Monobind Inc.
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AccuBind
ELISA Microwells

Anti-Rubella IgM (Rubella IgM) Test System

Product Codes: 6425-300

1.0 INTRODUCTION

Intended Use: The Semi-Quantitative Determination of Anti-Rubella Specific Antibodies of the IgM type by Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Rubella, also known as "German measles", is a human pathogen of the rubivirus genus that is most known for causing childhood rashes, low-grade fever, and myalgia.[1-2] However, rubella infections affecting pregnant women, also known as congenital rubella syndrome (CRS), may cause miscarriage, fetal death, or congenital defects.[3-4] Even with a working vaccine, there still remains approximately 100,000 cases of rubella infection globally due to its infectious nature.[5] Since humans are the only known host and the virus has only one serotype that does not cross-react with similar viruses, a blood test can be used to screen for antibodies.[1]

Due to rubella being highly contagious, there have been recent large outbreaks in countries with vaccination programs because the programs remained incomplete.[5] Currently, ELISA is the most frequently used and preferred method for rubella antibody screening. Tests for immunoglobulin M (IgM) antibodies are particularly useful because IgM indicates an active, acute, or recent infection.[5] Since Rubella infection during gestation is associated with high risk to the fetus, antibodies against Rubella should be screened at regular intervals throughout the course of a pregnancy to enable rapid medical intervention.

The Anti-Rubella IgM Accubind® ELISA Test System is a semi-quantitative test designed to produce highly sensitive and specific results with a simple and brief protocol. The test utilizes recombinant chimeric rubella antigen conjugated to horseradish peroxidase to detect native antibodies in the patient sample in a sequential sandwich type method.

3.0 PRINCIPLE

Sequential Sandwich ELISA Method (TYPE 10):

The reagents required for the sequential ELISA assay include immobilized antibody, circulating antibody to Rubella, and enzyme-linked Rubella antigen.

Upon adding a sample containing the anti-rubella antibody, reaction results between the anti-human IgM antibody that has been immobilized on the microwell and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

$$\text{h-Ab}_{(\text{X-rubella})} + \text{Ab} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} \text{h-Ab}_{(\text{X-rubella})} - \text{Ab}$$

Ab = Immobilized Antibody (Constant Quantity)
$\text{h-Ab}_{(\text{X-rubella})}$ = Human Antibody (Variable Quantity)
$\text{h-Ab}_{(\text{X-rubella})}$ - Ab= Immune Complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked disease-specific antigen is then added to the microwells. This conjugate binds to the immune complex that formed.

$$\text{IC}_{(\text{h-IgM})} + {}^{\text{ENZ}}\text{Ag}_{(\text{Rubella})} \Rightarrow {}^{\text{ENZ}}\text{Ag}_{(\text{Rubella})} - \text{IC}_{(\text{h-IgM})}$$

$\text{IC}_{(\text{h-IgM})}$ = Immobilized Immune complex (Variable Quantity)
${}^{\text{ENZ}}\text{Ag}_{(\text{Rubella})}$ = Enzyme-antigen Conjugate (Constant Quantity)
${}^{\text{ENZ}}\text{Ag}_{(\text{Rubella})}$ - I.C. $_{(\text{h-IgM})}$ = Ag-Ab Complex (Variable)

The antigen enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing a serum reference equivalent to the positive-negative cut-off value, the absorbance value can be compared to the cut-off to determine a positive or negative result.

4.0 REAGENTS

Materials provided:

A. Anti-Rubella IgM Controls – 2ml/vial - Icons PC, NC, CC
Three (3) vials of ready-to-use references for Anti-Rubella IgM at positive, negative, and cut-off levels of IgM. Store at 2-8°C. A preservative has been added.

B. Rubella IgM Enzyme Reagent – 13ml/vial - Icon
One (1) vial of chimeric recombinant rubella (E1+E2) horseradish peroxidase (HRP) conjugate in a buffering matrix. A preservative has been added. Store at 2-8°C.

C. Anti-hIgM Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with anti-human IgM antibody and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Serum Diluent Concentrate – 20ml
One (1) vial of concentrated serum diluent containing buffer salts and a dye. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C

F. Substrate Reagent– 12ml/vial - Icon S^N
One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial – Icon
One (1) vial contains a strong acid (0.5 M H_2SO_4). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8ºC. Kit and component stability are identified on the label.**

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Fixed volume or variable volume pipette capable of delivering volumes ranging from 10 to 1000 µl with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050 ml, 0.100 ml, and 0.350 ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens used should be serum or plasma from blood. The usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA, heparin, or citrate (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of seven (7) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.200ml of the diluted specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Serum Diluent**
   Dilute contents of Serum Diluent Concentrate to 200ml (1:10 Dilution) in a suitable container with distilled or deionized water. Store at 2-8°C.
2. **Wash Buffer**
   Dilute contents of wash solution concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
3. **Patient Sample Dilution (1/100)**
   For example, dispense 0.010ml (10µl) of each patient specimen into 0.990 ml (990 µl) of serum diluent or 0.0101 ml (10.1 µl) into 1 ml (1000 µl). Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note : Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each control sample and patient specimen to be assayed in duplicate. Dilute the patient or any external control samples 1/100 (see Reagent Preparation **Section 8.0) Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.100 ml (100µl) of the appropriate control or diluted patient specimen into the assigned well for IgM determination.
   DO NOT SHAKE THE PLATE AFTER SAMPLE ADDITION
3. Cover and incubate 30 minutes at room temperature**.**
4. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
5. Add 350µl of wash buffer (see Reagent Preparation Section 8.0), decant (blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
6. Add 0.100 ml (100µl) of Rubella IgM Enzyme Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
7. Cover and incubate for thirty (30) minutes at room temperature.
8. Wash the wells five (5) times with 350 µl wash buffer by repeating steps (4 & 5) as explained above.
9. Add 0.100 ml (100µl) of Substrate Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells. Do not use the Substrate Reagent if it looks blue.**
   DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
10. Incubate at room temperature for twenty (20) minutes.
11. Add 0.050ml (50µl) of stop solution to each well and swirl the microplate gently for 15-20 seconds to mix. **Always add reagents in the same order to minimize reaction time differences between wells.**
12. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within fifteen (15) minutes of adding the stop solution.**

10.0 INTERPRETATION OF RESULTS

A Cut-Off Control (CC) and kit specific Cut-Off Factor is used to ascertain the positivity or negativity of samples. Follow the following procedure to interpret the sample results.

1. Record the absorbance of all samples obtained from the printout of the microplate reader as outlined in Example 1.
2. Multiply the average absorbance of the Cut-Off Control by the Cut-Off Factor to obtain the Cut-Off Value.
2. Divide the average absorbance of each sample by the Cut-Off Value and multiply by 10 to obtain the relative value unit (RV).
3. If RV <9, the sample is negative for Anti-Rubella IgM and if RV >10, the sample is positive for Anti-Rubella IgM.
4. Samples with RV that fall within the range of 9-10 are considered borderline and should be retested with a new blood draw for reevaluation.

Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

Interpretation of Samples

IgM < 9 RV	Negative
IgM 9-10 RV	Borderline
IgM > 10 RV	Positive

EXAMPLE 1
(Cut Off Factor = 1.00)

COV = MeanCC x COF
COV = Cut-Off Value
MeanCC = Mean Absorbance of Cut-Off Control
COF = Cut-Off Factor (See Certificate of Analysis)
COV = 0.490 x 1.00 = 0.490

Sample I.D.	Abs	Mean Abs	RV	Pos/Neg
Negative	0.060 0.067	0.064	÷0.490 x 10 = 1.3	Negative
Cut-Off	0.485 0.495	0.490	÷0.490 x 10 = 10.0	Cut-Off
Positive	2.446 2.551	2.499	÷0.490 x 10 = 51.0	Positive
Patient 1	0.473 0.491	0.482	***÷0.490 x 10 = 9.83***	***Borderline***
Patient 2	0.204 0.203	0.203	***÷0.490 x 10 = 4.15***	***Negative***
Patient 3	0.844 0.866	0.855	***÷0.490 x 10 = 17.45***	***Positive***

*The data presented in Example 1 is for illustration only and **should not** be used in lieu of a Cut-Off Control run and Cut-Off Factor with each assay. **In this example, since the Cut-Off Factor = 1.00, the average absorbance of the Cut-Off Value = 1.00 x Cut-Off Control.**

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. Maximum Absorbance (Positive control) > 1.5
2. Positive control RV > 15
3. Negative control RV < 6

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the Cut-Off control.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Very high concentration of anti-rubella in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
10. The Anti-Rubella IgM AccuBind® ELISA Test System is a semi-quantitative assay and gives quantities of IgM only in relative units to a cut-off that is traceable to an international standard.
11. Samples, which are contaminated microbiologically, should not be used.
12. Any patient samples used in manufacturing have been heat inactivated prior to handling. However, treat all samples, including the control samples, as potentially hazardous or infectious.
13. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
14. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
15. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
16. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. The clinical significance of the result should be used in evaluating the possible presence of rubella infection. However, **clinical inferences should not be solely based on this test** but rather as an adjunct to the clinical manifestations of the patient and other relevant tests such as Histology, nasophyrangeal swab, etc. A positive result does not indicate and does not distinguish between infection or contagiousness of rubella. Similarly, a negative result does not eliminate the absence of a rubella infection but rather a very low titer of antibody that may be related to the early stages of disease.

13.0 EXPECTED RANGES OF VALUES

Known negative and positive Rubella IgM samples were used to determine expected values for the Anti-Rubella Accubind® ELISA Test System. Based on the international standards, the following cut-off point was established.

Presence of rubella antibodies Confirmed

IgM > 10 RV

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The precision of the Anti-Rubella IgM AccuBind® ELISA Test System was determined on six different patient sera of varying levels. The data summary is collected in the tables below.

Table 1: Total Precision

Sample	Mean Value (RV)	Within-Run Precision		Total Precision (n=80)	
		SD	CV%	SD	CV%
Patient 1	37.88	1.0	2.71	2.57	6.77
Patient 2	23.22	0.53	2.27	1.21	5.23
Patient 3	0.68	0.06	9.27	0.13	19.56
Patient 4	1.91	0.12	6.44	0.33	17.20
Patient 5	10.19	0.26	2.53	1.08	10.62
Patient 6	0.60	0.07	11.66	0.10	16.52

The above data was collected using three different kits in forty assays in duplicate over 20 days.

Table 2: Reproducibility of Interpretation

Sample	Number Negative	Number Borderline	Number Positive
Patient 1	0/80	0/80	80/80
Patient 2	0/80	0/80	80/80
Patient 3	80/80	0/80	0/80
Patient 4	80/80	0/80	0/80
Patient 5	10/80	30/80	40/80
Patient 6	80/80	0/80	00/80

14.2 Sensitivity and Specificity

The sensitivity and specificity of the Anti-Rubella IgM AccuBind® ELISA Test system was determined by measuring 130 different samples from a random population on the Monobind kit and another commercially available ELISA test. The results are tabulated below.

Commercial Interpretation	Monobind Interpretation		Total
	Positive	Negative	
Positive	16	0	16
Negative	3	111	114
Total	19	111	130

Monobind Interpretation	Proportion	Wilson 95% CI
True Positives (Sensitivity)	100%	79.4-100%
True Negatives (Specificity)	97.4%	92.5-99.5%
False Positives	2.6%	0.5-7.5%
False Negatives	0.0%	0.0-20.6%

14.3 Linearity

The linearity of the Anti-Rubella IgM Accubind® ELISA test system was tested by diluting human serum samples containing high levels of IgM against Rubella (11.8 to 29.7 RV) with the serum diluent solution. The system produces excellent linearity up to 29.7 RV and as low as 1.3 RV.

16.0 REFERENCES

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MP6425 **Product Code: 6425-300**

Size		96(A)	192(B)
Reagent (fill)	A)	2ml set	2ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	2 (20ml)
	E)	1 (20ml)	1 (20ml)
	F)	1 (13ml)	2 (13ml)
	G)	1 (8ml)	2 (8ml)

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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)