Foresight®

CMV IgM EIA Test Kit
Package Insert

REF I231-1141	English

An enzyme immunoassay (EIA) for the qualitative detection of IgM antibodies to Cytomegalovirus (CMV) in human serum or plasma.
For professional in vitro diagnostic use only.

INTENDED USE

The CMV IgM EIA Test Kit is an enzyme immunoassay for the qualitative detection of IgM antibodies to CMV in human serum or plasma. It is intended as an aid in the diagnosis of possible CMV infection.

SUMMARY

Cytomegalovirus (CMV) is a member of the Herpes virus family which includes Herpes Simplex virus (HSV) type 1 and 2, Varicella Zoster virus (VZV) and Epstein-Barr virus (EBV). It is a ubiquitous human pathogen transmitted through saliva, sexual contact, perinatally, organ transplantation or blood transfusion.

In majority of the cases, the infection remains asymptomatic. However, CMV infection can cause serious illness in newborns and immunosuppressed individuals such as patients with AIDS, HIV, cancer or patients that received organ transplants.[1] During immunosuppressive therapy, a reactivation of the latent virus or primary infection occurs frequently. For most newborns, CMV infections can be acquired before birth, during birth and later in life. The infection may cause severe congenital abnormalities such as microcephaly, motor disability, and mental retardation.[2, 3, 4] Therefore, determining primary maternal infections and distinguishing primary from latent infection is of great importance. The presence of IgM antibodies indicates the presence of primary infection, while presence of IgG antibodies indicates immune status of patients.

The CMV IgM EIA Test Kit is an immunoassay for the qualitative detection of the presence of IgM antibodies to CMV in serum or plasma specimen. The test utilizes recombinant CMV antigens to selectively detect IgM antibodies to CMV in serum or plasma.

PRINCIPLE

The CMV IgM EIA Test Kit is a solid phase enzyme immunoassay based on immunocapture principle for the qualitative detection of IgM antibodies to CMV in human serum or plasma. The microwell plate is coated with anti-human IgM antibodies. During testing, the specimen diluent and the specimens are added to the antibody coated microwell plate and then incubated. If the specimens contain IgM antibodies to CMV, it will bind to the antibodies coated on the microwell plate to form immobilized anti-human IgM antibody-CMV IgM antibody complexes. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme-conjugated recombinant CMV antigens are added to the microwell plate and then incubated. The enzyme-conjugated recombinant CMV antigens will bind to the anti-human IgM antibody-CMV IgM antibody complexes present. If the specimens do not contain IgM antibodies to CMV, the complexes will not be formed. After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of CMV IgM antibodies present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of CMV IgM antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.

HEALTH AND SAFETY INFORMATION

- Human specimens should be considered potentially hazardous and handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Specimen Diluent, Substrate, Calibrators and Controls. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not pipette by mouth.
- Avoid any contact of the Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution contains 0.5M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

STORAGE AND STABILITY

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and remove the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch with desiccant supplied at 2-8°C and can be used within 3 months of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seal closure to seal the pouch completely and immediately store at 2-8°C.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The CMV IgM EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.
- Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS AND COMPONENTS

Materials Provided

No.	Reagent	Component Description	Quantity		
			96 wells/kit	480 wells/kit	48 wells/kit
	CMV IgM Microwell Plate	Microwell plate coated with anti-human IgM antibodies	1 plate (96wells/plate)	5 plates (96 wells/plate)	1 plate (48wells/plate)
1	Conjugate	Recombinant CMV antigens bound to peroxidase; Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL	1 x 6 mL
2	Concentrated Wash Buffer (25x)	Tris-HCl buffer containing 0.1% Tween 20; Preservative: 0.1% ProClin™ 300	1 x 50 mL	5 x 50 mL	1 x 25 mL
2A	Specimen Diluent	Tris buffer; Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL	1 x 6 mL
3	Substrate A	Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL	1 x 4 mL
4	Substrate B	Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL	1 x 4 mL
5	Stop Solution	0.5M Sulfuric acid	1 x 8 mL	5 x 8 mL	1 x 4 mL
6	CMV IgM Negative Control	Buffer; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
7	CMV IgM Cut-Off Calibrator	Buffer containing IgM antibodies to CMV; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
8	CMV IgM Positive Control	Buffer containing IgM antibodies to CMV; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
	Plate Sealers		3	15	3
	Package Insert		1	1	1

Materials Required But Not Provided

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 37°C ± 2°C
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Calibrated micropipettes with disposable tips capable of dispensing 5, 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Timer
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter
- Automated processor (optional)

DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the controls so that well A1 is the Blank well. From well A1, arrange the controls in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

Step	Detailed Procedure	Simplified Procedure
	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle containing the concentrated wash buffer in a graduated cylinder and fill it with freshly distilled or deionized water to 1250 mL for 96 wells/plate testing, or 625 mL for 48 wells/plate testing. The Working Wash Buffer is stable for 2 weeks at 15-30°C. **Note:** If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve. • Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 • Remove and store unused strips at 2-8°C
0	• Leave A1 as Blank well.	• Leave A1 as Blank well
1	• Add 100 µL of Negative Control in wells B1 and C1. (Blue Reagent) • Add 100 µL of Cut-Off Calibrator in wells D1 and E1. (Blue Reagent) • Add 100 µL of Positive Control in wells F1 and G1. (Red Reagent)	• B1 and C1: Add 100 µL Negative Control • D1 and E1: Add 100 µL Cut-Off Calibrator • F1 and G1: Add 100 µL Positive Control
2	• Add 100 µL of Specimen Diluent to assigned wells starting at H1. (Green Reagent). • Add 5 µL of specimen to assigned wells starting at H1. Then a color change from green to blue will occur to verify that the specimen has been added.	• Starting H1: Add 100 µL Specimen Diluent • Starting H1: Add 5 µL specimen
3	• Mix gently by swirling the microwell plate on a flat bench for 30 seconds. • Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.	• Mix gently • Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
4	• Remove the Plate Sealer.	• Remove the Plate Sealer

	• Wash each well 5 times with 350 µL of Working Wash Buffer per well, then remove the liquid. • Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. Note: Improper washing may cause false positive results.	• Wash each well 5 times with 350 µL of Working Wash Buffer • Turn the microwell plate upside down on absorbent tissue
5	• Add 100 µL of Conjugate to each well except for the Blank well. (Red Reagent)	• Add 100 µL of Conjugate to each well except for the Blank well
6	• Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.	• Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
7	• Repeat Step 4.	• Repeat Step 4
8	• Add 50 µL of Substrate A to each well. (Clear Reagent) • Add 50 µL of Substrate B to each well. (Clear Reagent) Then a blue color should develop in wells containing Positive specimens.	• Add 50 µL of Substrate A to each well • Add 50 µL of Substrate B to each well
9	• Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator at 37°C ± 2°C for 10 minutes ± 1 minute.	• Mix then cover microwell plate with Plate Sealer and incubate at 37°C for 10 min
10	• Remove the Plate Sealer. • Add 50 µL of Stop Solution to each well. (Clear Reagent). Then a yellow color should develop in wells containing Positive specimens.	• Remove Plate Sealer • Add 50 µL of Stop Solution to each well
11	• Read at 450/630-700 nm within 30 minutes. Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.	• Read at 450/630-700 nm within 30 min

AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

1. Calculate the Mean Absorbance of Negative Control, Cut-Off Calibrator, and Positive Control by referring to the table below.

Example of Cut-Off Calibrator Calculation

Item	Absorbance
Cut-Off Calibrator: Well D1	0.249
Cut-Off Calibrator: Well E1	0.263
Total Absorbance of Cut-Off Calibrator	0.249 + 0.263 = 0.512
Mean Absorbance of Cut-Off Calibrator	0.512/2 = 0.256

2. Check the validation requirements below to determine if the test results are valid.

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm Note: It should be < 0.100 if read at 450 nm
Negative Control	Mean Absorbance after subtraction of Blank Absorbance should be < 0.150
Cut-Off Calibrator	Mean Absorbance after subtraction of Blank Absorbance should be > 0.150
Positive Control	Mean Absorbance after subtraction of Blank Absorbance should be > 0.500

NOTE: The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

INTERPRETATION OF RESULTS

Qualitative

Calculate the Index Value to obtain qualitative specimen results.

1. If the test is valid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean Absorbance of Cut-Off Calibrator. See an example of Cut-Off calculation below.

Item	Absorbance
Blank Absorbance: Well A1	0.011
Cut-Off Value: Mean Absorbance of Cut-Off Calibrator – Blank Absorbance	0.256 – 0.011 = 0.245

2. Calculate the Index Value by dividing the Specimen Absorbance by the Cut-Off Value, then read the results by referring to the Interpretation of Results table below.

Item	Absorbance
Specimen: Well H1	1.037
Cut-Off Value	0.245
Index Value: Specimen/Cut-Off Value	1.037/0.245 = 4.233

Interpretation of Results - Qualitative

Results	Qualitative Index Value
Negative	< 0.9
Positive	> 1.1
Equivocal*	≥ 0.9 and ≤ 1.1

***NOTE**: For Equivocal results, the specimen should be retested. Specimens that are repeatedly Equivocal after retest should be confirmed using an alternate method. If the results remain Equivocal, collect a new specimen in two weeks. If the new specimen is Positive, the specimen is presumed to be Positive.

LIMITATIONS

1. The CMV IgM EIA Test Kit is used for the detection of IgM antibodies to CMV in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test results. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A negative test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. As with other sensitive immunoassays, there is the possibility that the positive result cannot be repeated due to inadequate washing from initial testing. The results may be affected due to procedural or instrument error.
4. The Positive Control in the test kit is not to be used to quantify assay sensitivity. The Positive Control is used to verify that the test kit components are capable of detecting a Positive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The CMV IgM EIA Test Kit has correctly identified specimens of a mixed titer performance panel (PTC202, Boston Biomedica Inc) when compared to a leading commercial CMV IgM EIA test. It has also been compared to a leading commercial CMV EIA test using clinical specimens. The results show that the clinical sensitivity of the CMV IgM EIA Test Kit is 97.9%, and the clinical specificity is 98.8%.

CMV IgM EIA vs. Other EIA

Method		**Other EIA**		**Total Results**
CMV IgM EIA	**Results**	Positive	Negative	
	Positive	137	12	149
	Negative	3	965	968
Total Results		140	977	1117

Clinical Sensitivity: 97.9% (93.9-99.6%)* Clinical Specificity: 98.8% (97.9-99.4%)*
Overall Agreement: 98.7% (97.8-99.3%)* *95% Confidence Interval

Reproducibility

Intra-Assay: Within-run precision has been determined by using 15 replicates of three specimens: a low positive, a medium positive, and a high positive.
Inter-Assay: Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, a medium positive, and a high positive. Three different lots of the CMV IgM EIA Test Kit have been tested using these specimens over a 5-day period.

Specimen	**Intra-Assay**			**Inter-Assay**		
	Mean Absorbance/ Cut-Off	**Standard Deviation**	**Coefficient of Variation (%)**	**Mean Absorbance/ Cut-Off**	**Standard Deviation**	**Coefficient of Variation (%)**
1	2.024	0.139	6.867	2.125	0.154	7.247
2	5.107	0.366	7.167	5.004	0.409	8.173
3	9.355	0.698	7.461	9.722	0.704	7.241

Interferences

Interferences are not observed up to a concentration of 1 mg/mL Acetaminophen, 0.2 mg/mL Gentistic Acid, 0.1 mg/mL Ascorbic Acid, 0.1 mg/mL Acetosalisilyc Acid, 0.1 mg/mL Caffeine, 0.6 mg/mL Oxalic Acid, 2 mg/mL Bilirubin, 2 mg/mL Hemoglobin, 1% Methanol and 1% Ethanol. Rheumatoid factors do not interfere with the test.
Cross-Reactivity are not observed in Syphilis, HBsAg, HIV, HCV, HSV IgM, Toxo IgM, and Rubella IgM positive specimens.

BIBLIOGRAPHY

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3. Reynolds, DW, Stagno, S, Stubbs, KG, Dabte, AJ, Livingston, NM, Saxon, SS, Alford, CA. *Inapparent Congenital Cytomegalovurus*. N. Engl. J. Med. (1974) 790:291-296.
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Index of Symbols

	Consult instructions for use		Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by	EC REP	Authorized Representative
2°C 8°C	Store between 2-8°C		Lot Number	REF	Catalog #
CMV IgM	CMV IgM	Substrate A	Substrate A	Substrate B	Substrate B
Wash Buffer 25x	Wash Buffer (25x)	Conjugate	Conjugate	Control +	Positive Control
Calibrator Cut-Off	Cut-Off Calibrator	Control −	Negative Control	Package Insert	Package Insert
Microwell Plate	Microwell Plate	Plate Sealer	Plate Sealer		
Specimen Diluent	Specimen Diluent	Stop Solution	Stop Solution		

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