_{rr}	0.22	0.11	0.05
CV (%)	1.8	2.9	2.7

XIII. LIMITS OF THE TEST

- It is inadmissible to make a diagnosis only on the basis of the anti-CMV IgM testing results. 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 urine or positive PCR result in serum).
- Poor correlation of results obtained with different commercial kits for IgM testing may be caused by distinctions in immunological responses depending on the type of CMV antigens used.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION ★

- **Shelf life is 18 months.** Storage and transportation conditions for the kit, working solutions and unused reagents are specified in Table 3.
- Transportation should be done by covered transport at specified temperature in accordance with established transportation regulations. Kits transported at improper temperature cannot be used.
- Kits stored improperly cannot be used.

Table 3

1	**Storage conditions**
	Keep in a dark dry place at (2-8) °C. Freezing is prohibited.

2	**Transportation conditions**		
	at (2-8) °C		
	at (9-25) °C	not more than 10 days	
3	**Conditions and terms of storage for Working solutions** Keep in a dark dry place and in a chemically neutral vial or in reagent container used in an open type automated analyzer.		
	Working washing solution	at (2-8) °C	for up to 28 days
		at (18-24) °C	for up to 14 days
4	**Conditions and terms of storage of unused reagents after opening**		
	Keep in a dark dry place at (2-8) °C.		
	CMV-Ag Coated Strips	Place the unused strips back into the bag, reseal the foil-lined package in Zip-Lock plastic bag.	until the kit expiration date
	Positive control, Negative control, Preliminary Sample Diluent, Sample Diluent, Washing solution (concentrated 25-fold), Stopping reagent	Close the vials tightly with screw caps and store them in the manufacturer`s package.	until the kit expiration date
	Conjugate, TMB-Substrate	Close the vials tightly with screw caps and store them in the manufacturer`s package	for 3 months

XV. GUARANTEE

- Manufacturer guarantees conformity of the product to the requirements of regulatory and technical documentation.
- Quality and safety of the kit is guaranteed within established shelf life.
- Please contact Manufacturer, if you have any questions.
- Any serious incident occurred in relation to the kit shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

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XVI. REFERENCES

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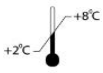
XVII. EXPLANATION OF SYMBOLS ★

	Manufacturer		Temperature limit
	Date of manufacture YYYY -MM		Consult Instructions for use
	Expiry date YYYY-MM-DD	**Danger!**	Signal word
	Batch code	**Warning!**	Signal word
	Catalogue number		Symbol “corrosion”
	Do not use if package is damaged and consult instructions for use		Symbol “health hazard”
	Fragile, handle with care		Symbol “exclamation mark”
	Top		In vitro diagnostic medical device
	Keep away from sunlight		Contains sufficient for <n> tests
	Keep dry	★	Changes highlighted

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
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 450 nm