Monobind Inc.
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AccuBind
ELISA Microwells

Anti-SARS-CoV-2 (COVID-19) IgM Test System

Product Codes: 11725-300

1.0 INTRODUCTION

Intended Use: The Qualitative Determination of Anti-SARS-CoV-2 Specific Antibodies of the IgM type in Human Serum or Plasma by Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), discovered at the end of 2019, is the cause of the disease COVID-19.[1-2] Both SARS-CoV-2 and SARS-CoV, the cause of the 2002 SARS epidemic, are of the genus betacoronavirus and are closely related.[2] Transmission of SARS-CoV-2 is primarily through close contact with infected patients via expelled respiratory droplets, usually from coughing or sneezing.[1-2]

Due to its high transmission rate and severeness, COVID-19 has emerged as a global pandemic that has forced lockdowns and quarantine protocols from countries all over the world.[3] Though diagnoses are primarily conducted using viral nucleic acid detection via real-time reverse transcriptase PCR, many false negatives have been reported and there is urgent need for serological antibody screening as a more robust and reliable test methodology.[4-5]

Tests for immunoglobulin M (IgM) antibodies are of importance as as an early detection of infection.[6] The body's primary defense against a pathogen (antigen) is to produce antibodies. Specifically, IgM appears first and wanes over time as IgG antibodies begin to rise and appear at detectable levels 10-20 days after symptom onset.[7-8]

The Anti-SARS-CoV-2 (COVID-19) IgM AccuBind® ELISA test kit is a qualitative test designed to produce highly sensitive and specific results with a simple and brief protocol. The test utilizes a recombinant nucleocapsid protein (rNCP) in the Enzyme Reagent and Anti-human IgM antibodies coated on microwells to capture native antibodies in the sample. In the first step, prediluted samples are added directly to the wells. After the first incubation, excess sample material is washed out and a rNCP labeled with an enzyme is added to the wells to detect IgM against SARS-CoV-2. After the second incubation, excess material is washed out again and substrate is added to produce a measurable color through the reaction with the enzyme and hydrogen peroxide.

3.0 PRINCIPLE

Sequential Sandwich ELISA Method (TYPE 10):

The reagents required for the sequential ELISA assay include immobilized antibody, circulating antibody to SARS-CoV-2, and enzyme-linked SARS-CoV-2 antigen.

Upon adding a sample containing the anti-SARS-CoV-2 antibody, reaction results between the antibody that has been immobilized on the microwell and the antibody to form an immune-complex. The interaction is illustrated by the following equation:

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

$\text{Ab}_{(\text{x-IgM})}$ = Immobilized Antibody (Constant Quantity)
$\text{h-Ab}_{(\text{IgM})}$ = Human Antibody (Variable Quantity)
$\text{h-Ab}_{(\text{IgM})} - \text{Ab}_{(\text{x-IgM})}$ = 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 SARS-CoV-2 antigen is then added to the microwells. This conjugate binds to the immune complex that formed.

$$\text{IC}_{(\text{h-IgM})} + {}^{\text{ENZ}}\text{Ag}_{(\text{SARS-CoV-2})} \Rightarrow {}^{\text{ENZ}}\text{Ag}_{(\text{X-SARS-CoV-2})} - \text{IC}_{(\text{h-IgM})}$$

$\text{IC}_{(\text{h-IgM})}$ = Immobilized Immune complex (Variable Quantity)

${}^{\text{ENZ}}\text{Ab}_{(\text{X-SARS-CoV-2})}$ = Enzyme-antibody Conjugate (Constant Quantity)

${}^{\text{ENZ}}\text{Ab}_{(\text{X-SARS-CoV-2})} - \text{I.C.}_{(\text{h-IgM})}$ = Ag-Ab Complex (Variable)

The anti-h-IgM 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-SARS-CoV-2 IgM Controls – 1ml/vial - Icons PC, NC, CC
Three (3) vials of ready-to-use references for anti-SARS-CoV-2 at positive, negative, and cut-off levels of IgM. Store at 2-8°C. A preservative has been added.

B. SARS-CoV-2 IgM Enzyme Reagent – 12 ml/vial - Icon
One (1) vial of nucleocapsid protein from SARS-CoV-2 labeled with horseradish peroxides (HRP) 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 – 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 2-8°C. 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

Any components containing human serum from COVID-19 patients have been heat inactivated prior to handling and manufacturing. 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 shall be blood; serum or plasma in type and 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 or heparin (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Please note that there has been no evidence of COVID-19 transmission through blood handling, but technicians should always exercise caution and treat all patient samples as potentially hazardous.[9]

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 2-8°C.**
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 two (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 two (2) additional times.**
6. Add 0.100 ml (100µl) of SARS-CoV-2 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 to develop sufficient color.
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.**

Note: The relationship of absorbance to cut-off value is not necessarily linear so samples need not be diluted further if the absorbance is higher than the plate reader's capability (usually 3.0). However, these samples should be interpreted as strongly positive.

10.0 INTERPRETATION OF RESULTS

A Cut-Off Control 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-SARS-CoV-2 IgM and if RV >10, the sample is positive for Anti-SARS-CoV-2 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 within 4-7 days 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.

EXAMPLE 1
(Cut-Off Factor = 1.0)

COV = MeanCC x COF
COV = Cut-Off Value
MeanCC = Mean Absorbance of Cut-Off Control
COF = Cut-Off Factor (See Certificate of Analysis)
COV = 0.230 x 1.0 = 0.230

Sample I.D.	Abs	Mean Abs	RV	Pos/Neg
Negative	0.059	0.060	÷0.230 x 10 =2.6	Negative
	0.061			
Cut Off	0.216	0.230	÷0.230 x 10 =10	Cut-Off
	0.244			
Positive	2.805	2.845	÷0.230 x 10 =124	Positive
	2.884			
Patient 1	0.104	0.105	***÷0.230 x 10 =4.6***	***Negative***
	0.106			
Patient 2	1.534	1.603	***÷0.230 x 10 =69.7***	***Positive***
	1.671			
Patient 3	0.225	0.217	***÷0.230 x 10 =9.4***	***Borderline***
	0.209			

*The data presented in Example 1 is for illustration only and **should not** be used in lieu of a Cut-Off sample run with each assay. **In this example, since the Cut-Off Factor = 1.0, the average absorbance of the Cut-Off Control = Cut-Off Value**

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-SARS-CoV-2 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-SARS-CoV-2 (COVID-19) IgM AccuBind® ELISA Test System is a qualitative assay and does not necessarily give an indication of quantities of IgM antibodies.
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 SARS-CoV-2 infection or COVID-19. 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 COVID-19 and does not distinguish between infection or contagiousness of COVID-19. Similarly, a negative result does not eliminate the absence COVID-19 infection but rather a very low titer of antibody that may be related to the early stages of disease.
7. Since this test utilizes the nucleocapsid protein of SARS-CoV-2, antibodies against any part of the spike protein are not detected. The nucleocapsid protein is produced in high levels during infection and is very immunogenic. Therefore, a positive result confirms a current or previous *contraction* of COVID-19. Patients who have been vaccinated against the spike protein of SARS-CoV-2 but have not been exposed to the live virus will not react with the test.

13.0 EXPECTED RANGES OF VALUES

A study of apparently healthy population (>150) from prior to December 2019 was undertaken to determine expected values for the Anti-SARS-CoV-2 Accubind® ELISA test system. Based on the data, the following cut-off point was established.

Presence of SARS-CoV-2 antibodies Confirmed

IgM > 10 RV

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Anti-SARS-CoV-2 (COVID-19) AccuBind® ELISA Test System were determined by analyses on two different levels of pool control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented below.

TABLE 1
Within Assay Precision (Values in RV)

Sample	N	X	σ	C.V.
Negative	20	2.1	0.11	5.24%
Borderline	20	9.2	0.23	2.50%
Positive	20	30.5	0.54	1.77%

TABLE 2*
Between Assay Precision (Values in RV)

Sample	N	X	σ	C.V.
Negative	16	1.9	0.16	8.42%
Borderline	16	9.3	0.45	4.84%
Positive	16	29.6	1.38	4.66%

*As measured in eight experiments in duplicate.

14.2 Sensitivity

The sensitivity of the Anti-SARS-CoV-2 IgM AccuBind® ELISA Test System was determined by testing samples from 41 patients who had previously tested positive for SARS-CoV-2 via RT-PCR. The patient samples were sourced from three different blood banks. 40 out of the 41 patients tested positive indicating that the sensitivity of the test is at least 97.6% Positive Percent Agreement.

14.3 Accuracy

The Anti-SARS-CoV-2 (COVID-19) IgM AccuBind® ELISA test system was used to test samples drawn at various time intervals from 41 patients who tested PCR and IgM positive for SARS-CoV-2. The data is shown in Table 3 below.

TABLE 3

Days from Symptom Onset	Number of Subjects Tested	Candidate Test Results: Total Antibody Positive results	Candidate Test Results: Total Antibody PPA	Candidate Test Results: 95% CI
0-7 days	7	7	100%	64.6%-100%
8-14 days	14	14	100%	78.5%-100%
15-30 days	9	9	100%	70.1%-100%
Unknown	11	10	90.9%	62.3%-98.4%
Total Subjects	41	N/A	N/A	N/A

Overall IgM PPA: (97.6% 40/41); [(95% CI (87.4% - 99.6%)]

14.4 Specificity

>150 different patient samples drawn prior to December 2019 were assayed to determine the prevalence of false positives. No false positive samples were detected indicating the Anti-SARS-CoV-2 (COVID-19) IgM AccuBind® ELISA Test System has a 100% Specificity.

16.0 REFERENCES

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Glossary of Symbols
(EN 980/ISO 15223)