DIAKEY COVID-19 Ag Rapid Test

Rapid differential detection kit for antigen from COVID-19 in nasopharyngeal swab

1. INTENDED USE

The DIAKEY COVID-19 Ag Rapid Test is a lateral flow immunochromatographic assay intended for the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in nasopharyngeal swab specimens from individuals who are suspected of COVID-19 by their healthcare provider.

Results are for the identification of the SARS-CoV-2 nucleocapsid protein antigen. The antigen is generally detectable in nasopharyngeal swab specimen during the acute phase of infection. Positive results indicate the presence of viral antigens, but the clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out a bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19, and confirmed with a molecular assay, if necessary for patient management.

The DIAKEY COVID-19 Ag Rapid Test is intended for use by trained clinical laboratory personnel specifically instructed and trained in vitro diagnostic procedures.

2. SUMMARY

A novel coronavirus (SARS-CoV-2) belongs to the β genus, which also includes Severe Acute Respiratory Syndrome coronavirus (SARS-CoV, 2003) and Middle East Respiratory Syndrome coronavirus (MERS-CoV, 2012). The novel coronavirus consists of four viral protein named spike (S), envelop (E), membrane (M), and nucleocapsid (N).

Common signs of a person infected with a coronavirus include fever, respiratory symptoms, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. It has spread rapidly worldwide and can cause a variety of acute and chronic diseases. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source.

Particularly, the identification of subclinical or asymptomatic cases is important to reduce or stop the infection because these individuals may transmit the virus. Therefore, it is essential to use the DIAKEY COVID-19 Ag Rapid Test to diagnose infection quickly.

3. PRINCIPLE OF THE ASSAY

The DIAKEY COVID-19 Ag Rapid Test is a lateral flow immunochromatographic assay based on the principle of the double antibody-sandwich technique using two pre-coated lines of control line (C) and test line (T) on the surface of the nitrocellulose membrane. Both lines in the result window are not visible before testing.

Monoclonal mouse anti-SARS-CoV-2 antibody is coated on the test line region and goat anti-mouse IgG on the control line, respectively. When the test specimen is applied to the specimen well (S), a specimen migrates upward by capillary action.

The SARS-CoV-2 antigens if present in the specimen will react with monoclonal mouse anti-SARS-CoV-2 antibody making an immune complex. The complex is then captured on the membrane by the pre-coated monoclonal mouse anti-SARS-CoV-2 antibody making antigen-antibody color particle complex, and a visible colored line will show up in the test line region indicating a positive result.

In the absence of SARS-CoV-2 antigens, a colored line will not form in the test line region indicating a negative result.

The solution continues to migrate to encounter a control reagent that binds a control conjugate. To serve as a procedural control, a colored line will always appear at the control line region, indicating that the test is performed properly and the test reagents of the control line are working.

4. MATERIALS PROVIDED

1. Device
2. Sterilized Swab
3. Package insert
4. Extraction Buffer Tube
5. Nozzle cap
6. Extraction buffer

5. MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer
2. Medical mask and latex gloves
3. Micropipette and disposable pipette tips
4. Specimen collection container

6. STORAGE AND STABILITY

1. Store as packaged in the sealed pouch at temperature (4 to 30℃ or 40 to 86℉). 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.

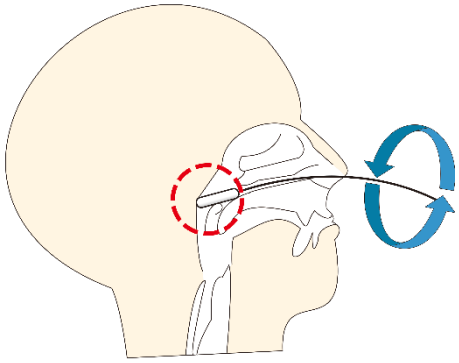
7. PREPARATION

1. Check the expiry date at the back of the device pouch. Do not use the kit, if expiry date has passed.
2. Allow the specimen and test device to equilibrate to room temperature (15 to 30°C or 59 to 86°F) 15 to 30 minutes prior to the test.
3. Check the test device and the desiccant pack in the pouch.

8. SPECIMEN COLLECTION AND PREPARATION

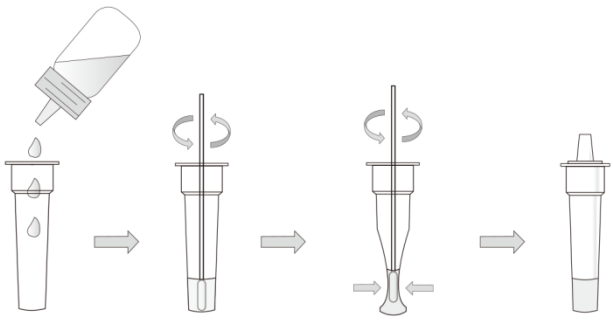
Nasopharyngeal swab

1. To collect nasopharyngeal swab sample, insert a sterilized swab into the nostril of the patient, until it reaches the nasopharynx.
2. Gently rub and roll the swab a few times against the nasopharyngeal wall.
3. Leave the swab from the nostril carefully. Specimens can be collected from both sides using the same swab, but it is not necessary.



Nasopharyngeal swab collection

4. Add 7 drops (0.3 mL) of the extraction buffer into an extraction buffer tube, and insert the swab sample into the extraction buffer tube.
5. While pressing the bottom and side of the extraction buffer tube, stir the swab more than 5 times.
6. **Leave the swab in the extraction buffer tube for one minute**. The extraction solution will be used as test specimen.
7. Remove the swab squeezing the sides of the tube to release the liquid from the swab.
8. Insert a nozzle cap into the extraction buffer tube tightly.
9. Specimen should be tested as soon as possible upon collection.



(The picture is for references only, please refer to the material object.)

VTM/UTM sample

1. Fill the extraction buffer tube with the extraction buffer up to 3 drops (~100 µl).
2. Using pipette, add 100 µl VTM (or UTM) sample in the extraction buffer tube and mix it.
3. Insert a nozzle cap into the extraction buffer tube tightly.
4. The specimen solution should be tested as soon as possible.

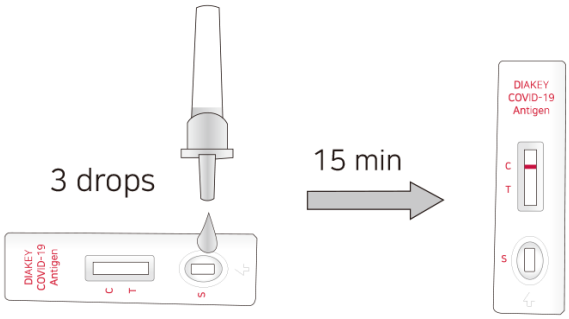
※ Please do not use the Nucleic Acid Preservation & Transport (NAPT) Medium.

※ In case of using VTM/UTM samples, avoid multiple freeze/thaw cycles.

9. TEST PROCEDURE

1. Remove the test device from the sealed pouch.
2. Reverse the tube prepared with the test specimen, holding the tube upright, transfer 3 drops (approximately 100µl) to the specimen well(S) of the test device, then start the timer. See illustration below.
3. Wait for colored lines to appear. Interpret the test results at **15 minutes**.

※ Do **not** read test results **after 20 minutes**.



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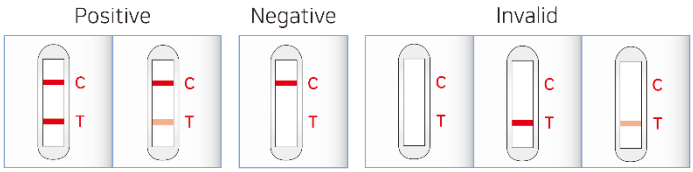
10. INTERPRETATION OF THE RESULTS



Positive: Two lines appear. One colored line should be in the control region (C), and another apparent colored line adjacent should be in the test region (T). Positive for the presence of SARS-CoV-2 nucleocapsid antigen.
Positive results indicate the presence of viral antigens but clinical correlation with patient history and other diagnostic information is necessary to determine infection status Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative: One colored line appears in the control region (C). No line appears in the test region (T). Negative results are presumptive. Negative test results do not preclude infection and should not be used as the sole basis for treatment or other patient management decisions, including infection control decisions, particularly in the presence of clinical signs and symptoms consistent with COVID-19, or in those who have been in contact with the virus. It is recommended that these results be confirmed by a molecular testing method, if necessary, for patient management.

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 using a new test device.
If the problem persists, discontinue using the lot immediately and contact your local distributor.

11. QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Control standards are not supplied with this kit. However, it is recommended that positive and negative controls be tested as good laboratory practice to confirm the test procedure and to verify proper test performance.

12. PERFORMANCE CHARACTERISTICS

1. Clinical evaluation

For the evaluation of diagnostic performance, 118 positive and 162 negative COVID-19 specimens were tested by the DIAKEY COVID-19 Ag Rapid Test. The sensitivity and specificity of the DIAKEY COVID-19 Ag Rapid Test was compared to a commercialized molecular assay.

Table 1: Summary of the sensitivity and specificity of the DIAKEY COVID-19 Ag Rapid Test compared to Real-Time PCR.

COVID-19 specimen (Medium: VTM)		Real-Time PCR		Total
		Positive	Negative	
DIAKEY COVID-19 Ag Rapid Test	Positive	114	0	114
	Negative	4	162	166
Total		118	162	280

- Positive percent agreement (PPA)
  = **96.6%** (114/118, 95% CI 91.6 – 98.7%)
- Negative percent agreement (NPA)
  = **100%** (162/162, 95% CI 97.7 – 100%)
- Overall percent agreement
  = **98.6%** ((114+162) / 280, 95% CI 96.4 – 99.4%)

2. Analytical sensitivity

Limit of Detection (LoD): $1.44x10^3$ $TCID_{50}$/mL (Heat-inactivated SARS-CoV-2)

Table 2: Analytic sensitivity of the DIAKEY COVID-19 Ag Rapid Test

Dilution	Concentration ($TCID_{50}$/mL)	Positive/Valid	Pos.%
1/50	$2.30x10^5$	3/3	100
1/100	$1.15x10^5$	3/3	100
1/200	$5.75x10^4$	3/3	100
1/400	$2.87x10^4$	3/3	100
1/800	$1.43x10^4$	3/3	100
1/1,600	$7.18x10^3$	3/3	100
1/3,200	$3.59x10^3$	3/3	100
1/6,400	$1.79x10^3$	3/3	100
1/6,993	$1.64x10^3$	20/20	100
1/8,000	**$1.44x10^3$**	**20/20**	**100**
1/9,009	$1.28x10^3$	8/20	40
1/10,000	$1.15x10^3$	0/20	0
1/12,800	$8.98x10^2$	0/3	0

3. Cross-reactivity

There was no cross-reaction with potential cross-reactive substances except SARS-CoV.

Table 3: Cross-reactivity of the DIAKEY COVID-19 Ag Rapid Test.

Organism	Concentration	Results
Influenza A (H1N1)	0.1 mg/mL	NEG
Influenza A (Michigan)	6.9 EID_{50}/mL	NEG
Influenza A (Hong Kong)	6.7 EID_{50}/mL	NEG
Influenza B	7.3 EID_{50}/mL	NEG
Influenza Antigen A	35 µgHA/mL	NEG
Influenza Antigen A (H3N2)	36 µgHA/mL	NEG
Human Coronavirus (229E)	$1.6x10^6$ $TCID_{50}$/mL	NEG
Human Coronavirus (NL63)	$1.6x10^4$ $TCID_{50}$/mL	NEG
Human Coronavirus (OC43)	$4.5x10^4$ $TCID_{50}$/mL	NEG
Inactivated SARS-CoV	2.0 µg/mL	POS
Inactivated MERS-CoV	$1.7x10^5$ $TCID_{50}$/mL	NEG
Human Metapneumovirus	$1.4x10^4$ $TCID_{50}$/mL	NEG
Human Parainfluenza virus 1	$1.6x10^4$ $TCID_{50}$/mL	NEG
Human Parainfluenza virus 2	$1.0x10^4$ $TCID_{50}$/mL	NEG
Human Parainfluenza virus 3	$1.6x10^4$ $TCID_{50}$/mL	NEG
Human Parainfluenza virus 4a	$1.6x10^4$ $TCID_{50}$/mL	NEG
Human Parainfluenza virus 4b	$5.0x10^4$ $TCID_{50}$/mL	NEG
Human Respiratory Syncytial Virus	$2.8x10^5$ $TCID_{50}$/mL	NEG
Human Respiratory Syncytial Virus (B1)	$4.5x10^4$ $TCID_{50}$/mL	NEG
Measles Virus	$1.6x10^4$ $TCID_{50}$/mL	NEG

4. Interfering substances

There was no interference for potential interfering substances listed below.

Table 4: Interfering substance of the DIAKEY COVID-19 Ag Rapid Test.

Compound	Concentration	Results
Albumin, human	2,000 mg/dL	NEG
Bilirubin	5 mg/mL	NEG
Hemoglobin, human	500 mg/dL	NEG
Triglyceride mixture	20 mg/mL	NEG
K3-EDTA	20 mg/mL	NEG
Sodium Citrate	10 mg/mL	NEG
Sodium Heparin	30 mg/mL	NEG
Whole blood	20 mg/mL	NEG
Viral Transport medium(VTM)	1.5 mg/mL	NEG
Phenylephrine hydrochloride	10 mg/mL	NEG
Dexamethasone	600 ng/mL	NEG
Beclomethasone	500 ng/mL	NEG
Benzocaine	1 mg/mL	NEG
Flunisolide	3 mg/mL	NEG
Tobramycin	20 mg/mL	NEG
Zanamivir	10 mg/mL	NEG
Oseltamivir phosphate	10 mg/mL	NEG
Ethanol	10 mg/mL	NEG
Methanol	10 mg/mL	NEG
Salicylic Acid	20 mg/dL	NEG
Acetaminophen	1 mg/mL	NEG
Aspirin	1 mg/mL	NEG
Ibuprofen	1 mg/mL	NEG

5. Hook effect

There was no hook effect at $1.15x10^4$ $TCID_{50}$/mL of heat-inactivated SARS-CoV-2.

Table 5: Hook effect of the DIAKEY COVID-19 Ag Rapid Test.

Dilution	Concentration ($TCID_{50}$/mL)	Positive/Valid	Pos.%
1/10	$1.15x10^6$	3/3	100
1/100	$1.15x10^5$	3/3	100
1/1,000	$1.15x10^4$	3/3	100
1/10,000	$1.15x10^3$	0/3	0
1/100,000	$1.15x10^2$	0/3	0

13. WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only.
2. For healthcare professionals and professionals at point of care sites.
3. The results of this product alone cannot diagnose SARS-CoV-2 infection, and it must be identified as a licensed or emergency use approved RT-PCR product, and the expert must make a final judgment considering clinical symptoms, etc.
4. Do not use after the expiration date.
5. Please read all the information in this leaflet before performing the test.
6. The test device should remain in the sealed pouch until use.
7. All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
8. The used test device should be discarded according to federal, state and local regulations.
9. When using samples from viral/universal transport media, it may cause inaccurate results due to decreasing the sensitivity of the test.
10. When using swab for collecting specimen, DO NOT use Nucleic Acid Preservation & Transport (NAPT) Medium.
11. Do not re-use the test kit.

14. 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>

15. LIMITATIONS OF THE TEST

1. The DIAKEY COVID-19 Ag 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 antigen of the specimens.
2. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions.
3. A physician must interpret the results in conjunction with the patient's history, physical findings, and other diagnostic procedures.
4. A negative result can occur if the quantity of antigens for the SARS-CoV-2 virus present in the specimen is below the detection threshold of the assay, or the virus has undergone minor amino acid mutation(s) in the target epitope region recognized by the monoclonal antibodies utilized in the test.
5. Proper specimen collection is critical, and failure to follow the procedure may give inaccurate results. Improper specimen collection, improper specimen storage or repeated freezing and thawing of specimens can lead to inaccurate results.

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Technical Assistance

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