Instructions for use
InviScreen® SARS-CoV-2 RT-PCR Test for Wastewater

INVITEK diagnostics

ALS Life Sciences Portugal, S.A.
Zona Industrial de Tondela, ZIM II, Lote 6,
3460-070 Adiça - Tondela, Portugal

Phone: +351 232 817 817
info@invitek.com
invitek.com

REF 6011002200 | Σ 100 tests | -25 °C -15 °C | RUO

1. Intended use

InviScreen® SARS-CoV-2 RT-PCR Test for Wastewater is designed for the qualitative detection of SARS-CoV-2 RNA in wastewater samples. It provides a reliable and sensitive method to identify the presence of the SARS-CoV-2 virus in wastewater, aiding in the monitoring and surveillance of viral spread within communities. This kit is intended for use by trained laboratory professionals in authorized laboratories or facilities equipped with appropriate biosafety measures. It is specifically developed for the detection of SARS-CoV-2 RNA in wastewater samples, which can serve as an indicator of viral shedding from infected individuals in a population. The kit utilizes a real-time reverse transcription polymerase chain reaction (RT-PCR) technique to amplify and detect specific target regions of the SARS-CoV-2 genome. The recommended targets and primers are based on established guidelines from the World Health Organization and Centers for Disease Control and Prevention. The InviScreen® SARS-CoV-2 RT-PCR Test for Surfaces is intended for research use only and should not be used for diagnostic or therapeutic purposes.

2. Product description

This kit has been developed to specifically detect highly conserved regions of the SARS-CoV-2 genome, namely nucleocapsid (N) phosphoprotein gene. In addition, the kit includes a set of primers and probes to detect process control virus provided with the kit to be used as extraction control. The detection of amplified DNA fragments is achieved using different fluorescence channels (FAM and HEX) that are available in common real-time thermocyclers. The multiplex detection system enhances amplification accuracy, simplifies the reaction set-up, and ensures sensitivity and specificity specifically for SARS-CoV-2, differentiating it from other known coronaviruses.

3. Kit contents

REF.	COMPONENT	FUNCTION	CAP COLOR	QUANTITY
B02.01	Primer/Probe Mix[1,2]	Targeted detection		1 tube, 500 µL
B02.02	Enzyme Mix [2]	Reverse Transcription Amplification		1 tube, 1000 µL
B02.03	Negative Control	Negative Control		1 tube, 100 µL
B02.04	Positive Control	Positive Control		1 tube, 100 µL
B02.05	Process Control Virus	Extraction Control		4 tubes, 250 µL

[1] N gene and process control virus primer/probe mix

[2] Reagents are supplied with a 5% of extra volume

4. Storage

Reagents should be stored sealed at -20 ± 5°C and may be used until the expiration date shown on the package label. Expiry date refers to the product under rightful handling and storage conditions. It is not recommended the use of the kit after the expiry date stated on the box. Avoid unnecessary repeated freeze/thawing cycles. Protect reagents from light exposure to prevent degradation.

5. Equipment and materials required (not provided)

- Viral RNA Extraction Kit [3]
- Real-Time PCR instrument [4]
- Plates and/or tubes for RT-PCR
- 1.5 mL microcentrifuge tubes
- PCR cabinet [5]
- Micropipettes (10, 200 and 1000 µL) and filter tips
- Vortex
- Microcentrifuge

[3] It is the user's responsibility to choose extraction methods relevant to the type of samples tested.
[4] The assay was validated on a Bio-Rad CFX96.
[5] To minimize the risk of contamination and/or degradation of RNA, it is recommended that the PCR reaction set-up is performed in a controlled environment.

6. Warnings and/or precautions to be adopted

- Carefully read the Instructions for Use before using the kit. This product is intended for Research Use Only.
- The assay must be performed by competent personnel, qualified in molecular biology laboratory techniques applied to diagnosis.
- The InviScreen® SARS-CoV-2 RT-PCR Test for Wastewater is intended for the detection of SARS-CoV-2 viral RNA and is not intended for use for the detection of any other viruses or organisms.
- Biological samples must be handled as being potentially infectious, following appropriate biosafety precautions in accordance with requirements and/or applicable legislation.
- Do not use the kit or any of its components after the expiration date.
- Discard plates immediately after testing is complete. Plates should always be disposed of in a suitable biohazard container after use.
- To extract RNA from the environmental samples, the use of RTP® Pathogen Kit and InviSorb® Spin Universal Kit is highly recommended.

7. Test Procedure

a. Sample Preparation

For the sample preparation, add 10 µl of process control virus to 100 mL of water sample before centrifugation and/or filtration. This must be performed before RNA extraction. At the same time, it is highly recommended to prepare a process blank by adding 10 µL of process control virus to a SARS-CoV-2-free water and process along with the samples to be analysed.

b. PCR Reaction Preparation

Allow all reagents to thaw at room temperature and centrifuge briefly to avoid entrapment of droplets in the tube cap. For each reaction, prepare the reaction mixture on ice according to the table below:

REAGENT	VOLUME
Enzyme Mix	10 µL
Primer/Probe Mix	5 µL
Total Volume	**15 µL**

1. Homogenize the reaction mixture and pipette 15µL into individual wells according to the predicted PCR plate configuration.

2. Add 5µL of viral RNA extract to each well.

At least one positive control reaction and one negative control reaction must be included in the PCR run, replacing the sample in these wells with 5 µL of Positive Control and 5 µL of RNase/DNase free water, respectively. If a process blank was performed, add 5 µL of RNA extract to a specific well.

It is recommended to prepare the reaction mixture carefully in a controlled environment, preferably in a nucleic acid-free zone. The addition of the positive control and sample RNA should preferably be carried out in a separate room.

c. Amplification Protocol

The amplification conditions are as follows:

	STEPS	TEMPERATURE	TIME	CYCLES
1	Reverse Transcription	50 °C	20 min	1
2	Enzymatic Activation	95 °C	5 min	1
3	Denaturation	95 °C	10 seg	40
4	Hybridization/extension plate reading *	58 °C	45 seg	

*Fluorescence data must be obtained during this step through FAM (N gene) and HEX (process control virus).

d. Results Interpretation

A result is considered positive when Ct ≤ 36.

RESULT	N Gene (FAM)	Process Control Virus (HEX)	Actions
Positive	+	+	—
Negative	-	+	—
Inconclusive	-	-	Dilute sample 1:5 or repeat Real-Time PCR reaction using a superior amount of RNA template.

Analytical sensitivity: 100 copies/100 mL

Note: Analytical sensitivity depends on sample volume, elution volume, viral RNA extraction method, and other factors.

8. Quality Control

The test can only be considered valid under the following control conditions:

CONTROLS	N Gene (FAM)	Process Control Virus (HEX)
Positive Control	+	+
Negative Control	-	-
Process Blank [6]	-	+

[6] Highly recommended

The negative control and positive control play essential roles as calibrators in this kit and should be included in all assays. Failure to meet any of the mentioned criteria renders the test invalid. In such instances, it is necessary to verify the conditions of the equipment, reagents, and protocol, and repeat the assay accordingly.

9. Performance Characteristics

Specificity/Selectivity: The specificity of the kit is first and foremost ensured through the selection of primers and hydrolysis probes, as well as optimization of reaction conditions. Additionally, method specificity was evaluated using internal reference materials and specimens received in the laboratory for analysis and identified by other techniques. The list of internal reference materials includes a control group of pathogens (not detected by the kit) that may be associated with clinical respiratory specimens. None of the pathogens tested with the kit were cross-reactive, such as Infectious Bronchitis Virus (D388 strain), Infectious Bronchitis Virus (1/96 strain), Avian Orthoreovirus, Porcine reproductive and respiratory syndrome virus (DV strain), Porcine reproductive and respiratory syndrome (Leystad Virus - EU strain), Porcine reproductive and respiratory syndrome (VR-2332 - US strain), Infectious Bursal disease Virus (D78 strain), Infectious Bursal disease Virus (W2512 strain), Newcastle disease virus (LaSota strain), Fowl aviadenovirus (Type 1 and 2), Avian rhinotracheitis (ART), Infectious laryngotracheitis (ILT), Adenovirus, Escherichia coli (NCTC 9001), *Legionella pneumophila* (ATCC 33152), *Klebsiella pneumophila* (MR35 isolate), Hepatovirus A (HAV) and Norovirus (Group I and II).

Analytical Sensitivity and Limit of Detection: The Limit of detection (LOD) of the method was determined using quantified SARS-CoV-2 positive sample. The SARS-COV-2 RNA in positive samples was quantified by Digital PCR. The SARS-CoV-2 positive sample was adjusted to $1x10^5$ copies/µL using nuclease free water and serial diluted to $1x10^2$ copies/µL of SARS-CoV-2. Different concentration of SARS-CoV-2 sample were spiked with the respective process control virus sample($1x10^5$ copies/µL).
The LOD is often matrix dependent, and the sensitivity of the analysis may be reduced depending on the total RNA extracted, but also its quality. Under optimal conditions, the lowest amount of SARS-CoV-2 RNA detected in 100% of the experiments was at least $1x10^2$ copies/100 ml of wastewater.

10. References

[1] https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance
[2] https://www.cdc.gov/coronavirus/2019-ncov/lab/virus-requests.html
[3] https://www.dgs.pt/directrizes-da-dgs/orientacoes-e-circulares-informativas/orientacao-n-0152020-de-23032020-pdf.aspx