NanoEntek

NESPI-FRCOG-001EN(V.0.1)

CE IVD

FREND™ COVID-19 Ag

Qualitative assay for COVID-19 Nucleocapsid protein

Intended use

The FREND™ COVID-19 Ag is an *in vitro* diagnostic medical device based on fluorescence immunoassay (FIA) for use with the FREND™ System. It is designed for the qualitative detection of the nucleocapsid protein of SARS-CoV-2 directly from nasopharyngeal swab specimens from individuals suspected with novel coronavirus disease 2019 (COVID-19) by their healthcare provider.

Principle of the assay

FREND™ COVID-19 Ag is a single-use fluorescence immunoassay (FIA) kit that can detect the presence of the nucleocapsid protein of SARS-CoV-2 in nasopharyngeal swab specimen via sandwich immunoassay. The lysis buffer extracts virus from the swab specimen and releases viral proteins. Within FREND™ COVID-19 Ag cartridge, the released nucleocapsid proteins are captured by antibody specific to the nucleocapsid protein from SARS-CoV-2 and detected by antibody conjugated to fluorescent micro-particles. The FREND™ System analyzes fluorescence intensity of control zone for validity of the test and the test zone for the presence of the nucleocapsid protein and displays the result on the screen after about 4 minutes of process.

Material provided

Q'ty	Contents	Catalogue number
20	Cartridges	FRCOG 020P
20	Lysis tubes	
20	Filter dropper with a specimen cup	
20	Disposable pipette tip	
20	Disposable sterile nasopharyngeal swabs*	
01	COVID-19 Ag Positive control swab	
01	COVID-19 Ag Negative control swab	
01	Code chip	
01	Package insert	

Product description manufactured by other companies*

Product name: Disposable sterile nasopharyngeal swab (Sterile flocked swab)
Model name: NFS-1
Manufacturer: Noble Biosciences, Inc.
Certificate: CE marked(Class IIa) under the supervision of Notified Body 2292
EC REP: S.B Pharma GMBH (Address: Max-Planck Str. 39a D-50858, Köln, Germany / Tel. +49-(0)2234–988-1521 / Fax. +49(0)-2234-988-1523)
Description: Disposable sterile nasopharyngeal swab is a sterile flocked swab, it is intended to collect specimens in the nasal cavity of patients being suspected of any disease. This is a single-use device and sterilized by gamma irradiation sterilization process.

Warning and Precautions

- The FREND™ COVID-19 Ag cartridges are intended for *in vitro* diagnostic use only.
- The FREND™ COVID-19 Ag cartridges are only to be used on the NanoEntek FREND™ System.
- The FREND™ COVID-19 Ag cartridges, Lysis tubes and filter droppers are disposable, single use devices. Do not reuse them under any circumstances.
- Allow sealed cartridges and Lysis tubes to come to room temperature for 15-30 minutes prior to use when stored in refrigerator.
- Cartridges and Lysis tubes should not be frozen.
- Assure the humidity in the laboratory is in the 10-80% range when tests are run.
- Avoid cross-contamination between samples by using a new pipette tip or a filter dropper for each new specimen.
- Avoid high humidity, direct sunlight or heat in the area used for cartridge storage.
- Inaccurate results are possible if the sample used is contaminated in any way.
- Inadequate pipetting or inappropriate use of the filter dropper may occur insufficient or excessive volume of lysed specimen into the cartridge which may affect test results.
- Discard and do not use any damaged or dropped cartridges.
- Do not use the cartridge after the expiration date indicated on the pouch.
- Do not use the cartridge if the pouch is damaged or the seal is broken.
- Do not use Lysis tube if leakage is found.
- Perform testing as specified in the package insert and the user manual.
- Inadequate or inappropriate sample collection, storage, and transport may bring false test results.
- Use only the provided swab for specimen collection.
- Keep the cartridge sealed in the pouch until ready to use.
- Use the cartridge immediately after opening the pouch.
- For professional use only.
- Use Universal Precautions when handling all specimens and controls. Wear disposable gloves when handling the cartridges and the samples.
- Wash hands thoroughly and often after handling reagent cartridges or samples.
- Do not ingest the silica gel packet found in the cartridge pouch.
- Do not bend cartridges.

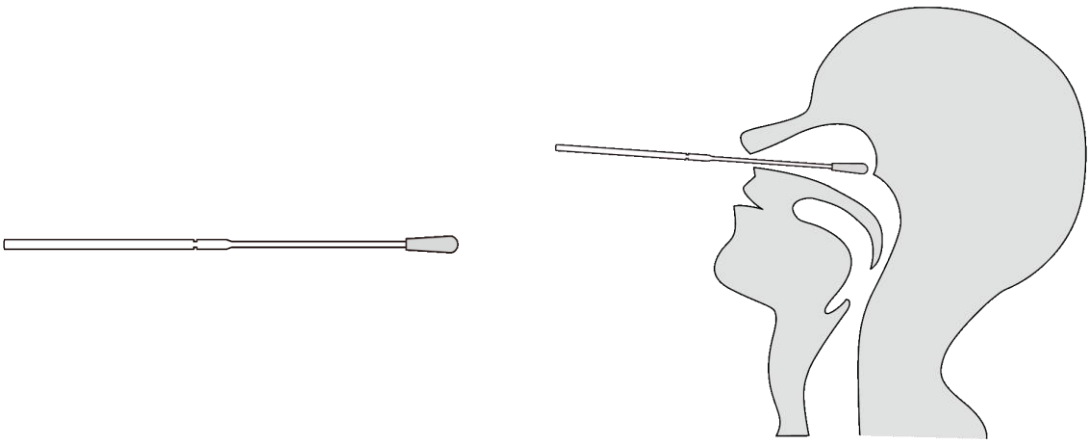
Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at 2-30℃. Reagent stability has been demonstrated for twelve months from the date of manufacture. The expiration date is clearly indicated on the product box and the cartridges.

Specimen collection and handling

1) Nasopharyngeal swab
   (1) Tilt patients' head back 70°.
   (2) Open nasopharyngeal swab in the FREND™ COVID-19 Ag kit.
   (3) Carefully insert flexible shaft through nares parallel to palate (not upwards) until resistance is met, or distance is equivalent to half the distance from the patient's ear to their nostril.

(4) Gently rub and roll the swab several times.
(5) Leave the swab in place for several seconds to absorb secretions.
(6) Slowly remove the swab while rotating it.



Procedure

Code chip installation

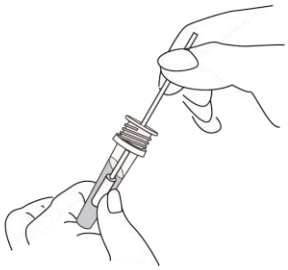
A lot-specific Code chip is supplied with each kit of FREND™ COVID-19 Ag. When using a new lot of reagent, the Code chip of the same lot must be installed in the FREND™ System. Please refer to the FREND™ System User manual for more detailed instructions relative to the Code chip installation. Abbreviated instructions are as follows:

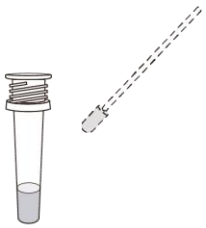
(1) Insert the FREND™ System electrical cord into an appropriate outlet.
(2) Insert the Code chip into the Code chip slot at the rear of the system following the arrows.
(3) Press the 'Setup' button on the 'Main' screen.
(4) Press the 'Code chip' button on the 'Setup' screen.
(5) The information embedded on the FREND™ COVID-19 Ag Code chip is automatically saved on the FREND™ System.
(6) When the Code chip installation is completed, press the 'OK' button to go back to the 'Setup' screen.
(7) Press the 'Item' button on the 'Setup' screen.
(8) Click the FREND™ COVID-19 Ag cartridge and check the installed lot number and the installation date of the Code chip.
(9) Press the 'Home' button to go to the 'Main' screen to begin running external quality control and specimen swabs.

Specimen processing

Allow the sealed pouches containing the FREND™ COVID-19 Ag cartridges and lysis tubes to come to room temperature for 15-30 minutes prior to use when stored in the refrigerator.
If using refrigerated patient samples, remove them from the refrigerator and allow them to reach the room temperature prior to testing.

Assay Procedure





STEP 01.

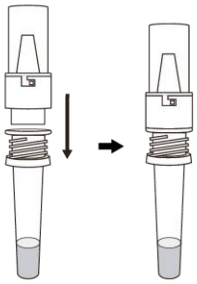
Put the swab from which the specimen was collected into the lysis tube.

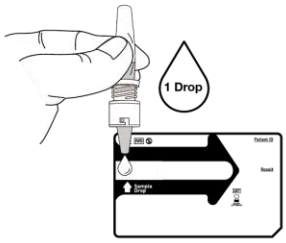
STEP 02.

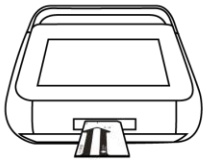
Stir the swab up and down more than 10 times to dissolve its content in the lysis buffer.

STEP 03.

Remove liquid from the swab as much as you can, and dispose the used swab in bio-hazardous waste.







STEP 05-1.

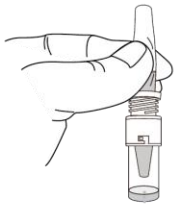
Remove the specimen cap by rotating clockwise and invert the lysis tube. Gently squeeze the tube in vertical and load only **one drop** of the sample.

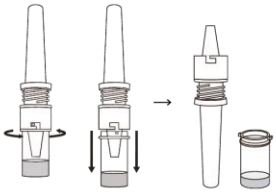
STEP 06.

Insert the cartridge into the FREND™ System.

STEP 04.

Gently push the filter dropper with a specimen cup against the lysis tube and close it tight.



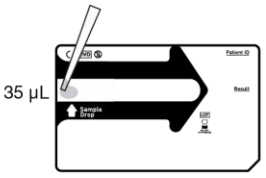


STEP 05-2.

Invert the lysis tube and gently squeeze the lysis tube until all the specimen solution inside the lysis tube dropped and collected in the specimen cup.

STEP 06.

Separate the specimen cup that is tight with lysis tube and the filter dropper by rotating counterclockwise. Take off the lysis tube from the specimen cup. Dispose the lysis tube in biohazrd waste and leave the specimen cup for the next step.



STEP 07.

Take 35 µL of a sample from the specimen cup using a pipette.

STEP 08.

Load the sample into the cartridge inlet.

STEP 09.

Insert the cartridge into the FREND™ System.

(1) Take out one lysis tube and one filter dropper and remove the cap of the tube by rotating.
(2) Put the swab from which the specimen was collected into the lysis tube following the procedure according to "Specimen collection and sampling".
(3) Stir the swab up and down more than 10 times to dissolve its content in the lysis buffer.
(4) Remove the swab riding the sides of the tube wall to remove liquid absorbed in the swab.
(5) Discard the used swab in biohazard waste.
(6) Gently push the filter dropper with a specimen cup against the lysis tube and close it tight.
Note : Do not rotate and remove the specimen cup attached on the dropper. It is required to be attached for the following procedures.
(7) In case of sample loading into the cartridge directly using filter dropper, remove the specimen cup from the filter dropper and gently squeeze the lysis tube vertically pin-pointing the tip of the dropper toward the cartridge inlet. Allow only a single drop of the specimen solution.
Note: Squeeze the lysis tube gently to prevent bubbles. A clear single drop without any bubbles should be dropped from the dropper. When loading, ensure that the tip of the dropper does not touch the cartridge inlet otherwise, the wrong volume may be loaded and cause the misreading.
(8) In case of sample loading by pipette, invert the lysis tube and gently squeeze the lysis tube until all the specimen solution inside the lysis tube dropped and collected in the specimen cup.
(9) Separate the specimen cup that is tight with lysis tube and the filter dropper by rotating counterclockwise. Take off the lysis tube from the specimen cup. Dispose the lysis tube in biohazard waste and leave the specimen cup for the next step.
(10) Take 35 µL of a sample from the specimen cup using a pipette and pipette tip and then load the sample into the cartridge inlet.
(11) Press the 'Test' button on the 'Main' screen of the FREND™ System.
(12) The system moves to the Patient ID screen automatically.
(13) Type the Patient ID and press the 'Enter' button to begin the test.
(14) Insert the cartridge into the cartridge slot using the cartridge arrows as a guide.
Caution: Please check the direction of the cartridge before insertion and assure the insertion is complete.
Caution: Please insert the cartridge into the FREND™ System after loading the sample into the cartridge.
(15) When the reaction in the cartridge is complete, the FREND™ System will automatically start the reading.
(16) After the reaction ends, cartridge will be ejected and the result will be displayed and saved.
Caution: Do not remove power from the FREND™ System while a cartridge is in the reading chamber. This may cause a system error.
(17) If the FREND™ System is connected to the printer *(optional)*, press 'Print' button and the result will be printed out.
(18) For more detailed instructions, please refer to the 'FREND™ System User manual'.

Display and Interpretation of results

Displayed result	Description
	COVID-19 Ag : "Negative" → Negative result on the presence of SARS-CoV-2 nucleocapsid protein. *Note : Negative result does not rule out false negative. Negative results from patients with symptoms should be treated as presumptive and confirmation with a molecular assay may be performed.*

	COVID-19 Ag : "Positive" → Positive result on the presence of SARS-CoV-2 nucleocapsid protein. *Note : A positive result does not rule out co-infections with other pathogens.*

Performance evaluation

Limit of Detection

The limit of detection (LoD) of the FREND™ COVID-19 Ag was determined using limiting dilutions of heat-inactivated SARS-CoV-2, USA-WA1/2020 isolate from ZeptoMetrix. To reflect the procedure of using swabs directly to collect specimen, the diluted virus (in phosphate buffer) was spiked on the nasopharyngeal swab, dried and used following the procedures in the package insert.

Sample type	LoD (Limit of Detection)
Heat inactivated SARS-CoV-2	1.47×10^2 $TCID_{50}$/mL

Interference

In the FREND™ COVID-19 Ag test, the potential chemicals as a drug or biological products that may be found in the upper respiratory tract were selected and it was confirmed that the following interfering agents did not interfere the assay on the stated concentration.

No.	Substances	Concentration	Unit
1	Whole Blood (Type A)	4	% (*w/v*)
2	Whole Blood (Type B)	4	% (*w/v*)
3	Whole Blood (Type AB)	4	% (*w/v*)
4	Whole Blood (Type O)	4	% (*w/v*)
5	Mucin from bovine submaxillary gland	1	µg/mL
6	4-Acetamidophenol	1	mg/mL
7	Acetylsalicylic acid	1	mg/mL
8	Albuterol	0.083	mg/mL
9	Amantadine Hydrochloride	900	ng/mL
10	Afrin	50	µg/mL
11	Beclomethasone	500	ng/mL
12	Budesonide	500	ng/mL
13	Benzocaine	1.5	mg/mL
14	Chlorpheniramine maleate	5	mg/mL
15	CVS Nasal Drops	1	mg/mL
16	CVS Nasal Spray	1	mg/mL
17	Dexamethasone	2.5	mg/ml
18	Diphenhydramine HCl	5	mg/mL
19	Fexofenadine	500	ng/mL
20	Fluticasone	500	ng/mL
21	guaifene sin	5	mg/mL
22	Ibuprofen	500.3	µg/mL

23	L-ascorbic acid	29.9	µg/mL
24	Loratidine	100	ng/mL
25	Mometasone	500	ng/mL
26	Mupirocin	1	mg/mL
27	Oseltamivir Phosphate	500	ng/mL
28	Pseudoephedrine HCl	2	mg/mL
29	Tobramycin	500	ng/mL
30	Triamcinolone	500	ng/mL
31	Zanamivir	1	mg/mL
32	Galphimia glauca	10	mg/mL
33	Histaminum hydrochloricum	10	mg/mL
34	Flunisolide	250	µg/mL
35	Sodium Chloride with Preservatives	0.9	% (*w/v*)
36	Phosphate buffer (pH 7.4)	50	mM

Cross-reactivity

Potential virus or the bacteria that may show similar symptoms as COVID-19 were selected and evaluated at the concentration as indicated did not show cross-reactivity in the FREND™ COVID-19 Ag test.

No.	Substances (Virus/Bacteria)	Concentration	Unit
1	Human adenovirus B (Adenovirus type 3)	3.00×10^5	PFU/mL
2	Human adenovirus B (Adenovirus type 11)	2.20×10^6	PFU/mL
3	Human adenovirus C (Adenovirus type 1)	4.00×10^7	PFU/mL
4	Human adenovirus C (Adenovirus type 5)	2.00×10^8	PFU/mL
5	Human adenovirus E (Adenovirus type 4)	4.00×10^8	PFU/mL
6	Human enterovirus A (Enterovirus Type 71)	1.00×10^5	PFU/mL
7	Human enterovirus D (Enterovirus Type 70)	4.40×10^7	PFU/mL
8	Human Rhinovirus A, Human Rhinovirus 7	8.00×10^4	PFU/mL
9	Human Rhinovirus A, Human Rhinovirus 8	1.30×10^6	PFU/mL
10	Human Rhinovirus B, Human Rhinovirus 14	6.00×10^4	PFU/mL
11	Human Rhinovirus B, Human Rhinovirus 42	5.50×10^3	PFU/mL
12	Measles virus	7.00×10^3	PFU/mL
13	Parainfluenza virus 1	1.85×10^5	PFU/mL
14	Parainfluenza virus 2	1.00×10^7	PFU/mL
15	Parainfluenza virus 3	8.00×10^5	PFU/mL
16	Parainfluenza virus 4a	4.50×10^4	PFU/mL
17	Parainfluenza virus 4b	2.60×10^5	PFU/mL
18	Human Respiratory syncytial virus A	1.20×10^6	PFU/mL
19	Human Respiratory syncytial virus B	2.30×10^5	PFU/mL
20	Human Respiratory syncytial virus	4.00×10^5	PFU/mL
21	Human Influenza A H1N1	1.55×10^5	PFU/mL
22	Human Influenza A H3N2 (A/Aichi/2/1968)	2.50×10^6	PFU/mL
23	Human Influenza A H3N2 (A/Brisbane/09/2006)	1.00×10^5	PFU/mL
24	Human Influenza B virus	6.00×10^5	PFU/mL
25	Streptococcus pneumoniae (Klein) Chester 262[CIP 104340]	1×10^6	CFU/mL
26	Mycoplasma pneumoniae Somerson et al. FH Strain of Eaton Agent [NCTC 10119]	1×10^6	CFU/mL
27	Legionella pneumophila subsp. pneumophila Brenner et al. Philadelphia-1	1×10^6	CFU/mL

28	Human alphacoronavirus (229E)	2.09 X 10^5	$TCID_{50}$/mL
29	Human corona virus Betacoronavirus (OC43)	5.25 X 10^5	$TCID_{50}$/mL
30	Human corona virus alphacoronavirus (NL63)	7.05 X 10^4	$TCID_{50}$/mL
31	MERS-CoV (heat-inactivated)	1.78 X 10^5	$TCID_{50}$/mL

Precision

As a result of the repeatability and reproducibility test for FREND™ COVID-19 Ag, all negative samples were negative, and all positive samples were positive, which met the criteria.

Clinical performance

The total of 109 clinical specimens (34 positive and 75 negative) were collected in domestic (Korea). Patients were confirmed with RT-PCR were tested with the FREND™ COVID-19 Ag.

		RT-PCR		
		Positive	Negative	Total
FREND™ COVID-19 Ag	Positive	32	0	32
	Negative	2	75	77
Total		34	75	109

- Positive Percent Agreement: 94.12% (32/34), 95% CI (78.94-98.97)
- Negative Percent Agreement: 100% (75/75), 95% CI (93.93-100.00)
- Positive Predictive Value: 100% (32/32), 95% CI (86.66-100.00)
- Negative Predictive Value: 97.40% (75/77), 95% CI (90.07-99.55)

The total of 100 clinical specimens (60 positive and 40 negative) were collected at Italy. Patients were confirmed with RT-PCR were tested with the FREND™ COVID-19 Ag.

		RT-PCR		
		Positive	Negative	Total
FREND™ COVID-19 Ag	Positive	57	0	57
	Negative	3	40	43
Total		60	40	100

- Positive Percent Agreement: 95.00% (57/60), 95% CI (85.18-98.70)
- Negative Percent Agreement: 100% (40/40), 95% CI (89.09-100.00)
- Positive Predictive Value: 100% (57/57), 95% CI (92.13-100.00)
- Negative Predictive Value: 93.02% (40/43), 95% CI (79.88-98.18)

Glossary of symbols

	Caution, warning, Consult accompanying documents	IVD	*In vitro* diagnostic medical device
REF	Catalogue number /Reference number		Temperature limitation
LOT	Lot number /Batch number		Contains sufficient for <n> tests
	Use by YYYY-MM-DD or YYYY-MM		Do not reuse
	Manufacturer		Do not use if package is damaged
EC REP	Authorized representative in the European Community		For prescription use
CE	CE marking		Irritant

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