INSTRUCTIONS FOR USE

EN

Anti-Measles Virus IgG

REF 9Z9271G
SM9Z9271G
9Z9271GB

IVD CE Rx Only

Σ 96
Σ 480

INTENDED USE

The Anti-Measles Virus IgG is designed for the qualitative detection of IgG antibodies to Measles (Rubeola) virus in human serum and is for *In Vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Measles (Rubeola) is a highly contagious viral disease resulting from infection with a paramyxovirus (genus *Morbillivirus*). A prodromal phase of measles begins eight to twelve days following infection. Symptoms of the prodromal phase include fever, cough, coryza, and conjunctivitis. In many cases, the onset of the prodromal symptoms is followed (two to three days) by the appearance of a specific enenthem (Koplik's spots) and a generalized maculopapular eruption (three to four days after onset) (1). In uncomplicated measles, a peak in temperature one to two days later and a rapid defervescence follows the appearance of the rash on the third/fourth day of the rash.

Under normal circumstances, the appearance of the prodromal symptoms, especially the highly specific and pathognomonic Koplik's spots, is sufficient for clinical diagnosis. Since the introduction of the measles vaccine in 1963 however, the incidence of measles has dramatically decreased (2). As a result, medical professionals have had less experience in the clinical diagnosis of the disease and may require laboratory assistance for confirmation. Further complicating the diagnosis of measles is the appearance of an atypical form of the disease. When re-infected with the wild-type virus, the previously immunized people (who received an inactivated measles vaccine between 1963 and 1967), were severely and clinically confused to have Rocky Mountain Spotted Fever (3). Additional complications may include acute measles from secondary bacterial infections of the respiratory tract and middle ear, post-infectious encephalitis, and a rare, but oftentimes fatal disease, subacute sclerosing panencephalitis (SSPE) (1).

Antibodies to measles virus begin to appear with the development of the rash. A transient IgM antibody response (three to six 6 weeks) may appear first, or in conjunction with IgG. IgG antibodies peak in two to six weeks, decline gradually over six months, and remain relatively stable thereafter. Following administration of live, attenuated measles vaccine, antibodies can be detected 11 - 14 days after inoculation (1). Subclinical re-infections can occur in persons with either vaccine-induced or natural immunity resulting in a boost in measles-specific IgG titer (1). In spite of the wide-spread vaccination program, many individuals remain susceptible to measles as a result of primary vaccine failure or non-immunization. Serology is a useful tool for ascertaining the immune status of previously vaccinated individuals and detection of seroconversion in recently vaccinated individuals. In addition, measles serology can be a valuable tool in the diagnosis of subacute sclerosing panencephalitis that may occur years after the original measles infection (3).

PRINCIPLE OF THE ASSAY

The Anti-Measles Virus IgG is designed to detect IgG antibodies to Measles virus in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with Measles antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG is added to the wells and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped, and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE:** The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator, and SAVe Diluent®.

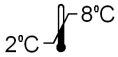
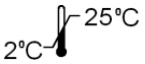
Kit Component	Quantity Σ96	Quantity Σ480	Description
PLATE	1	5	Plate: 96 wells configured in twelve, 1x8-well, strips coated with inactivated Measles antigen (Edmonston Strain). The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	1	5	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc specific) in 15mL, white-capped bottle(s). Ready to use.
CTRL +	1	2	Positive Control (Human Serum): 0.35mL, red-capped vial(s). 21X concentrate.
CAL	1	4	Calibrator (Human Serum): 0.5mL, blue-capped vial(s). 21X concentrate.
CTRL -	1	2	Negative Control (Human Serum): 0.35mL, green-capped vial(s). 21X concentrate.
DIL SPE	1	4	SAVe Diluent®: 30mL, green-capped, bottle(s) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.**
SOLN TMB	1	5	TMB: 15mL, amber-capped, amber bottle(s) containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN STOP	1	3	Stop Solution: 15mL, red-capped, bottle(s) containing 1M H2SO4, 0.7M HCl. Ready to use.
WASH 10X	1	5	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped, bottle(s) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

NOTE: The following components are not Test System Lot Number dependent and may be used interchangeably within the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer. SAVe Diluent® may be used interchangeably with any ZEUS ELISA Test System utilizing Product No. 005CC.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One-liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e., 10% household bleach - 0.5% sodium hypochlorite).

STORAGE CONDITIONS

	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening, strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remain blue.
	Conjugate – DO NOT FREEZE.
	Unopened Kit, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent.
	Stop Solution: 2 – 25 °C Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days Wash Buffer (10X): 2 - 25°C

PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (9).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The SAVe Diluent®, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.

25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (8, 9). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (7).

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 - 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Negative Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Positive Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent®) of the Negative Control, Calibrator, Positive Control, and each patient serum. **NOTE: The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the diluent.**
4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of SAVe Diluent® to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
6. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
   a. **Manual Wash Procedure**:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps 1. and 2. for a total of 5 washes.
      4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.
   b. **Automated Wash Procedure**:

If using an automated microwell wash system, set the dispensing volume to 300 - 350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. ⟶ *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. ⟶ *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. ⟶ *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

	OD Range
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	≥0.500

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
   c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay Cutoff.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. **Calculations:**
   a. *Correction Factor:* The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-

day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.

b. *Cutoff OD Value*: To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above. *(CF x Mean OD of Calibrator = Cutoff OD Value)*

c. *Index Values/OD Ratios*: Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.

Example:	Mean OD of Calibrator	=	0.793
	Correction Factor (CF)	=	0.25
	Cutoff OD	=	0.793 x 0.25 = 0.198
	Unknown Specimen OD	=	0.432
	Specimen Index Value/OD Ratio	=	0.432/0.198 = 2.18

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

	Index Value/OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥1.10

a. An OD ratio ≤0.90 indicates no significant amount of IgG antibodies to Measles detected.

b. An OD ratio ≥1.10 indicates that IgG antibodies specific to Measles were detected.

c. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimens using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

LIMITATIONS OF THE ASSAY

1. Do not make a diagnosis based on the Anti-Measles Virus IgG alone. Interpret test results for anti-Measles in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. Do not use the antibody titer of a single serum specimen to determine a recent infection. Collect and test paired samples (acute and convalescent) concurrently to demonstrate seroconversion.
3. Samples collected too early in the course of an infection may not have detectable levels of IgG. In such cases, collect a second sample after two to seven weeks and test concurrently with the original sample to look for seroconversion.

EXPECTED RESULTS

The incidence of Measles infection varies with age, socioeconomic factors and the usage of measles vaccine (1). In the absence of widespread measles vaccination, infection of the majority of children (>95%) occurs before the age of 15 years. In developing countries, the age of incidence of measles shifts to earlier age groups: infection of more than 50% of children occurs before the age of two years, and nearly 100% by the age of five years (1). With the introduction of the measles vaccine, the age-specific incidence of measles infection has shifted upwards as the overall incidence of measles has declined (2).

PERFORMANCE CHARACTERISTICS

1. **Comparative Study**

A study was conducted to compare the Anti-Measles Virus IgG to another commercial ELISA test system for detection of IgG antibodies to Measles virus. The study consisted of 93 serum specimens. The results are summarized in Tables 1 and 2 below:

Table 1: Comparative Results

		Anti-Measles Virus IgG			
		Positive	Negative	Equivocal	Total
Commercial ELISA Kit	Positive	42	4*	3	49
	Negative	1*	36	1	38
	Equivocal	0	4	2	6
	Total	43	44	6	93

*Represents discrepant specimens.

Calculation of Sensitivity/Specificity after Evaluation of Discrepant Samples:

Sensitivity = 42/45 = 93.3% Specificity = 37/38 = 97.4%

% Agreement = 79/83 = 95.2%

Table 2: Resolution of Discrepant Samples Summary

Sample ID	Anti-Measles Virus IgG Ratio	Commercial ELISA Results	ZEUS IFA Measles IgG Results
ND6	1.20	Negative	Negative
ND2	0.90	Positive	Positive
ND9	0.81	Positive	Positive
ND4	0.61	Positive	Positive
G16	0.09	Positive	Negative

2. **Reproducibility**

To assess the intra-assay and inter-assay variability of the test procedure, technicians tested eight serum samples ranging from strong positive to negative. Technicians tested each sample eight times on each of three days. The mean OD ratio and coefficient of variation (CV) were calculated for each sample. A responsible party then calculated the mean OD ratio and coefficient of variation from the resulting data. Depiction of the results of the experiment is below.

Table 3:	Intra-Assay (n=8)									Inter-Assay (n=24)		
	Day One			Day Two			Day Three					
Sample	Mean Ratio	StD	% CV	Mean Ratio	StD	% CV	Mean Ratio	StD	% CV	Mean Ratio	StD	% CV
1	0.11	0.039	N/A	0.10	0.031	N/A	0.05	0.036	N/A	0.09	0.044	N/A
2	0.69	0.031	4.5	0.65	0.083	13	0.62	0.048	7.7	0.65	0.062	9.5
3	2009	0.185	8.9	1.99	0.146	7.3	1.99	0.099	5.0	2.02	0.149	7.4
4	1.29	0.058	4.5	1.18	0.107	9.1	1.22	0.075	6.2	1.23	0.095	7.7
5	2.95	0.280	9.5	2.75	0.222	8.1	2.82	0.174	6.2	2.84	0.234	8.2
6	43.7	0.325	7.4	4.15	0.244	5.9	4.22	0.274	6.5	4.25	0.287	6.8
7	7.94	0.580	7.3	7.95	0.501	6.3	8.09	0.445	5.5	7.99	0.494	6.2
8	9.83	0.490	4.9	9.72	0.526	5.4	10.1	0.213	2.6	9.90	0.455	4.6

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GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product or products associated with this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device	PLATE	Plate
REF	Catalogue number	CONJ	Conjugate
	Sufficient for *n* tests	CTRL +	Positive Control
LOT	Batch code	CTRL -	Negative Control
	Use by	CAL	Calibrator
	Temperature limitation	DIL SPE	Sample Diluent
CONT	Contents	SOLN TMB	TMB
UDI	Unique Device Identifier	SOLN STOP	Stop Solution
	Consult the warnings and precautions	WASH 10X	Wash Buffer Concentrate (10X)
	Consult electronic instructions for use	EN	English
	Store in the upright position	Made in the USA	Made in the USA
RX Only	Applicable for U.S.A: Prescription *in vitro* diagnostic product		Corrosive
	Hazardous Communication	EC REP	European Commission Authorized Representative
	Conformity with Directive 98/79		

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