



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

	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">Wash each well 5 times with 350 µL of Working Wash BufferTurn the microwell plate upside down on absorbent tissue
5	<ul style="list-style-type: none">Add 100 µL of Conjugate to each well except for the Blank well. (Red Reagent)	<ul style="list-style-type: none">Add 100 µL of Conjugate to each well except for the Blank well
6	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
7	<ul style="list-style-type: none">Repeat Step 4.	<ul style="list-style-type: none">Repeat Step 4
8	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">Add 50 µL of Substrate A to each wellAdd 50 µL of Substrate B to each well
9	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">Mix then cover microwell plate with Plate Sealer and incubate at 37°C for 10 min
10	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">Remove Plate SealerAdd 50 µL of Stop Solution to each well
11	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">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

- 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.
- As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
- 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.
- 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		Other EIA		Total Results
CMV IgM EIA	Method	Positive	Negative	
	Results			
	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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- Reynolds, DW, Stagno, S, Stubbs, KG, Dabte, AJ, Livingston, NM, Saxon, SS, Alford, CA. *Inapparent Congenital Cytomegalovirus*. N. Engl. J. Med. (1974) 790:291-296.
- Stern, H. Cytomegalovirus Vaccine: Justification and Problems. In: Waterson AP (ed.) Recent Advances in Clinical Virology (1977) 117-134.

Index of Symbols			
	Consult instructions for use		Tests per kit
	For <i>in vitro</i> diagnostic use only		Use by
	Store between 2-8°C		Lot Number
	CMV IgM		Substrate A
	Wash Buffer (25x)		Conjugate
	Cut-Off Calibrator		Negative Control
	Microwell Plate		Plate Sealer
	Specimen Diluent		Stop Solution

	Manufacturer
	Authorized Representative
	Catalog #
	Substrate B
	Positive Control
	Package Insert



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