For in vitro diagnostics use only

DIAKEY COVID-19 IgM/IgG Rapid Test

Rapid differential detection kit for IgM and IgG against COVID-19 in human serum, plasma and whole blood

Code No: PF09, 10 Tests / PF092, 20 Tests / PF0910, 100 Tests

1. INTENDED USE

DIAKEY COVID-19 IgM/IgG Rapid Test Kit is a solid phase immunochromatographic assay intended for qualitative detection of IgG and IgM antibodies to SARS-CoV-2 in human serum, plasma (sodium citrate, heparin, or dipotassium EDTA), and venous whole blood (sodium citrate, heparin, or diptassium EDTA).

The DIAKEY COVID-19 IgM/IgG Rapid Test Kit is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, to perform moderate or high complexity tests.

Results are for the detection of SARS CoV-2 antibodies. IgM/IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

The sensitivity of DIAKEY COVID-19 IgM/IgG Rapid Test Kit early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for DIAKEY COVID-19 IgM/IgG Rapid Test Kit may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Due to the risk of false positive results, confirmation of positive results should be considered using second, different IgG or IgM assay.

The DIAKEY COVID-19 IgM/IgG Rapid Test Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

The DIAKEY COVID-19 IgM/IgG Rapid Test Kit has not been reviewed by FDA.

The DIAKEY COVID-19 IgM/IgG Rapid Test Kit is not for screening of donated blood.

For Prescription Use only.

2. INTRODUCTION

Coronaviruses are enveloped RNA viruses that are distributed broadly among humans, other mammals, and birds and that cause respiratory, enteric, hepatic, and neurologic diseases. Seven coronavirus species are known to cause human disease. Four viruses - 229E, OC43, NL63, and HKU1 - are prevalent and typically cause common cold symptoms in immunocompetent individuals. The three other strains - severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and 2019 Novel Coronavirus (COVID-19) - are zoonotic in origin and have been linked to sometimes fatal illness. IgG and IgM antibodies to 2019 Novel Coronavirus can be detected with 2-3 weeks after exposure. IgG remains positive, but the antibody level drops overtime.

3. PRINCIPLE OF THE ASSAY

DIAKEY COVID 19 IgM/IgG Rapid Test device is a qualitative membrane strip based immunoassay for the detection of antibodies (IgG and IgM) to Novel coronavirus in human Whole Blood/Serum/Plasma. The test device consists of: 1) a burgundy colored conjugate pad containing Novel coronavirus recombinant envelope antigens conjugated with Colloid gold (Novel coronavirus conjugates), 2) a nitrocellulose membrane strip containing two test lines (IgG and IgM lines) and a control line (C line). The IgM line is pre-coated with the Mouse anti-Human IgM antibody, IgG line is coated with Mouse anti-Human IgG antibody. When an adequate volume of test specimen is dispensed into the sample well of the test device, the specimen migrates by capillary action across the device. IgM anti-Novel coronavirus, if present in the specimen, will bid to the Novel coronavirus conjugates. The immunocomplex is then captured by the reagent pre-coated on the IgM band, forming a burgundy colored IgM line, indicating a Novel coronavirus IgM positive test result. IgG anti-Novel coronavirus if present in the specimen will bind to the Novel coronavirus conjugates. The immunocomplex is then captured by the reagent coated on the IgG line, forming a burgundy colored IgG line, indicating a Novel coronavirus IgG positive test result. Absence of any T lines (IgG and IgM) suggests a negative result. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

4. MATERIALS PROVIDED

Contents Name	Quantity (in a kit)
Device	20 ea
Sample buffer	4ml/vial x 1 ea
10ul Dropper	20 ea (10 ul)
Package insert	1 ea

5. MATERIALS REQUIRED BUT NOT PROVIDED

1. Blood collection tube
2. Centrifuge and Pipette
3. Pair of gloves
4. Timer

6. WARNINGS AND PRECAUTIONS

1. For professional In Vitro diagnostic use only. Do not use after expiration date.
2. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
3. Do not use it if the pouch is damaged or broken.
4. Test is for single use only. Do not re-use under any circumstances.
5. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal of specimens.
6. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
7. Humidity and temperature can adversely affect results.
8. Do not perform the test in a room with strong air flow, ie. Electric or strong air-conditioning.

7. STORAGE AND STABILITY

1. Store as packaged in the sealed pouch at the temperature (4-30℃). DO NOT FREEZE.
   The kit is stable within the expiration date printed on the labeling.
2. Once open the pouch, the test should be used within one hour.
   Prolonged exposure to hot and humid environment will cause product deterioration.
3. The LOT and the expiration date were printed on the labeling.

8. PROCEDURE

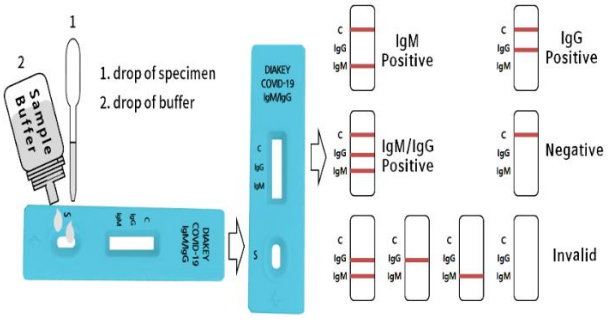
● SPECIMEN COLLECTION AND STORAGE

1. Specimen to be tested should be obtained and handled by standard methods for their collections.
2. Serum: Allow the blood to clot, then centrifuge to separate the serum.
3. Plasma: Collect the whole blood into the tube containing anticoagulants such as heparin, citrate, or EDTA. Centrifuge the blood and separate the plasma.
4. Whole blood: whole blood should be collected over heparin, citrate, or EDTA. Mix the blood by inversion and use it to the test.

● TEST PROCEDURE

1. Allow the test device and specimens (Whole Blood/Serum/Plasma) to equilibrate to temperature(15-30℃ or 59-86℉) prior to testing.
2. Remove the test device from the sealed pouch.
3. Hold the dropper vertically and transfer 1 drop of specimen (approximately 10ul) to the specimen well (S) of the test device, then add 2 drops buffer to dilute sample (approximately 70ul) and start the timer. See the illustration below.
4. Wait for colored lines to appear. Interpret the test results in 10-15 minutes. Do not read results after 20 minutes.

9. INTERPRETATION OF THE RESULTS



Positive: Control line and at least one test line appear on the membrane. The appearance of IgG test line indicates the presence of Novel coronavirus specific IgG antibodies. The appearance of IgM test line indicates the presence of Novel coronavirus specific IgM antibodies. And if both IgG and IgM line appear, it indicates that the presence of both Novel coronavirus specific IgG and IgM antibodies.

Negative: One colored line appears in the control region(C). No apparent colored line appears in the test line region.

Invalid: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Table 1: Interpretation of Results

No.	C Line	M Line	G Line	Test Result Interpretation
1	Not present	Any	Any	Invalid Test:, The specimen must be retested with another device
2	+	–	-	Valid Test, Negative for antibodies for SARS-CoV-2
3	+	+	-	Valid Test, IgM positive for antibodies for SARS –CoV-2
4	+	+	+	Valid Test, IgM and IgG positive for antibodies for SARS-CoV-2
5	+	-	+	Valid Test, IgG positive for antibodies for SARS-CoV-2

10. QUALITY CONTROL

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance. Additional controls may be required according to guidelines or local, state, and/or federal regulations (such as 42 CFR 493.1256) or accrediting organizations.

1. Internal Control: This test contains a built-in control feature, the C Line. The C Line develops after addition of the specimen and sample diluent. If the C Line does not develop, the test is invalid. Review the procedure and repeat the test with a new device.
2. Positive and Negative Control: Positive and negative controls should be tested to ensure the proper performance of the assay, particularly under the following circumstances:
   a) A new operator uses the kit.
   b) A new lot of test kits is used.
   c) A new shipment of kits is used.
   d) The temperature used during storage of the kit falls outside of 4-30℃
   e) The temperature of the test area falls outside of 15-30℃
   f) To verify a higher than expected frequency of positive or negative results.

11. LIMITATIONS OF THE TEST

1. For use under an Emergency Use Authorization only.
2. Use of the DIAKEY COVID-19 IgM/IgG Rapid Test is limited to laboratory personnel who have been trained. Not for home use or point of care (POC) use.
3. The COVID-19 IgM/IgG Rapid Test device is limited to provide a qualitative detection. The intensity of the test line does not necessarily correlate to the concentration of the antibody in the blood.
4. The test results should be interpreted 15 minutes after starting the test. The test results should not be interpreted after 20 minutes.
5. This test can only be used for the analysis of human serum, plasma (sodium citrate, sodium heparin, or dipotassium EDTA), and venous whole blood (sodium citrate, sodium heparin, or dipotassium EDTA) samples. Do not use the DIAKEY COVID-19 IgM/IgG Rapid Test with fingerstick (capillary) whole blood samples.
6. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. IgM antibodies may not be detected in the first several days of infection; the sensitivity of the Rapid COVID-19 IgM/IgG Combo Test Kit early after infection is unknown. False positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
7. A negative result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or if the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody used in the test.
8. The test may have lower sensitivity for IgG and IgM detection in symptomatic individuals prior to 15 days since symptom onset.
9. The results obtained from this test are intended to be an aid in diagnosis only. Each physician must interpret the results in conjunction with the patient's history, physical findings, and other diagnostic procedures.
10. Direct testing with a molecular diagnostic test should be performed to evaluate for acute SARS-CoV-2 infection in symptomatic individuals.
11. Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection or to determine infection status.
12. It is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity.
13. Positive results may not indicate previous SARS-CoV-2 infection. Consider other information including clinical history and local disease prevalence, in assessing the need for an alternative serology test to confirm an adaptive immune response. Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
14. A negative test result indicates that antibodies to Novel coronavirus are either not present or at levels undetectable by the test.
15. This test will only indicate the presence of SARS-CoV-2 IgM and/or IgG antibodies in the specimen.
16. The detection of SARS-CoV-2 IgM/IgG antibodies is dependent upon proper specimen collection, handling, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
17. This device has been evaluated for use with human specimen material only.
18. This test cannot rule out diseases caused by other bacterial or viral pathogens.
19. This device should not be used for the screening of donated blood.

12. PERFORMANCE CHARACTERISTICS

1. Assay Analytical Sensitivity

For samples with concentrations of 73.13 ng/mL, 39.06 ng/mL, 19.53 ng/mL, 9.77 ng/mL, and 4.88 ng/mL, add COVID-19 IgG antibody to negative serum. Test the samples with one LOT of DIAKEY COVID 19 IgM/IgG Rapid Test Kits for 5 days. The minimum concentration confirmed with 95% positive results by repeating 20 tests per sample was as follows. The limit of detection(LoD) identified was 19.53 ng/mL.

Table 2: Assay Analytical Sensitivity (IgG)

Sample con. (ng/mL)	Repeat	Number of Pos. judgments	Pos. judgment rate (%)
73.13	20	20	100
39.06	20	20	100
19.53	20	20	100
9.77	20	8	40
4.88	20	0	0

For samples with concentrations of 39.06 ng/mL, 19.53 ng/mL, 9.77 ng/mL, 4.88 ng/mL, and 2.44 ng/mL, add COVID-19 IgM antibody to negative serum. Test the samples with one lot of DIAKEY COVID 19 IgM/IgG Rapid Test Kit for 5 days. The minimum concentration confirmed with 95% positive results by repeating 20 tests per sample was as follows. The limit of detection(LoD) identified was 9.77 ng/mL.

Table 3: Assay Analytical Sensitivity (IgM)

Sample con. (ng/mL)	Repeat	Number of Pos. judgments	Pos. judgment rate (%)
39.06	20	20	100
19.53	20	20	100
9.77	20	20	100
4.88	20	5	25
2.44	20	0	0

2. Assay Cross Reactivity

Cross-reactivity of the COIVD-19 IgM/IgG Rapid Test (Whole blood/Serum/Plasma) was evaluated using serum samples which contain antibodies and antigens to the pathogens listed below. A total of 65 specimens from 13 different categories were tested. No false Positives were found with the following (Table 4):

Table 4: Assay Cross Reactivity Results

Sample Categories	Tested Sample Number
Influenza A (H1N1) virus/New Caledonia/20/99	5
Influenza A/Michigan/45/2015	5
Influenza A/Hong Kong/4801/2014	5
Influenza B/Brisbane/60/2008	5
Influenza A Virus (NP) Antibody (IgG)	5
Influenza A Virus (NP) Antibody (IgG)	5
Influenza B Virus (NP) Antibody (IgG)	5
Influenza B Virus (NP) Antibody (IgG)	5
Influenza Antigen A/California/2009	5
Influenza Antigen A/Victoria/210/2009 (H3N2)	5
Adenovirus Monoclonal Antibody (1E11) (IgG/IgM)	5
Anti-influenza A [T1-3B] (IgM)	5
Anti-influenza B [3-10B] (IgM)	5

3. Interfering Substances

COIVD-19 antibody positive serum and COVID-19 antibody negative serum were spiked with one of the following substances to specified concentrations and tested in multiple replicates. No false Positive or false Negatives were found with the following (Table 5).

Table 5: Assay Interfering Substance Results

Name of Substances	Concentration
Albumin human	2,000 mg/dL
Salicylic Acid	20 mg/dL
Hemoglobin human	500 mg/dL
Ethanol	10 mg/mL
Acetaminophen	1,000 ug/mL
Caffeine	1,000 ug/mL

Aspirin	1,000 ug/mL
Ibuprofen	1,000 ug/mL
Conjugated bilirubin	5 mg/dL
Unconjugated bilirubin	15 mg/dL
Triglycerides	500 mg/dL

4. Class Specificity

A Class Specificity Study was conducted to determine the impact of DTT treatment on the detection of IgM and/or IgG positive samples by the DIAKEY COVID-19 IgM/IgG Rapid test device (Whole blood/Serum/Plasma). IgM samples treated with DTT showed no visible IgM line with the COVID-19 IgM/IgG Rapid Test (Whole blood/Serum/Plasma), whereas the IgG samples were not affected by DTT treatment. Test results with IgM positive samples after DTT treatment showed 100% agreement to the expected results. Test results with IgG positive samples after DTT treatment showed 100% agreement to the expected results. The results observed confirm the class specificity of the test.

5. Clinical Agreement

The clinical performance of DIAKEY COVID-19 IgM/IgG Rapid Test was evaluated using retrospectively collected SARS-CoV-2 serum and plasma samples at one site in the Republic of Korea. A total of 356 samples, 243 serum samples, 49 plasma samples and 65 whole blood samples. The samples were collected from patients at one site in Republic of Korea at a time when the acute SARS-CoV-2 infection was prevalent. The DIAKEY COVID-19 IgM/IgG Rapid Test results for IgM and IgG detection were compared to the results of RT-PCR assays for SARS-CoV-2. 120 samples of positive and 236 negative samples were selected from the RNA samples that were diagnosed by the SD Biosensor STANDARD M nCoV Real-Time Detection Kit RT-PCR assays.

Respiratory samples were collected for PCR testing mostly between 1 and 7 days after symptom onset. Serum, plasma and whole blood samples were collected from the same patients for serology testing between 1 day and 30 days following PCR sample collection.

For IgM antibody detection, the positive percent agreement (PPA) of DIAKEY COVID-19 IgM/IgG Rapid Test was 80.0% (96/120) (95% CI of 72.0% - 86.2%) and negative percent agreement (NPA) was 99.6% (235/236) (95% CI of 97.6% - 99.9%). For IgG antibody detection, the PPA was 90.8% (109/120) (95% CI of 84.3% - 94.8%) and NPA was 99.2% (234/236) (95% CI of 97.0% - 99.8%).

The overall PPA (either IgM positive or IgG positive counted as positive) was 93.3% (112/120) (95% CI of 87.4% - 96.6%) and overall NPA was 98.7% (233/236) (95% CI of 96.3% - 99.6%).

Table 6: IgM Positive results stratified by days post-onset of symptoms

Infectious period (days)	PCR positive at any time	DIAKEY COVID-19 Rapid Test positive	IgM PPA	95% CI
≤ 7	21	8	38.1%	(20.8% – 59.1%)
8 – 14	23	16	69.6%	(49.1% – 84.4%)
≥ 15	76	72	94.7%	(87.2% – 97.9%)

Table 7: IgG Positive results stratified by days post-onset of symptoms

Infectious period (days)	PCR positive at any time	DIAKEY COVID-19 Rapid Test positive	IgG PPA	95% CI
≤ 7	21	13	61.9%	(40.9% - 79.2%)
8 – 14	23	21	94.3%	(73.2% - 97.6%)
≥ 15	76	75	98.7%	(92.9% - 99.8%)

Table 8: Combined Antibody Results by days post-onset of symptoms

Infectious period (days)	PCR positive at any time	DIAKEY COVID-19 Rapid Test positive	IgM/IgG Combined Antibody PPA	95% CI
≤ 7	21	15	71.4%	(50.0% - 86.2%)
8 – 14	23	21	91.3%	(73.2% - 97.6%)
≥ 15	76	75	98.7%	(92.9% - 99.8%)

Table 9: Negative results

	PCR Negative	DIAKEY COVID-19 Rapid Test negative	NPA	95% CI
IgM	236	235	99.6%	(97.6% - 99.9%)
IgG		234	99.2%	(97.0% - 99.8%)
Overall		233	98.7%	(96.3% - 99.6%)

13. SYMBOL INFORMATION

Symbol	Used for
	Caution
	Do not re-use
	Operator's manual; operating instructions
	Temperature limit
	Batch code
	Use-by date
	In vitro diagnostic(IVD) device
	Contain sufficient for <n>

14. REFERENCE

1. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. Adv Virus Res 2011; 81: 85-164.
2. Masters PS, Perlman S. Coronaviridae. In: Knipe DM, Howley PM, eds. Fields virology. 6th ed. Lippincott Williams & Wilkins, 2013: 825-58.
3. Su S, Wong G, Shi W, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 2016; 24: 490-502.
4. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019; 17: 181-192.
5. "Naming the coronavirus disease (COVID-19) and the virus that causes it". World Health Organization. Archived from the original on 28 February 2020. Retrieved 28 February 2020.
6. "Coronavirus Disease 2019 (COVID-19) Symptoms". Centers for Disease Control and Prevention.United States. 10 February 2020. Archived from the original on 30 January 2020.

15. ORDERING INFORMATION

Cat. No.	Name	Size
MF01	DIAKEY REALcheck COVID-19(nCOV) Detection Kit	100 Tests/Kit
PF09	DIAKEY COVID-19 IgM/IgG Rapid Test	20 Tests/Kit
DP01	DIAKEY REALcheck Viral RNA/DNA Prep Kit	100 Preps/Kit

Technical Assistance

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