CovIgM-Assay

(SARS CoV-2 Spike S1-RBD IgM ELISA Detection Kit)

The operator should read technical manual carefully before using this product. This product is for Research use only and not for diagnostic use.

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https://prsciencetrust.org/the-covigm-assay-kit/

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I. SUMMARY

COVID-19 is the disease caused by the novel severee acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (Ref). Persons infected with SARSCoV-2 may develop fever or chills, dry cough, shortness of breath, fatigue, loss of appetite, body aches, body aches and mucus or phlegm (Ref). Antibodies are produced gradually by the immune system after infection. IgM is the first antibody class that is elicited in response to SARS-CoV-2 and generally could be detectable in blood approximately 2 days after initial infection (Ref). Although some studies report that IgM peak occurs approximately 20-22 days after symptoms onset (Ref), the correct timing to detect IgM response after infection with SARS-CoV-2 is unclear since it is unknown how long these antibodies persist in circulation after infection and the few studies avalilable show divergent results (Ref). The sensitivity of the IgM antibody detection is directly related to the time after infection when the blood sample are collected.

II. INTENDED USE

The CovIgM-Assay ELISA is an indirect enzyme immunoassay (ELISA) intended for qualitative detection of IgM antibody class to SARS-CoV-2 in human plasma from anticoagulated blood (heparin, EDTA and sodium citrate), or serum. The CovIgM-Assay is intended for use as an aid in indentifying individuals with an acute adaptive immune response to SARS-CoV-2, indicating recent infection. **This product is for research only and it is intended onnly for professional persons use**.

Results are for the detection of SARS-CoV-2 IgM antibody class. Negative results do not exclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary. False positive results for CovIgM-Assay may occur due to cross-reactivity from pre-existing antibodies or other possible causes including the presence of rheumatoid factor (RF).

III. MEASUREMENT PRINCIPLE

CovIgM-Assay is an indirect ELISA that uses the 96-well plate format on which a recombinant Spike S1-RBD protein is immmobilized. The sample is applied to the test wells. The IgM antibodies present in the patient's sample are bound to the solid phase making an antigen-antibody complex. This complex is detected by the addition of a of an anti-human IgM-Fc antibody conjugated with HRP followed by an specific substrate solution to produce a measurable product colorimetrically.

IV. KIT CONTENT

Component	Quantity
Polystyrene flat bottomed 96-wells plate or 12 x 8-well strips coated with recombinant Spike-1-RBD protein	1 plate or 12 x 8-wells strips
IgM Highly positive control (HPC) (80µg/ml)	1 x 1 mL
IgM Negative control (0.078µg/ml)	1 x 1mL
10X Sample Dilution / Wash Solution	2 x 50 mL
HRP conjugated mouse anti-Human IgM-mu chain	1 x 1 ml
TMB-substrate	1 x 12 ml
Stop solution	1 x 6 mL
User manual	1

V. STORAGE

Store the kit 2-8°C upon arrival.

VI. MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Microplate reader suitable for the measurement of absorbance at 492nm
- Automatic microplate washer
- Distilled water to dilute 10X Sample Solution / Wash Solution
- Graduate cylinder to prepare Wash Solution
- Incubator 37°C
- Precision pipettes to deliver volumes of 10µL, 100µL, 200µL and 1000µL
- 10µL, 100µL, 200µL and 1000µL pipette tips
- Multichannel pipettes
- Disposable reagent reservoir
- Paper towel
- Laboratory timer
- Refrigerator to store samples and kit components
- Disposable tubes
- Centrifuge

VII. PROTOCOL

All reagents in the kit and test samples should be equilibrated to room temperature before test.

Reagent Preparation

- **Wash Solution:** Dilute 10-fold the Wash Solution with distilled water. For example, dilute 50 mL of 10X Wash Solution with 900 mL of distilled water to make 500 mL of 1X Wash Solution. Store at 2-8ºC.
  Note: If any precipitate is found in the 10× Wash Solution, incubate the bottle in water bath (up to 50°C) with occasional mixing until all the precipitate is dissolved.

- **Conjugate Preparation:** Dilute 40-fold the anti-Human IgM-mu-HRP conjugate. For example, dilute 50µL of the conjugate solution with 1950µL of 1X Wash Solution. Discard the unused conjugate solution.

Samples Preparation

Handle serum or plasma samples in accordance with NCCLS (National Committee for Clinical Laboratory Standards) guidelines for preventing transmission of blood borne infections.

- **Human Serum:** Use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000g. Run assay immediately, otherwise store aliquot sample below -20°C. Avoid repeat freeze-thaw cycle. When the human serum is tested, it should be diluted 1:100.

- **Human Plasma:** Treat blood with an anticoagulant such as citrate, EDTA or heparin. Centrifuge for 10 minutes at 1000g within 30 minutes for plasma collection. Run assay immediately, otherwise store aliquot sample below -20°C. Avoid repeat freeze-thaw cycle. When the human plasma is tested, it should be diluted 1: 100.

Controls

Two controls are provided: A high positive control (HPC) and a negative control (NC). Both are supplied ready to use. Therefore, they do not require any preparation before using in the CoVIgM-Assay. Controls should be storage at 4ºC.

Protocol

Read the instructions for use before performing the assay. Result reliability depends on strict adherence to the instruction for use. This assay is only validated for manual procedure and not for ELISA automatic systems. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates

recommended) should be carefully established on the plate layout. Perform all assay steps in the order given and paying attention to incubation times.

Handle serum or plasma samples in accordance with NCCLS (National Committee for Clinical Laboratory Standards) guidelines for preventing transmission of blood borne infections.

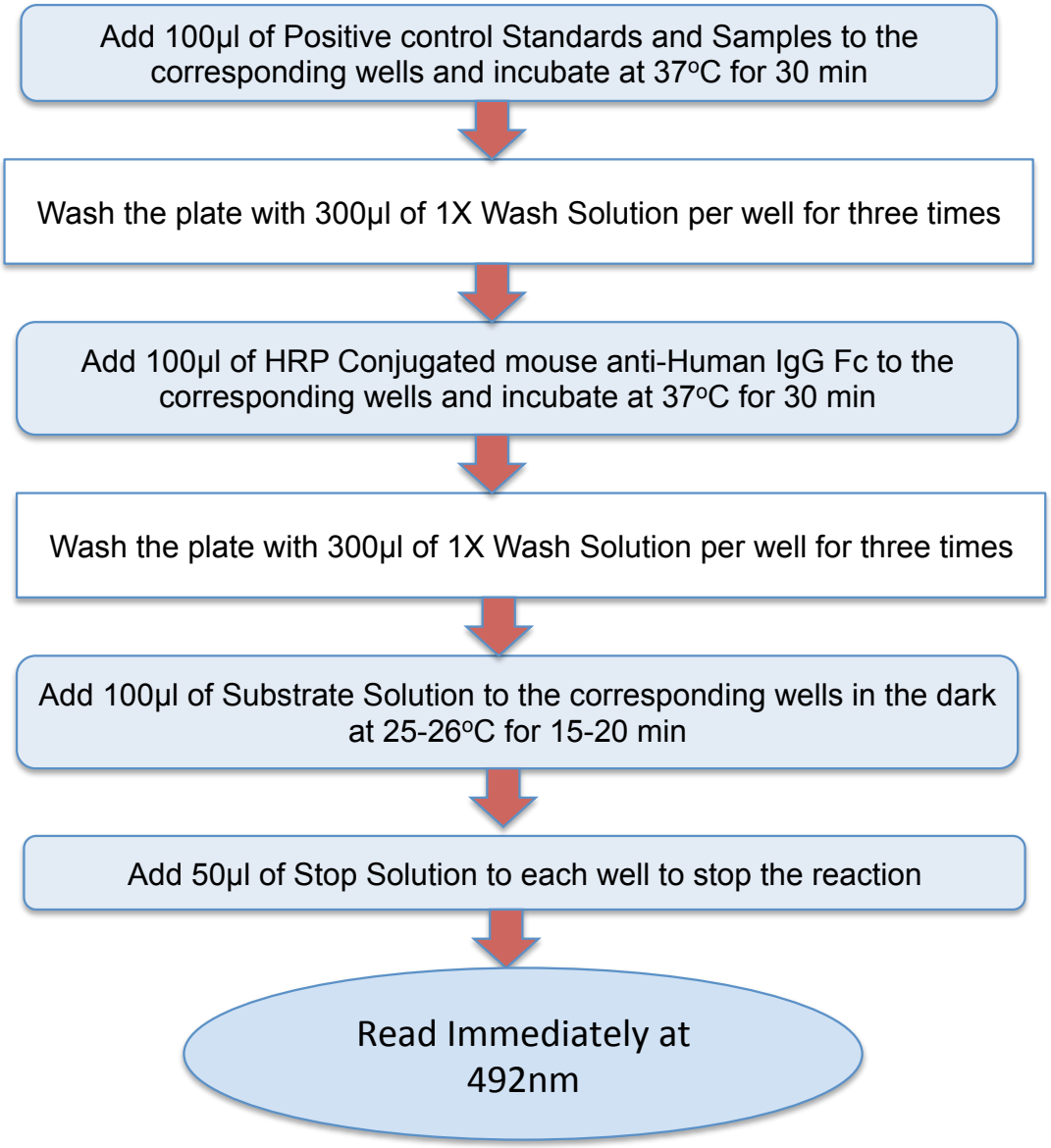
Human Serum or plasma: Use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000g. Run assay immediately, otherwise store aliquot sample below -20°C. Avoid repeat freeze-thaw cycle. When the human serum or plasma is tested, it should be diluted 1:100. The minimal amount of serum or plasma per test per subject is 1 microliter (µl).

1. Plates Sensitization
    a. Coat a 96 well plate[1] wells with 2.0µg/mL of SARS CoV-2 Spike protein[2] [100µL/well] in Coating buffer[3] ON (≤18h)
        i. Spike (S) stock |Lot P50462003| [1.2 mg/µL]
        ii. For 12.0 mL use 20µL in coating buffer
2. Wash 3 times with 300µL/well of PBS[4] T-20[5] [0.05%]
3. Blocking
    a. Add 250µL of blocking solution (BSA[6]) in PBS T-20 [0.05%] + 10% Glycerol[7] to each well
    b. Cover with sealing tape and incubate 37°C/30 min.
4. Decant/DO NOT WASH
5. Add serum or plasma dilutions
    a. Add 100µLwell of **1:100** diluted serum or plasma in PBS T-20 [0.05%] + 3% BSA
    b. For blank, add 100µL of PBS T-20 [0.05%] + 3% BSA
    c. Cover and incubate 37°C/30 min
6. Wash 3 times with 300µL/well of PBS[4] T-20[5] [0.05%]
7. Add Conjugate (IgM-HPR) dilution
    a. For IgM[8]- 1:50,000 in PBS[4] T-20[5] [0.05%]
    b. Cover with sealing tape and incubate 37°C/30 min
8. Wash 3 times with 300µL/well of PBS[4] T-20[5] [0.05%]
9. Substrate[9]
    a. Add 100µL/well of TMB solution
    b. Incubate for 15-20 min/RT/dark
    c. Add 50µL of stop solution 3M (10%) HCl[10]
10. Read at 492 nm

[1] Millipore Sigma CLS 3361
[2] GenScript Z03483-100
[3] Sigma 08058
[4] Gibco 21300-058
[5] Sigma P1379
[6] Bovine Serum Albumin, Sigma 10735086001
[7] Sigma G5516
[8] Abcam ab97205
[8] Sigma P4809
[9] Sigma Millipore ES001
[10] Sigma 258148

ASSAY PROCEDURE SUMMARY

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK											
B	BLK											
C	HPC											
D	HPC											
E	HPC											
F	NC											
G	NC											
H	NC											



VIII. TYPICAL ASSAY DATA

IgG Positive Control (µg/ml)	OD 492nm		
	Duplicate-1	Duplicate-2	Average
HPC (80.00)	1.0935	1.114	1.1037
NC (0.078)	0.006	0.005	0.0055

IX. ANALYTICAL SPECIFICITY AND SENSITIVITY

Potentially Cross-reacting Antibodies

The CovIgM-Assay was evaluated for potentially cross-reacting antibodies. We completed the cross-reactivity test by using a total of 132 specimens from 7 different categories from which 122 had been collected prior December-2019 and 10 were collected during the COVID-19 pandemic (specimens from Mycoplasma-IgM-positive subjects). One hundred twenty (120) specimens were negative and twelve (12) specimens were positive.

The data are summarized in the following table.

CATEGORY	N	POSITIVE	NEGATIVE
NO RESPIRATORY OR VIRAL INFECTION	81	4	77
RESPIRATORY ALLERGY	13	3	10
ZIKA VIRUS	5	0	5
DENGUE VIRUS	5	0	5
INFLUENZA A/B	12	1	11
RESPIRATORY SYNCYTIAL VIRUS	6	0	6
MYCOPLASMA*	10	4	6
SUB-TOTAL	**132**	**12**	**120**
COVID-19	61	55	6
TOTAL	**193**	**67**	**126**

* Samples collected during COVID-19 pandemic

This data was used to estimate the sensitivy, specificity, accuracy and positive percent agreement (PPA) and the negative percent agreement between the CovIgM-Assay and the PCR or Rapid tests comparator using a 95% confidence interval (CI). Sera from subjects with confirmed Mycoplasma infection were excluded from analysis because the possibility that these subjects have been exposed to SARS-CoV-2 is high. Although the patients had respiratory symptoms no tests were applied to rule out COVID-19.

CLINICAL AGREEMENT RESULT

		Positive Samples by PCR-based assay (n=61) / Negative samples collected before August 2019 (n= 122)		
		Positive	**Negative**	**Total**
CovIgM-Assay	**Positive**	55	8	**63**
	Negative	6	114	**120**
	Total	**61**	**122**	**183**

Sensitivity: 91.04%
Specificity: 93.89%
Positive Predictive Value: 88.41%
Negative Predictive value: 95.35%
Accuracy: 92.93%

X. PRESICION

HPC and NC were replicated 12 times on the same plate on 2 different days to evaluate intra- and inter-assay precision. Moreover 4 negative and 4 COVID-19 specimens were also evaluated in replicates of 3 in different plate positions on 3 different days.

			Repeatability (Within-Run)		**Within-Laboratory** [a]	
Sample	**N**	**Mean A_{492}**	**SD**	**% CV**	**SD**	**% CV**
NC	24	0.0058	0.002	N/A[b]	0.0026	N/A[b]
HPC	24	1.051	0.065	6.66	0.10	9.51
Negative sample-1	10	0.1302	0.059	N/A[b]	0.014	N/A[b]
Negative sample-2	10	0.0733	0.02	N/A[b]	0.022	N/A[b]
Negative sample-3	10	0.0929	0.026	N/A[b]	0.045	N/A[b]
Negative sample-4	10	0.0229	0.014	N/A[b]	0.032	N/A[b]
Positive sample-1	10	1.450	0.118	8.13	0.15	10.3
Positive sample-2	10	1.446	0.147	10.1	0.102	7.05
Positive sample-3	10	1.263	0.052	4.11	0.07	5.54
Positive sample-4	10	1.725	0.138	8.0	0.13	7.5

[a] Includes repeatability (Within-run), between-run and between-day variability

[b] Not applicable

The precision study was run based on guidance from CLSI EP05-A3 as described before[1].

[1]Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline—Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.

XI. RESULT EVALUATION

Interpretation for a Negative Result

When the OD value of a serum or plasma sample at the working dilution (1:100) is equal or less to the cut-point (OD492= 0.221), the CovIgM-Assay in the sample is inferred to be negative. A negative result in the CovIgM-Assay does not make impossible acute SARS-CoV-2 infection and should not be used as the sole basis for patients' management decisions. IgM antibody generally become detectable beginning 2-4 days following infection but may occur later and patients may remain infectious during acute infection even if IgM antibody is not present. For this reason results of CovIgM-Assay should be combined with IgG antibody detection, clinical observation, patient history, and epidemiological information.

Interpretation for a Positive Result

When the OD492 of an specimen at the dilution 1:100 is greater than the cut-point (0.221) but lower than 0.31 the sample is reported as borderline and may require re-drawn at least 21 days apart and retested with CovIgM-Assay. Samples with OD492 >0.31 are inferred as positive and if their OD492 are equal or greater than the accepted HPC range, then the sample is inferred to be strongly positive.

The table below illustrate the acepted OD492 ranges for both controls provided

Table-1. Accepted OD range for the Positive Controls	
Controls	**Valid OD Values**
HPC	0.99-2.8
NC	< 0.1

XII. TROUBLSHOOTING

Problem	Probable Cause	Solution
Poor Precision	Wells are not properly washed	Make sure that washer apparatus works properly and wells are well aspirated after each washing
	Samples have some particulates	Remove any particulate by centrifugation prior assay
	Improper preparation of Controls	Prepare new controls as described in the manual
	Pipetting error	Check pipette calibration and repeat assay
	Components are used from other lots or source	Never substitute any component from another kit
	Components are not brought to room temperature prior to assay	Repeat the assay with components that have been equilibrated at room temperature
	Incubation steps are not performed at wrong temperature	Perform incubation steps as described in the manual
Weak / No Signal	Substrate are not properly prepared, not added or added to wrong time	Follow the instruction in the manual for proper prepation and addition of substrate
	Volumen of reagents are not correct	Repeat the assay with the required volumes indicated in the manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat the assay
	The plate was not read immediately	Read the plate no later than 5 min after completing the assay

High Background	Plate is not washed properly	Make sure that washer apparatus works properly and wells are well aspirated after each washing
	Substrate buffer is contaminated	Prepare again the substrate and repeat the assay
	Evaporation of wells during incubation	Perform incubation steps with plate sealer in repeat assay or place the plate into an humidity chamber and then incubate at 37°C.