Instructions for Use

Novel Coronavirus COVID-19 IgM ELISA RUO

RUO

REF **EIA-6147R**

96

DRG International, Inc., USA
841 Mountain Ave., Springfield, NJ 07081
Phone: (973) 564-7555, Fax: (973) 564-7556
Website: www.drg-international.com
E-mail: corp@drg-international.com

Version 3.1_ca

Version 3.1_ca *(US/V2/2020-03)*
Effective, April 2020

Please use only the valid version of the Instructions for Use provided with the kit.

Table of Contents

For Research Use Only

Not for use in diagnostic procedures

1 INTENDED USE

This kit is intended for the **qualitative detection** of human anti-COVID-19 IgM antibody in human serum.

2 INDICATIONS FOR USE

This kit is used as an aid for the detection of novel COVID-19.

This kit is for research use only; not for use in diagnostic procedures.

3 ASSAY PRINCIPLE

This ELISA kit is designed, developed, and produced for the qualitative measurement of the COVID-19 IgM antibody in serum. This assay utilizes the “IgM capture” method on microplate based enzyme immunoassay technique.

Assay controls and samples are added to the microtiter wells of a microplate that was coated with an anti-human IgM specific antibody.

After the first incubation period, the unbound protein matrix is removed with a subsequent washing step. A horseradish peroxidase (HRP) labeled recombinant COVID-19 antigen is added to each well. After an incubation period, an immunocomplex of "Anti-hIgM antibody - human COVID-19 IgM antibody - HRP labeled COVID-19 antigen" is formed if there is novel coronavirus IgM antibody present in the tested materials.

The unbound tracer antigen is removed by the subsequent washing step. HRP-labeled COVID-19 antigen tracer bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antigen bound to the coronavirus IgM on the wall of the microtiter well is proportional to the amount of the coronavirus IgM antibody level in the tested materials.

4 REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 °C to 8 °C upon receipt.
For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

1. COVID-19 IgM Microplate
Microplate coated with anti-human IgM specific antibody.
Qty: 1 x 96 well microplate
Storage: 2 °C - 8 °C
Preparation: Ready to use

2. COVID-19 IgM Sample Diluent
A ready-to-use sample dilution buffer.
Qty: 1 x 15 mL
Storage: 2 °C - 8 °C
Preparation: Ready to use.

3. HRP Labeled COVID-19 Antigen
HRP labeled COVID-19 Antigen in a stabilized protein matrix.
Qty: 1 x 11 mL
Storage: 2 °C - 8 °C
Preparation: Ready to use.

4. ELISA Wash Concentrate
Surfactant in a phosphate buffered saline with non-azide preservative.
Qty: 1 x 30 mL
Storage: 2 °C - 25 °C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

5. ELISA HRP Substrate
Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 15 mL
Storage: 2 °C - 8 °C
Preparation: Ready to use.

6. ELISA Stop Solution
0.5 M sulfuric acid.
Qty: 1 x 15 mL
Storage: 2 °C - 25 °C
Preparation: Ready to use.

7. COVID-19 IgM Negative Control
Negative control with a bovine serum albumin based matrix with non-azide preservative.
Control products do not contain any serum from patients with new type of coronavirus infection.
Qty: 1 x 1 mL
Storage: 2 °C - 8 °C
Preparation: Ready to use.

8. COVID-19 IgM Positive Control
Positive control with a bovine serum albumin based matrix with non-azide preservative.
Control products do not contain any serum from patients with new type of coronavirus infection.
Qty: 1 x 0.5 mL
Storage: 2 °C - 8 °C
Preparation: Ready to use.

5 SAFETY PRECAUTIONS

The reagents are for research use only.

Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases.

Wear gloves while performing this assay and handle these reagents as if they were potentially infectious.

Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid.

Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes.

On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

6 MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 20 µL, 25 µL, 100 µL, and 1000 µL, etc.
2. Repeating dispenser suitable for delivering 100 µL
3. Disposable pipette tips suitable for above volume dispensing
4. Disposable 12 x 75 mm or 13 x 100 glass tubes
5. Disposable plastic 1000 mL bottle with cap
6. Aluminum foil
7. Deionized or distilled water
8. Plastic microtiter well cover or polyethylene film
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm
11. Incubator capable of holding the temperature at 37 ºC

7 SAMPLE COLLECTION & STORAGE

Only 20 µL of human serum is required for measurement in duplicate.

Samples should only be used on the same day.

Severe hemolytic samples should not be used.

8 ASSAY PROCEDURE

8.1 Reagent Preparation

1. Prior to use, allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate must be diluted to working solution prior to use.
   Please see REAGENTS section for details.

8.2 Assay Procedure

1. Place a sufficient number of microwell strips in a holder to run controls and samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Negative Control	SAMPLE 3	SAMPLE 7
B	Negative Control	SAMPLE 3	SAMPLE 7
C	Negative Control	SAMPLE 4	SAMPLE 8
D	Positive Control	SAMPLE4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	SAMPLE 9
F	SAMPLE 1	SAMPLE 5	SAMPLE 9
G	SAMPLE 2	SAMPLE 6	SAMPLE 10
H	SAMPLE 2	SAMPLE 6	SAMPLE 10

3. Add **100 µL** of controls into the designated microwells.
4. Add **10 µL** of samples into the designated microwells.
5. Add **100 µL** of *COVID-19 IgM Sample Diluent* to the microwells with the samples.
   Note: Do not add sample diluent to the wells with the controls!
6. Mix gently and cover the plate with one plate sealer and aluminum foil.
   Incubate at **37 °C** for **30 minutes.**
7. Remove the plate sealer. Aspirate the contents of each well.
   Wash each well **5 times** by dispensing **350 µL** of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
8. Add **100 µL** of the *HRP-labeled COVID-19 antigen* into the microwells.
9. Mix gently and cover the plate with one plate sealer and aluminum foil.
   Incubate at **37 °C** for **30 minutes.**
10. Remove the plate sealer. Aspirate the contents of each well.
    Wash each well **5 times** by dispensing **350 µL** of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
11. Add **100 µL** of the substrate into the microwells.
12. Mix gently and cover the plate with aluminum foil.
    Incubate at **room temperature (20 °C - 25 °C)** for **20 minutes.**
13. Remove the aluminum foil and add **100 µL** of stop solution into each of the microwells. Mix by gently tapping the plate.
14. Read the absorbance at **450 nm** within **10 minutes** with a microplate reader.

9 PROCEDURAL NOTES

1. It is recommended that all samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original bottles and avoid unnecessary exposure to the light.
3. Store any unused antibody-coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

10 QUALITY CONTROL

To assure the validity of the results each assay must include both negative and positive controls.

The average of the negative control absorbance values less than 0.25,
and the positive control absorbance value is **not less** than 0.50.

We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

11 INTERPRETION OF RESULTS

1. Calculate the average value of the absorbance of the negative control (xNC).
2. Calculate the Cutoff using the following formulas:

    - Positive Cutoff = 1.1 x (xNC + 0.10)
    - Negative Cutoff = 0.9 x (xNC + 0.10)

3. Determine the interpretation of the sample by comparing the OD to the following table:

Interpretation	Interval	Results
Negative	Measured value ≤ Negative Cutoff	The sample does not contain the new coronavirus (COVID-19) IgM related antibody.
Positive	Measured value ≥ Positive Cutoff	The sample contains novel coronavirus (COVID-19) IgM associated antibodies.
Borderline	Negative Cutoff < Measured value < Positive Cutoff	Retest the sample.

12 LIMITATIONS OF THE PROCEDURE

1. This test is only for qualitative detection. For Research Use Only.
2. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
3. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.
4. Not for the screening of donated blood.

SYMBOLS USED

Symbol	English
	Consult instructions for use
	Catalogue number
	Batch code
	Contains sufficient for <n> tests
	Temperature limit
	Use-by date
	Manufacturer
	Caution
RUO	For research use only
Distributed by	Distributed by
Content	Content
Volume/No.	Volume / No.

(06-Apr-2020_ia)