

Willi Fox – COVID-19

Antigen Test

(SARS-CoV-2)

Instructions for use

(IFU)

**Rapid test for the qualitative detection of SARS-CoV-2 antigen
in human nasopharyngeal and oropharyngeal specimens**

1. Intended use

The *Willi Fox* – COVID-19 antigen rapid test is a qualitative, membrane based immunoassay for the qualitative detection of SARS- CoV-2 viral nucleoprotein antigens in human nasopharyngeal and oropharyngeal specimens through visual interpretation of color development.

COVID-19 is the disease associated with SARS-CoV-2, which was identified in China at the end of 2019. Coronaviruses cause respiratory and intestinal infections in animals and humans.

Coronaviruses are enveloped RNA viruses that are distributed broadly among humans, other mammals as camels, cattle, cats and bats or birds. Corona viruses cause respiratory, enteric, hepatic and neurologic diseases. Six coronavirus species are known to cause human disease. Four viruses strains HCoV-NL63, HCoV-229E, HCoV-OC43 und HKU1 are prevalent and typically cause only mild upper respiratory diseases in immunocompetent individuals. Although some of them can cause severe infections in infants, young children and elderly individuals.

The two other strains SARS-CoV and MERS-CoV are highly pathogenic, zoonotic and can cause severe respiratory syndrome.

The virus is transmitted mainly via respiratory droplets that people sneeze, cough or exhale.

The incubation period for SARS-CoV-2 virus is currently estimated between two and 14 days.

Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause severe pneumonia, acute respiratory distress syndrome, kidney failure, sepsis and septic shock that can lead to the death of the patient. People with existing chronic conditions seem to be more vulnerable to severe illness.

The **Willi Fox** – COVID-19 antigen rapid test is designed for professional use only.

2. Test principle

The **Willi Fox** – COVID-19 antigen rapid test is a qualitative, membrane based immunoassay for the detection of anti-SARS-CoV-2 viral nucleoprotein antigens in human nasopharyngeal and oropharyngeal specimens through visual interpretation of color development.

Anti-SARS-CoV-2 antibodies are immobilized in the test line region (T) of the membrane. A specimen is added to an extraction tube containing buffer in order to release SARS-CoV-2 antigens. During the test, extracted antigens bind to anti-SARS-CoV-2 antibodies conjugated to colored particles and precoated onto the sample pad of the test cassette. The mixture then migrates along the membrane chromatographically by capillary action and interacts with the reagents on the membrane. The complexes are then captured by anti-SARS-CoV-2 antibodies in the test line region (T). Excess colored particles are captured in the control line region (C). The presence of a colored line in the test line region (T) indicates a positive result. The absence of a colored line in the test line region (T) indicates a negative result.

The presence of a red band(s) in the test region(s) indicates a positive result, while its absence indicates a negative result. A red band at the control region (C) serves as a procedural control, indicating that membrane wicking is working and that the proper volume of specimen has been added.

3. Material provided

- Individually packed test devices* *Kit with single-pouched test cassettes in foil pouches together with desiccant – The desiccant is not a test component; please give it to the waste!*
- Individually packed swaps
- 1 extraction Buffer**
- Extraction tubes
- Nozzles with filter
- Tube stand/ Workbench
- Package insert Information for user

*containing the preservative sodium azide: <0.1% **containing preservatives sodium azide: <0.1 mg/mL

4. Materials required but not provided

- Timer

5. Storage and stability

- The **Willi Fox** – COVID-19 antigen rapid test should be stored at 2-30°C and is stable until the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use.
- Do not freeze!
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

6. Precaution

- *For professional in-vitro diagnostic use only.*
- *Dispose the used test device according to the local regulations.*
- *Carefully read through the test procedure prior to testing.*
- *Wear appropriate personal protective equipment, such as face mask, isolation gown, gloves and eye protection during specimen collection, preparation and test procedure.*
- *Handle all specimens as if they contain infectious agents. Observe established precautions for microbiological risks throughout all procedures and standard guidelines for the appropriate disposal of specimens.*
- *Further specimen processing and patient management should follow local COVID-19 guidelines and regulations.*
- *Do not use kit or components after the expiration date.*
- *The device contains material of animal origin and should be handled as a potential biohazard.*
- *The test device should remain in the sealed pouch until ready to use.*
- *Do not use the buffer if it is discolored or turbid. Discoloration or turbidity may be a sign of microbial contamination.*
- *Do not use test of pouch, which has been damaged.*
- *Humidity and high temperature can adversely affect results.*
- *All specimens might be potentially infectious. Proper handling and disposal methods should be established. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.*
- *Do not eat, drink or smoke in the area where the specimens or kits are handled.*
- *Avoid skin contact with all components containing sodium azide which is a skin irritant.*
- *Tests are for single use only.*
- *Do not substitute or mix components from different test kits*
- *Viral isolation in cell culture and initial characterization of viral agents recovered in cultures of SARS-CoV-2 specimens are NOT recommended, except in a BSL3 laboratory using BSL3 work practices*

7. Specimen collection and preparation

Specimen Collection:

The **Willi Fox** – COVID-19 antigen rapid test is for testing of secretion samples from upper or respiratory tract.

Observe established precautions for microbiological risks throughout all procedures and standard guidelines for the appropriate disposal of specimens!

As sample for the **Willi Fox** – COVID-19 antigen rapid test you can use:

- Nasopharyngeal swab
- Oropharyngeal swab

Attention:

- Do not use specimens that are obviously contaminate with blood, as it may interfere with the flow of sample with the interpretation of test results.
- Use freshly collected specimens for best test performance.
- Rapid tests will have more reliable clinical performance when performed early in the course of infection.
- For best performance only use the swaps delivered with this kit.

7.1 Nasopharyngeal swap

Attention: Use the swaps provided with this kit.
Use only synthetic fiber swabs with plastic shafts.
Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit further testing.

Sample collection:

1. Insert the swab into the nostril with the most secretion.
2. Insert the swab into the nostril, parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. Gently rub and roll the swab. Leave swab in place for several seconds to absorb secretions.
3. Slowly remove the swab while rotating it. Specimens can be collected from both nostrils using the same swab, but it is not necessary to collect specimens from both sides if the tip is saturated with fluid from the first collection.

Specimen storage: If not tested immediately store the swap with the collected specimen in a sterile tube with max. 1ml viral transport media at 2-8°C for 24 hours after collection.
Store nasopharyngeal samples and oropharyngeal samples in separate tubes.

7.2 Oropharyngeal swap

Attention: Use the swaps provided with this kit.
Use only synthetic fiber swabs with plastic shafts.
Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit further testing.

Sample collection: Gently insert a sterile swab into the pharynx and collect secretions by brushing the swab several times against the reddened posterior pharyngeal wall and both tonsillar pillars. Avoid touching the tongue, teeth and gums.

Specimen storage: If not tested immediately store the swap with the collected specimen in a sterile tube with max. 1ml viral transport media at 2-8°C for 24 hours after collection.
Store nasopharyngeal samples and oropharyngeal samples in separate tubes.

Specimen storage and shipment:

- Swab specimens should be tested immediately after collection. Use freshly collected specimens for best test performance.
- If shipment is required, ship the specimen on ice pack

8. Test procedure and results

- **We recommend performing the COVID-19 test, if possible, in the early phase of infection.**
- **From the second day of the disease, the virus concentration in the patient should be sufficient for detection.**
- **At the moment it is considered certain that SARS-CoV-2 viruses can be detected in the throat swab 4 - 10 days after the onset of symptoms. If the disease is severe, this time can be significantly longer. (Robert Koch Institute Berlin)**

8.1. Preparation

1. Bring tests, specimens, buffer, and/or controls to room temperature (15-30°C) before use.

Remove the test device from the sealed pouch and place the **Willi Fox** – COVID-19 antigen test device on a clean and level surface.

For each specimen swab place a clean extraction tube into the designated area of the reagent holder and label the tube with the patient identification. For best results, the assay should be performed within one hour.

2. Mix the buffer gently by carefully swiveling the bottle. Holding the buffer bottle vertically and without touching the edge of the tube, add **10 drops** to the extraction tube



To prevent cross-contamination, avoid contact of the buffer vial with the swab or the extraction tube.

8.2. Test procedure

1. **Insert the sterilized swab with the specimen into the extraction tube.** Mix well by rotating the swab. Squeeze the swab against the inner wall of the extraction tube.

Let the swab soak up the liquid again and squeeze it out again. For best results repeat this process several times.



2. Let the solution **stand** with the swab **for 2 minutes**.
3. Now **remove the swab** by pressing it against the inner wall of the extraction tube again to keep **as much liquid as possible** in the tube.

Dispose of the swab in accordance with the regulations for infectious, biological waste!

4. Place the supplied nozzle with the built-in filter on the extraction tube.



5. Add 2 drops (approx. 100 µl) of the previously obtained extraction solution to the sample well of the test cassette by gently squeezing the tube.



Avoid air bubbles in the sample well of the test cassette and do not add liquids to the test result window.

8.3. Read Result

Read the results after **exactly 15 minutes**.

Do not read results after more than 15 minutes!

Interpretation of results

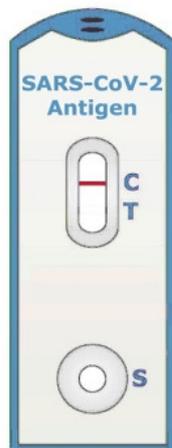


COVID-19 positive:

Two colored lines appear on the membrane.

One line appears in the control region (C) and another line appears in the test region (T).

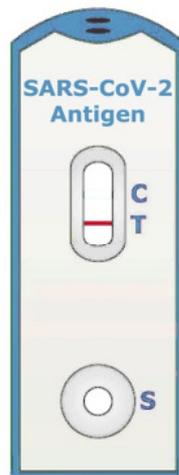
Attention: The intensity of the color in the test line region will vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered positive.



COVID-19 negative:

One colored lines appears on the membrane in the control line region (C).

No colored lines appear in the test regions.



Invalid:

No colored line appears in the control region (C).

The test is **not valid**. Review the procedure and repeat with a new test.

- Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.
- If the problem persists, discontinue using the test kit immediately and contact your local distributor.

9. Quality control

Internal procedural control:

As internal procedural control the *Willi Fox* – COVID-19 antigen rapid test includes the control line. It is only formed if sufficient specimen volume has been added and the chromatography has been finished successfully.

External procedural control:

Control standards are not supplied with this kit; yet, we recommend that positive and negative controls should be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

10. Limitations of the test

- The *Willi Fox* – COVID-19 antigen rapid test is for professional in vitro diagnostic use, and should only be used for the qualitative detection of SARS-CoV-2 antigens.
- Viable and nonviable SARS-CoV-2 viruses are detectable with the *Willi Fox* – COVID-19 antigen rapid test.
- No meaning should be inferred from the color intensity or width of any apparent lines.
- Improperly sample collections can give false negative results. The accuracy of the test depends on the quality of the sample. False negative results can result from poor specimen collection or storage.
- The *Willi Fox* – COVID-19 antigen rapid test should not be used as the sole criteria for the diagnosis of COVID-19. A negative result at any time does not preclude the possibility of SARS-CoV-2 infection.
- If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. Like with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
- As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
- Positive test results do not rule out co-infections with other pathogens.
- The *Willi Fox* – COVID-19 antigen rapid test performance has not been established for monitoring antiviral treatment or for identifying cell cultures.

- A high-dose "hook effect" can occur if the color intensity of the test line decreases while the antigen concentration increases. If a "hook effect" is suspected, diluting the samples can increase the color intensity of the test line.
- Failure to follow the instructions for use when performing and evaluating the test can affect the result and / or invalidate the result.

12. Expected Values

SARS-CoV-2 viral particles are normally present in the respiratory tracts of COVID-19 patients. A positive test result can indicate an acute infection. Virus concentrations in nasopharyngeal and oropharyngeal swab specimens may vary in the course of the disease and might fall below the detection limit of rapid tests, even though patients are still showing symptoms. Conversely, the virus might continue to be detectable over long periods of time even in convalescent patients. Possible infectiousness of test subjects cannot be ruled out based on negative test results.

12. Test Performance

A. Limit of detection

The limit of detection was determined with a quantified SARS-CoV-2 virus and has been evaluated at $2 \times 10^{2.4}$ TCID₅₀/ml.

The limit of detection was also determined with a recombinant SARS-CoV-2 nucleoprotein and has been evaluated at 0,4 ng/ml.

B. Analytical sensitivity and specificity

Clinical evaluation was performed to compare the results obtained by *Willi Fox* – COVID-19 antigen rapid test and RT-PCR. The results were summarized below:

		PCR positive	PCR negative	Total
<i>Willi Fox</i> – COVID-19 antigen rapid test	SARS-CoV-2 positive	120	0	120
	SARS-CoV-2 negative	3	161	164
	Total	123	161	284

Relative sensitivity: 97,6% (93,1%-99,2%)*
Relative specificity: >99,9% (97,7%-100%)*
Overall agreement: 98,9% (96,9%-99,6%)*

*95% Confidence Interval

C. Cross-Reactivity

Serum or plasma specimens from individuals with infections, which are common in regions where the **Willi Fox** – COVID-19 antigen rapid test would be used, were tested for interference. These infections included unrelated viral infections and other unrelated microbial infections.

The following organisms showed no interference with the **Willi Fox** – COVID-19 antigen rapid test:

HCoV-HKU1	Influenza A (H5N1)	Enterovirus (Coxsackie A virus, Coxsackie B virus, EV71)
HCoV-OC43	Influenza A (H7N9)	Norovirus
HCoV-NL63	Influenza A (H7N7)	Mumps virus
HCoV-229E	Influenza B Victoria lineage	<i>Legionella pneumophila</i>
MERS-CoV	Influenza B Yamagata lineage	<i>Mycoplasma pneumoniae</i>
Canine CoV	Respiratory syncytial virus	<i>Chlamydia pneumonia</i>
Feline CoV	Adenovirus	<i>Streptococcus pyogenes</i>
TGEV	Parainfluenza 1/2/3/4 virus	<i>Streptococcus agalactiae</i>
Influenza A (H1N1)pdm09	Human metapneumovirus	Group C <i>Streptococcus</i>
Influenza A (H3N2)	Rhinovirus	Epstein-Barr virus
<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Bordetella parapertussis</i>

None of the substances tested interfered in the assay.

D. Interfering Substances

The following substances, normally present in respiratory specimens or artificially introduced into the respiratory tract, were evaluated at the concentrations listed below and showed no interference with the *Willi Fox* – COVID-19 antigen rapid test.

Substances	Concentration	Substances	Concentration
3 OTC nasal sprays	10%	Guaiacol glycerol ether	20 mg/ml
3 OTC mouthwashes	10%	Mucin	1%
3 OTC throat drops	10%	Mupirocin	250 µg/ml
4-acetamidophenol	10 mg/ml	Oxymetazoline	10 mg/ml
Acetylsalicylic acid	20 mg/ml	Phenylephrine	10 mg/ml
Albuterol	20 mg/ml	Phenylpropanolamine	20 mg/ml
Chlorpheniramine	5 mg/ml	Relenza ® (zanamivir)	20 mg/ml
Dexamethasone	5 mg/ml	Rimantadine	500 ng/ml
Dextromethorphan	10 mg/ml	Tamiflu ® (oseltamivir)	100 mg/ml
Diphenhydramine	5 mg/ml	Tobramycin	40 mg/ml
Doxylaminesuccinate	1 mg/ml	Triamcinolone	14 mg/ml
Flunisolide	3 mg/ml		

13. Literature

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14. Symbols

REF	Article number		For single use only
LOT	Lot number		Expiry date
	Storage		Content
IVD	Only for in vitro diagnostics use		Instructions for use



All *Willi Fox* – COVID-19 antigen rapid tests are manufactured and distributed by:

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