

# SARS-CoV-2 Antigen Rapid Test Cassette (Nasal Swab)

## Instructions for Use

For Professional In-Vitro Diagnostic Use only  
Store at 2°C - 30°C



### 1. INTENDED USE

The novel coronaviruses belong to the  $\beta$  genus. COVID-19 is an acute respiratory infectious disease caused by SARS-CoV-2. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection: asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

The genome of coronavirus encodes spike protein, envelope protein, membrane protein and nucleocapsid. In the process of viral assembly, N protein binds to viral RNA and leads to the formation of spiral nucleocapsid. N protein is a highly immunogenic phosphoprotein, which is related to viral genome replication and cell signalling. Because of the conserved sequence of N protein, detection of SARS-CoV-2 N protein is of great clinical significance.

This rapid kit is used for the qualitative detection of SARS-CoV-2 nucleocapsid protein antigen (SARS-CoV-2 N-antigen) in anterior nasal swab samples.

### 2. TEST PRINCIPLE

The SARS-CoV-2 N-antigen in the sample forms a complex with the antibody labeled with colloidal gold. This complex migrates along the membrane and reaches the test region (T-line) on which a second antibody against the SARS-CoV-2 N-antigen is applied. Unbound colloidal gold migrate along the membrane to the control region (C-line) and are bound by the control region antibody. If both the T-line and the C-line are red, the test result is SARS-CoV-2 N-antigen positive; if only the C-line is red and no T-line becomes visible, the test result is SARS-CoV-2 N-antigen negative. If no C-line becomes visible, the test result is invalid and the sample must be retested with a new test cassette.

### 3. KIT COMPONENTS

Note: This kit comes in packs of 1, 5 or 25 tests. The number of items in the kit supplied will depend on which pack is purchased.

- Instructions for Use
- Test Cassettes
- Extraction Buffer Vials
- Swabs
- Extraction Tubes with Caps

### 4. WARNINGS AND PRECAUTIONS

- 4.1. For in vitro diagnostic use only. Do not use after expiration date.
- 4.2. Samples should be considered as potentially infectious. Operators should wear protective clothing, masks, gloves and are advised to take other appropriate safety precautions to avoid or reduce the risk of infection.
- 4.3. This test should be performed at 18-30°C. The test cassette and samples must be brought to room temperature before testing.
- 4.4. Follow the Instructions for Use carefully. The accuracy of the assay results cannot be guaranteed if there is any deviation from the instructions for Use.
- 4.5. Operators must handle the potentially contaminated materials safely according to local requirements.
- 4.6. Use a new clean disposable pipette/extraction tube for each sample to avoid cross contamination.

4.7. Although the test kit uses detergents in the extraction buffer which neutralize SARS-CoV-2, dispose of all samples and materials as if they were infectious waste in a biohazard waste container.

4.8. Once the test cassette is removed from the pouch, perform the test as soon as possible to avoid being humidified. The test cassette is sensitive to humidity as well as to heat.

4.9. Do not use the test cassette if the pouch is damaged or if the seal is broken.

4.10. The test cassette cannot be reused.

### 5. STORAGE CONDITIONS AND SHELF LIFE

The test kit can be stored at 2°C-30°C for 15 months from the date of manufacture. The test cassette inside the foil bag shall be used within 1 hour after opening.

### 6. APPLICABLE INSTRUMENTS

None.

### 7. SAMPLE REQUIREMENTS

- 7.1. Applicable to anterior nasal swab samples.
- 7.2. It is recommended that the samples are tested at the time of sample collection.
- 7.3. If not tested immediately, samples should be stored in a dry and clean tube tightly sealed.
- 7.4. The samples can be stored at 2-8°C for 24 hours.

### 8. MATERIALS REQUIRED BUT NOT PROVIDED

Timer.

### 9. SAMPLE COLLECTION

9.1. The test can be performed according to the standard anterior nasal swab collection procedure.

9.2. Sample collection:

- (1) Remove the swab from the pouch and hold with the handle only. Do not touch the swab tip.
- (2) Gently insert the swab up to 1 cm to 2 cm (1/2 to 3/4 of an inch) into the nostril of the patient. Roll the swab around the inside wall of nostril at least 4 times.
- (3) Repeat swab step for the other nostril with the same swab to ensure adequate sample is collected.



9.3. It is recommended that the sample is tested at the time of sample collection.

### 10. TEST PROCEDURE

Step 1: Break the tip of the single use vial with extraction buffer.

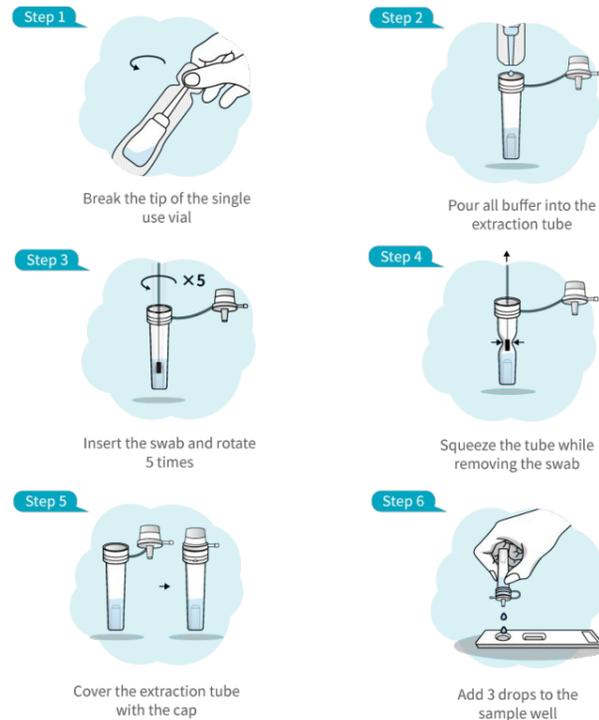
Step 2: Pour all the extraction buffer inside into the extraction tube.

Step 3: Insert the swab into the extraction tube. Rotate the swab 5 times and squeeze the sides of the extraction tube to mix the sample in the buffer.

Step 4: Remove the swab while squeezing the sides of the extraction tube to extract the liquid from the swab as much as possible. Discard the swab.

Step 5: Place the filter cap on the extraction tube and press firmly so that the cap sits tightly in the extraction tube.

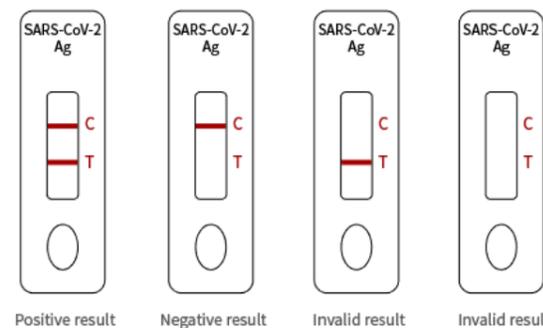
Step 6: Place the test cassette on a flat surface. Apply 3 drops of extracted sample to the sample well of the test cassette. Dispense the sample at 90 degree angle to avoid bubbles.



Step 7: After 15 minutes of development time, observe the result window for the result.

### 11. INTERPRETATION OF RESULTS

11.1 If a visible red line appears in the test region (T-line) and the control region (C-line) at the same time, the test is SARS-CoV-2 N-antigen positive; if a red line becomes visible in the control region (C-line), and no visible red line in the test region (T-line), the test is SARS-CoV-2 N-antigen negative; if there is no visible red line in the control region (C-line), regardless of whether there is a red line visible to in the test region (T-line), the test result is invalid and the sample needs to be tested again with a new test cassette.



11.2. Due to the complex structure of bioactive substances in samples and the difference of antigen antibody specificity, the possibility of false positive results cannot be completely ruled out when using this kit. If the test results are inconsistent with the clinical indications, other appropriate test methods should be used for confirmation.

11.3. The test results of this kit are only used as the basis of auxiliary diagnosis. Clinical diagnosis should be combined with clinical symptoms and other diagnostic methods.

### 12. LIMITATIONS

- 12.1 The test is intended for direct anterior nasal swab specimens. Using another sample may cause false results.
- 12.2. The accuracy of the test depends on the sample collection process. Improper sample collection, improper sample storage or repeated freezing and thawing of the sample may affect the test result.
- 12.3. A negative test result may occur if the level of antigen in a sample is below the detection limit of the test.
- 12.4. The performance of SARS-CoV-2 Antigen Rapid Test was evaluated using the procedures provided in the product insert only. Modifications to these procedures may alter the performance of the test.
- 12.5. False negative results may occur if swabs are stored in their paper sheath after specimen collection.
- 12.6. Positive test results do not rule out co-infections with other pathogens.
- 12.7. Negative test results are not intended to rule in other non-SARS viral or bacterial infections.
- 12.8. Swab sample after heat inactivation may affect the accuracy of the detection and may lead to erroneous results.

### 13. PERFORMANCE CHARACTERISTICS

13.1. Limit of Detection

13.1.1 Method

The SARS-CoV-2 Antigen Rapid Test Cassette (Nasal Swab) limit of detection (LoD) for SARS-CoV-2 was determined by evaluating different concentrations of heat inactivated viruses and recombinant N protein, respectively. Natural anterior nasal swab specimens were collected from healthy donors and confirmed negative for PCR test and were eluted in PBS. Swab elutes were combined and mixed thoroughly to create a negative clinical matrix pool to be used as the diluent. Inactivated SARS-CoV-2 virus and recombinant N protein were diluted in this natural anterior nasal swab matrix pool to generate virus and recombinant N protein dilutions, respectively, for testing. Contrived anterior nasal swab samples were prepared by adding 50 $\mu$ L of each virus dilution onto the sterile swab. The anterior nasal swab samples were tested according to the test procedure in the Instructions for Use.

13.1.2 LoD Screening

(1) LoD Screening of Recombinant N Protein

The recombinant N protein was added to the natural anterior nasal swab matrix pool to obtain samples with concentrations of 200  $\mu$ g/mL, 100  $\mu$ g/mL, 50  $\mu$ g/mL, 25  $\mu$ g/mL and 10  $\mu$ g/mL. Each sample was tested five times. The lowest concentration demonstrating 5 of 5 positives was chosen for LoD. Based on this testing, the concentration chosen was of 50  $\mu$ g/mL.

Test Concentration ( $\mu$ g/mL)	Test Cycles	Test Result
200	5	5/5 Positive
100	5	5/5 Positive
50	5	5/5 Positive
25	5	1/5 Positive
10	5	0/5 Positive

(2) LoD Screening of SARS-CoV-2 Virus

The SARS-CoV-2 virus (IVCAS 6.7512) was propagated and titrated in Vero E6 cell lines (ATCC CRL-1586). SARS-CoV-2 stock solution (2.0 $\times$ 10<sup>4</sup> TCID<sub>50</sub>/mL) (IVCAS 6.7512) that had been inactivated at 56°C for 30 minutes was diluted to 20000 TCID<sub>50</sub>/mL, 2000 TCID<sub>50</sub>/mL, 1000 TCID<sub>50</sub>/mL, 200 TCID<sub>50</sub>/mL, 100 TCID<sub>50</sub>/mL and 50 TCID<sub>50</sub>/mL anterior nasal swab samples extracts. Each sample was tested five times. The lowest concentration demonstrating 5 of

5 positives was chosen for LoD. Based on this testing, the concentration chosen was of 200 TCID<sub>50</sub>/mL.

Test Concentration (TCID <sub>50</sub> /mL)	Test Cycles	Test Result
20000	5	5/5 Positive
2000	5	5/5 Positive
1000	5	5/5 Positive
200	5	5/5 Positive
100	5	1/5 Positive
50	5	0/5 Positive

### 13.1.3 LoD Confirmation

#### (1) LoD Confirmation of Recombinant N Protein

The anterior nasal swab sample extract concentration of 50 pg/mL was tested twenty times to confirm. Twenty (20) of twenty (20) results were positive. Based on this testing, the LoD was confirmed to be 50 pg/mL.

#### (2) LoD Confirmation of SARS-CoV-2 Virus

The anterior nasal swab sample extract concentration of 200 TCID<sub>50</sub>/mL was tested twenty times to confirm. Twenty (20) of twenty (20) results were positive. Based on this testing, the LoD was confirmed to be 200 TCID<sub>50</sub>/mL.

### 13.2. Cross-reactivity Studies

Cross-reactivity of SARS-CoV-2 Antigen Rapid Test Cassette (Nasal Swab) was evaluated by testing a panel of high prevalence respiratory pathogens that could potentially cross-react with SARS-CoV-2 Antigen Rapid Test Cassette (Nasal Swab) in a negative sample. Each organism and virus were tested in triplicate. Results do not show any cross reactivity with the below listed microbial substances:

Microbial Substance	Test Concentration	Cross-reactivity (Yes/No)
Escherichia coli	1.0×10 <sup>6</sup> CFU/mL	No
Hepatitis C Virus (HCV)	1.12×10 <sup>5</sup> pfu/mL	No
Hepatitis B Virus (HBV)	1.54×10 <sup>5</sup> pfu/mL	No
Influenza B	0.7×10 <sup>6.67</sup> pfu/mL	No
Influenza A	1.05×10 <sup>5.67</sup> pfu/mL	No
Herpes Simplex Virus-1 (HSV-1)	1.12×10 <sup>5</sup> pfu/mL	No
Herpes Simplex Virus-2 (HSV-2)	1.47×10 <sup>5</sup> pfu/mL	No
Human Immunodeficiency Virus – 1 (HIV-1)	2.24×10 <sup>5</sup> pfu/mL	No
Enterovirus	2.52×10 <sup>5</sup> pfu/mL	No
Staphylococcus epidermidis	1.0×10 <sup>6</sup> CFU/mL	No
Legionella pneumophila	3.5×10 <sup>6</sup> CFU/mL	No
Chlamydia pneumoniae	1.7×10 <sup>6</sup> CFU/mL	No
Mycoplasma pneumoniae	1.5×10 <sup>6</sup> CFU/mL	No
Parainfluenza virus	1.26×10 <sup>5</sup> pfu/mL	No
Respiratory syncytial virus	1.47×10 <sup>5</sup> pfu/mL	No
Adenovirus	1.19×10 <sup>5</sup> pfu/mL	No
Cytomegalovirus (CMV)	1.4×10 <sup>5</sup> pfu/mL	No
Epstein-Barr Virus (EBV)	1.33×10 <sup>5</sup> pfu/mL	No
Varicella Zoster Virus (VZV)	1.05×10 <sup>5</sup> pfu/mL	No
Parvovirus B19	1.05×10 <sup>5</sup> pfu/mL	No
Streptococcus pneumoniae	1.0×10 <sup>6</sup> CFU/mL	No

Streptococcus pyogenes	1.6×10 <sup>6</sup> CFU/mL	No
Staphylococcus aureus	1.2×10 <sup>6</sup> CFU/mL	No
Human coronavirus 229E	1.26×10 <sup>5</sup> pfu/mL	No
Human coronavirus OC43	1.05×10 <sup>5</sup> pfu/mL	No
Human coronavirus (NL63)	1.47×10 <sup>5</sup> pfu/mL	No
MERS	1.61×10 <sup>5</sup> pfu/mL	No

### 13.3. Interference Studies

#### 13.3.1. Endogenous Interference Substances Studies

Endogenous Interference of SARS-CoV-2 Antigen Rapid Test Cassette (Nasal Swab) was evaluated by testing a panel of high prevalence respiratory pathogens that could potentially endogenous interference-react with SARS-CoV-2 Antigen Rapid Test Cassette (Nasal Swab) in a negative and a 3x LoD sample. Each organism and virus were tested in triplicate. The endogenous interference substances listed below do not interfere with the test results of the SARS-CoV-2 Antigen Rapid Test Cassette (Nasal Swab):

Interfering Substance	Concentration	Endogenous Interference (Yes/No)
Whole Blood	4%	No
Menthol	1.5 mg/mL	No
Naso GEL (NeilMed)	5% v/v	No
CVS Nasal Drops (Phenylephrine)	15% v/v	No
Afrin (Oxymetazoline)	15% v/v	No
CVS Nasal Spray (Cromolyn)	15% v/v	No
Zicam	5% v/v	No
Sore Throat Phenol Spray	15% v/v	No
Tobramycin	4 µg/mL	No
Fluticasone Propionate	5% v/v	No
Mucin	2% w/v	No
Homeopathic (Alkalol)	10% v/v	No
Mupirocin	10 mg/mL	No
Tamiflu (Oseltamivir Phosphate)	5 mg/mL	No

#### 13.3.2. Microbial Interference Studies

Microbial Interference of SARS-CoV-2 Antigen Rapid Test Cassette (Nasal Swab) was evaluated by testing a panel of high prevalence respiratory pathogens that could potentially microbial interference-react with SARS-CoV-2 Antigen Rapid Test Cassette (Nasal Swab) in a 3 x LoD sample. Each organism and virus were tested in triplicate. The following pathogens had no influence on the test results on SARS-CoV-2 antigen positive samples in the tested concentration:

Microbial Interfering Substance	Test Concentration	Interference (Yes/No)
Escherichia coli	1.0×10 <sup>6</sup> CFU/mL	No
Hepatitis C Virus (HCV)	1.12×10 <sup>5</sup> pfu/mL	No
Hepatitis B Virus (HBV)	1.54×10 <sup>5</sup> pfu/mL	No
Influenza B	0.7×10 <sup>6.67</sup> pfu/mL	No
Influenza A	1.05×10 <sup>5.67</sup> pfu/mL	No
Herpes Simplex Virus-1 (HSV-1)	1.12×10 <sup>5</sup> pfu/mL	No
Herpes Simplex Virus-2 (HSV-2)	1.47×10 <sup>5</sup> pfu/mL	No
Human Immunodeficiency Virus – 1 (HIV-1)	2.24×10 <sup>5</sup> pfu/mL	No
Enterovirus	2.52×10 <sup>5</sup> pfu/mL	No
Staphylococcus epidermidis	1.0×10 <sup>6</sup> CFU/mL	No
Legionella pneumophila	3.5×10 <sup>6</sup> CFU/mL	No

Chlamydia pneumoniae	1.7×10 <sup>6</sup> CFU/mL	No
Mycoplasma pneumoniae	1.5×10 <sup>6</sup> CFU/mL	No
Parainfluenza virus	1.26×10 <sup>5</sup> pfu/mL	No
Respiratory syncytial virus	1.47×10 <sup>5</sup> pfu/mL	No
Adenovirus	1.19×10 <sup>5</sup> pfu/mL	No
HAMA	1.4×10 <sup>5</sup> pfu/mL	No
Cytomegalovirus (CMV)	1.4×10 <sup>5</sup> pfu/mL	No
Epstein-Barr Virus (EBV)	1.33×10 <sup>5</sup> pfu/mL	No
Varicella Zoster Virus (VZV)	1.05×10 <sup>5</sup> pfu/mL	No
Parvovirus B19	1.05×10 <sup>5</sup> CFU/mL	No
Streptococcus pneumoniae	1.0×10 <sup>6</sup> CFU/mL	No
Streptococcus pyogenes	1.6×10 <sup>6</sup> CFU/mL	No
Staphylococcus aureus	1.2×10 <sup>6</sup> pfu/mL	No
Human coronavirus 229E	1.26×10 <sup>5</sup> pfu/mL	No
Human coronavirus OC43	1.05×10 <sup>5</sup> pfu/mL	No
Human coronavirus (NL63)	1.47×10 <sup>5</sup> pfu/mL	No
MERS	1.61×10 <sup>5</sup> CFU/mL	No

#### 13.4. Hook Effect

No high dose Hook effect was observed when tested with a concentration of 2.0x10<sup>6</sup> TCID<sub>50</sub>/mL of heat inactivated SARS-CoV-2 virus.

#### 13.5. Clinical Evaluation

The sensitivity of the test was determined with 129 PCR confirmed positive anterior nasal swab samples. The specificity was determined with 196 PCR confirmed negative swab samples. The sensitivity and specificity of the test were compared to a commercial PCR test. A sensitivity of 96.12% and a specificity of 99.49% were determined for the SARS-CoV-2 Antigen Rapid Test.

		PCR results	
		Positive	Negative
SARS-CoV-2 Antigen Rapid Test	Positive	124	1
	Negative	5	195
	Total	129	196
Sensitivity		96.12% (95CI: 91.19% - 98.73%)	
Specificity		99.49% (95CI: 97.19% - 99.99%)	

## 14. PROCEDURAL NOTES

- 14.1. Read the Instructions for Use carefully before performing the test.
- 14.2. Testing needs to be performed under proper testing conditions.
- 14.3. Protect the test cassette from moisture.
- 14.4. All reagents and samples should reach room temperature before use.
- 14.5. Do not use turbid or contaminated samples.

## 15. SYMBOLS

	In vitro diagnostic medical device
	Catalogue Number
	Batch Code
	Manufacturer
	Date of Manufacture
	Use by date
	Do Not Use if Package is Damaged
	Consult Instructions for Use
	Temperature Limit at 2°C 30°C.
	Contents Sufficient for <n> Cassettes
	Do Not Re-use
	Caution
	Keep Dry
	Protect from direct sunlight
	CE Mark

## 16. GENERAL INFORMATION

	Salofa Oy Örninkatu 15, 24100 Salo, Finland <a href="https://www.salofa.com">https://www.salofa.com</a>
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## 17. DATE OF ISSUE

SARS-CoV-2 Antigen Rapid Test Cassette (Nasal Swab) IFU  
Version 1, 24.05.2021