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bKIT SARS-CoV-2 Environmental

P/N bKTE-SCV2.01

V 1.7

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Index

Introduction	3
Principle.....	3
Method validation.....	3
bKTE-SCV2.01 content.....	4
Number of tests VS number of samples	4
Storage	4
Shelf life.....	4
Additional material/reagents.....	4
Workflow.....	5
Reading the results.....	9
Troubleshooting	10
Safety information	10
Important Use Information	11
What Can Sars-Cov-2 Environmental bKIT (bKTE-SCV2.01) NOT Do?.....	11

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Introduction

SARS-CoV-2 is a novel strain of the family of coronaviruses that was never identified before December 2019. SARS-CoV-2 is an ssRNA virus that appertain to the family of coronavirus, genus *Betacoronavirus* and that can induce in humans a disease called COVID-19.

The presence of SARS-CoV-2 in certain environments (surface and fomites) has been demonstrated and can be stable for several hours to days¹. For this reason, the availability of a rapid test method able to detect small viral traces in the environment is of extreme importance. Hyris Ltd developed a genetic assay based on the EUA sequences designed by CDC and approved by WHO to test environmental surfaces for the detection of SARS-CoV-2. The test can be used to screen industrial and working environments, to protect the health of the employees and to reduce the risk of an interruption of key services.

The genetic detection of pathogens on surfaces has some critical aspects, including the possible presence of sanitizer substances that can inhibit molecular reactions. Moreover, ensuring a correct sample handling before the analysis can be even more critical than with test on patients, where human genetic material can act as a known reference control.

The solution here proposed was developed to address all these attention points.

1 N VAN DOREMALEN, ET AL. AEROSOL AND SURFACE STABILITY OF HCoV-19 (SARS-CoV-2) COMPARED TO SARS-CoV-1. THE NEW ENGLAND JOURNAL OF MEDICINE. DOI: 10.1056/NEJMc2004973 (2020).

Principle

The procedure allows to detect the viral RNA of SARS-CoV-2 by a hydrolysis probe Real-Time RT PCR (qPCR) reaction. The product is intended for research purpose only and can be used to monitor the presence of SARS-CoV-2 on surfaces and. This kit is **NOT for human diagnostics**.

Primers and probes targeting Virus RNA are designed according to the CDC guidelines published on Feb 4th, 2020. An additional set of primers and probe is designed and added to detect a nuclease-resistant RNA that can be added to the sample before processing and acts as process control.

Method validation

The guidelines followed to develop this assay, including primers and probes sequences specification, have been [validated by the CDC](#) and [approved by WHO](#) on January 17th 2020.

The assay was successfully tested on the bCUBE platform, using as positive control samples both synthetic RNA sequences corresponding to the target regions of SARS-CoV-2 and viral RNA from real samples provided by “National Institute of Infectious Disease Lazzaro Spallanzani – IRCCS” (Rome, Italy).

The quality of the entire procedure was monitored thanks to the addition of an internal process control (IC) to the sample, consisting of synthetic heterologous RNA (IC-RNA), which is detected by a specific probe of this assay. This acts as amplification control, excluding false-negative results due to PCR inhibitors, and as performance control of the RNA extraction process.

The sampling on the surfaces was done using swabs containing a special medium created specifically to test areas possibly covered with substances like detergents, which might act as inhibitors, and prevents the possible detrimental effects on the whole testing process.

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bKTE-SCV2.01 content
Part number: bKTE-SCV2.01-32

Reaction Mix 1 (2 tubes, blue cap)	2 x 16 reactions (32 tests)
Reaction Mix 2 (2 tubes, yellow cap)	2 x 16 reactions (32 tests)
Internal control (2 tubes, white cap)	2 x 32 reactions (32 tests)
Positive Control (2 tubes, green cap)	2 x 16 reactions (8 tests)
Negative control (2 tubes, red cap)	2 x 16 reactions (8 tests)

Number of tests VS number of samples

The part number bKTE-SCV2.01-32 contains the reagents necessary to perform 32 tests. Each test requires the use of two reaction wells of a bCUBE® cartridge. It is possible to analyze 8 tests on the standard 16 wells cartridge. Each analysis requires to run 1 test for the positive control and 1 test for the negative control for both reaction mixes, leaving room to test up to 6 samples when using the 16 wells cartridge.

Following the above considerations, the part number bKTE-SCV2.01-32 is enough to test up to 24 samples (swabs), assuming the usage of fully loaded 16 wells cartridges.

Storage

Store the product at -20°C. bKTE-SCV2.01 is shipped in dry ice. The kit components must be frozen. If one or more components are not frozen upon receipt or if the tubes have been compromised during transport, contact Hyris Ltd for assistance. Avoid prolonged exposure to light and repeated freeze and thaw cycles.

Shelf life

If the bKIT is correctly stored, at constant-temperature freezer, its performance is guaranteed for 12 months and up to 3 freeze/thaw cycles. Please refer to the expiration date on the kit package.

Additional material/reagents
Required

- Swabs optimized for environmental sampling (e.g. MWE NRS II Transwab® or equivalent). Contact Hyris support at support@hyris.net for more options)
- Micropipettes and sterile tips with aerosol filter.
- bCUBE® instrument with filters calibrated for FAM™ and HEX™.
- bCUBE® disposable cartridge(s)

Recommended

- Cleaning/decontamination reagents (RNase Away®/RNaseZap™)
- Micro-centrifuge
- Vortex
- 1.5mL RNase/DNase Free (PCR Grade) tubes

Recommended minimum DPI*

- Disposable latex/nitrile powder free gloves.
- Goggles
- Disposable lab coat

Hyris can provide all the listed materials in a practical Starter kit which includes all the items listed above. Contact us at office.uk@hyris.net for more information!

* When working with chemical and biological samples wear always the appropriate protective equipment. For more information, consult the section **Safety information** of this datasheet or the safety data sheet (SDS). To obtain the appropriate SDS, please contact us at support@hyris.net.

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Workflow

Sampling

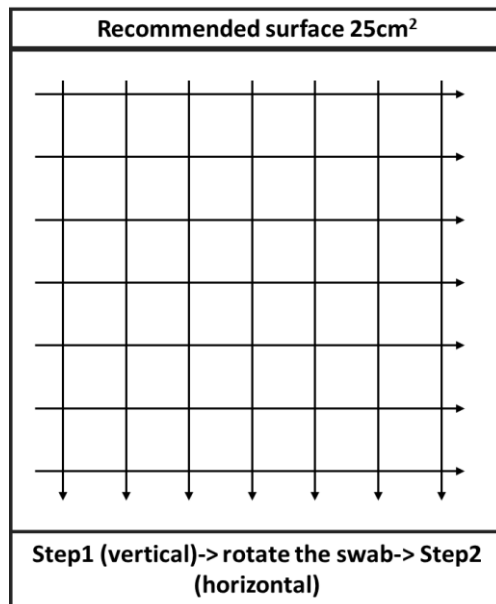


Figure 2: Sampling procedure



figure 1
MWE NRS II Transwab®

Commercially available swabs (e.g. MWE NRS II Transwab®, figure 1) can be used for collection, transport and preservation of specimens for subsequent molecular diagnostic analyses. NRS II Transwab® and similar contain a medium with a formulation created to neutralize the effect of any residual disinfectant which could be present on the sampling surface.

A typical sampling pattern, based on a tested surface of 25 cm², is shown in Figure 2. Different areas or sampling patterns can be adopted depending on the specific needs.

Store the tube at 4°C if you do not plan to analyze the sample immediately. Analyze the collected samples within two hours.

RNA extraction

The bKIT SARS-CoV-2 Environmental doesn't require an RNA extraction process to work properly. If the RNA extraction is required by your quality control procedure and/or your country/region/city regulation, the step by step protocol for RNA extraction and purification is available for download inside the bAPP.

Testing

1. Preliminary software setup

• **Analysis setup**

- Login on the bAPP
- Setup "New analysis" from the tab Analyses or on the bAPP home page.
- Compile the "Name" and "Description" field with the analysis information. If present, select your swarm.
- Flag bKITS and select "Virus Finder", then select "bKTE-SCV2.01 – SARS-CoV-2 Environmental" and press continue

Tips and tricks

- Scanning the QR code on the bKIT package, you'll be redirected directly to the "New analysis" section of the bAPP, with the correct product automatically selected.

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Tips and tricks

- If you are using the bPANEL, set-up “New Analysis” and select “SARS-COV-2 Environmental 1.x” (x indicates the number of the last version available) from the “Global recipes” list.

• Cartridge setup

- The cartridge layout is predefined for testing 6 samples as shown in **Figure 3**
- The wells with type **PosCtrl_Mix 1**, **PosCtrl_Mix 2**, **NegCtrl_Mix 1**, **NegCtrl_Mix 2** are reserved for the positive and the negative controls and should not be modified
- If less than 6 samples should be tested in the run, the corresponding wells can be deleted clicking on the button
- When the setup is completed, drag the selector to “Save for later”, then press “Finish” to save the analysis.



All samples and controls should be tested with both the reaction mixes. The two wells relative to each sample (type Sample_Mix 1 and Sample_Mix 2) should have the very same name for correct automatic results interpretation.



Figure 3



Tips and tricks

- To speed up the samples name editing you can press on the “Batch samples edit” button.
- To help yourself later in the experimental procedure you can download and print the cartridge layout. Press the “Download PDF cartridge layout” button (icon) on the top of the cartridge layout.

2. Sample preparation

Shake vigorously the Swab tube for 30s to release the genetic material into the Swab Buffer.

The testing procedure involves the pickup of 2 + 2 μL of the swab buffer, to be loaded directly into the wells of bCUBE cartridge. This pickup could be done with a “P10” pipette. However, given the typical size of this type of pipette and of the swab tube, contamination of the pipette touching the tube may occur.

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To avoid the pipette contamination we **recommend** performing the following procedure:

- Use a bigger pipette (P1000) and set it to a volume of 100 µL
- Open the tube and lay the hood/swab oriented upwards
- Carefully pipet 100 µL of the Swab Buffer into a 1.5 mL clean tube
- Use this aliquot for the analysis.
- Close the Swab tube and store at 4°C in case you need to repeat the analysis.

3. Cleaning procedures

To avoid any accidental contamination of the working surfaces or the instruments we recommend to performing the following cleaning and decontamination procedure:

- Spray the proper cleaning/decontamination reagent on the bench surface and wipe it with cleaning paper
- Spray the proper Cleaning/decontamination reagent on the micropipettes and wipe them with cleaning paper.
- Spray a bit of ethanol 70% or denatured ethanol on the micropipettes and wipe with cleaning paper, then wait a minute to dry.

4. Analysis reagent preparation

Allow the reagents to thaw. Vortex tubes when thawed and spin to collect contents at the bottom of the vial. Constitute the two reaction mixes following the volume reported in the table below. The procedure for preparing reagents for testing 6 samples is as following:

- **Mix 1 preparation for the analysis of 6 sample:** In a clean DNase/RNase tube **mix 144.5 µL of Reaction Mix 1 (blue cap) with 8.5 µL of Internal control (white cap)**. This solution will be enough to analyze 6 sample, one positive control and one negative control. In the **table 1** here below are reported the volume to use for the analysis of 1, 3 and 12 samples
- **Mix 2 preparation for the analysis of 6 sample:** In a clean DNase/RNase tube **mix 144.5 µL of Reaction Mix 2 (yellow cap) with 8.5 µL of Internal control (white cap)**. This solution will be enough to analyze 6 sample, one positive control and one negative control. In the **table 1** here below are reported the volume to use for the analysis of 1, 3 and 12 samples
- Vortex briefly and spin down in order to homogenize the mix.

N° of samples	N° of tests (including controls)	Reaction Mix 1 (blue cap)	Internal control (white cap)	Reaction Mix 2 (yellow cap)	Internal control (white cap)
1	3	59.5 µL	3.5 µL	59.5 µL	3.5 µL
2	4	76.5 µL	4.5 µL	76.5 µL	4.5 µL
3	5	93.5 µL	5.5 µL	93.5 µL	5.5 µL
4	6	110.5 µL	6.5 µL	110.5 µL	6.5 µL
5	7	127.5 µL	7.5 µL	127.5 µL	7.5 µL
6	8	144.5 µL	8.5 µL	144.5 µL	8.5 µL
7	11	195.5 µL	11.5 µL	195.5 µL	11.5 µL
8	12	212.5 µL	12.5 µL	212.5 µL	12.5 µL
9	13	229.5 µL	13.5 µL	229.5 µL	13.5 µL
10	14	246.5 µL	14.5 µL	246.5 µL	14.5 µL
11	15	263.5 µL	15.5 µL	263.5 µL	15.5 µL
12	16	280.5 µL	16.5 µL	280.5 µL	16.5 µL
			Mix 1		
			Mix 2		

Table 1


Tips and tricks

If you schedule to run more than 6 samples in a day, you can thaw all the necessary reagents, mix them as recommended and store up to 24h at 4°C until their use

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5. Cartridge loading

• **Reaction mixes loading**

Load the reaction mixes in the cartridge as following:

- Load 18 µL of **Mix 1** in each well highlighted in **BLUE** as shown in **Figure 5** below

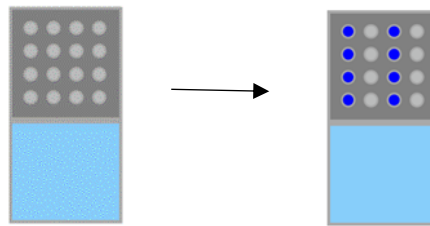


Figure 5
Mix 1 loading

White wells represent empty wells, whereas blue wells indicate wells filled with the Mix 1

- Load 18 µL of **Mix 2** in each well highlighted in **YELLOW** as shown in **Figure 6** below

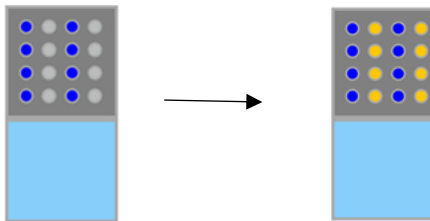


Figure 6
Mix 2 loading

White wells represent empty wells, whereas blue and yellow wells indicate wells filled with the Mix 1 and Mix 2 respectively.

• **Samples and controls loading**

- For each sample, load 2 µL in each of the two corresponding wells
- Load 2 µL of positive control in the wells of type **PosCtrl_Mix 1** and **PosCtrl_Mix 2**
- Load 2 µL of negative control in the wells of type **NegCtrl_Mix 1** and **NegCtrl_Mix 2**
- Carefully seal the cartridge with black adhesive aluminium foil provided with the cartridge in order to avoid any contamination.
- Load the cartridge onto the bCUBE®.

6. Run the experiment

Option A: From the “Edit Analysis” page, draw the bottom selector to “Launch now”, then:

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1. Click on the **Finish** button,
2. Select the bCUBE®
3. Click on **SEND**

Option B: from the “Analysis list” page, select the analysis, then:

1. Click on the “**Run Analysis**” button
2. Select the bCUBE®
3. Click on **SEND**

Reading the results

- a. The analysis can be monitored in real time using the bAPP. Depending from the mixture an amplification curve will/will not generate.
- b. At the end of analysis each well will be automatically labelled depending on the “Well type” as described in the tables below.

Well type	Possible labels	Label meaning
Positive control (PosCtrl_Mix 1 and PosCtrl_Mix 2)	OK	All parameter are in the range
	KO	At least one of the parameter is out of the range

Well type	Possible labels	Label meaning
Negative control (NegCtrl_Mix 1 and NegCtrl_Mix 2)	OK	All parameter are in the range
	KO	At least one of the parameter is out of the range

Well type	Target	Possible labels	Label meaning
Sample (Sample_Mix 1 and Sample_Mix 2)	SARS-COV-2 RNA	Negative	SARS-COV-2 RNA sequence not detected
		Positive	SARS-COV-2 RNA RNA sequence detected
		Inconclusive	IC control not detected
		Invalid Test	At least one of the control behaviors isn't within the expected range. Please repeat the test

When Negative or Positive control are **KO**, the test is **invalid**. Please re-test the samples from residual specimen. If this result persists, contact the Hyris technical support at support@hyris.net

Based on the results obtained for each locus under investigation, SARS-COV-2 RNA **detected** or **not detected** result can be assigned to each sample following instructions in the panel illustrated below.

Sample_Mix 1	Sample_Mix 2	Result
Positive	Positive	SARS-COV-2 RNA detected
Negative	Negative	SARS-COV-2 RNA not detected
At least one Inconclusive		Inconclusive results
Only one Positive		Inconclusive result

When **Inconclusive results** occurs re-test sample from residual specimen. If this results persist after re-test, contact Hyris technical support at support@hyris.net.

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The Kits may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. TaqMan® is a trademark of Roche Molecular Systems, Inc. FAM™, HEX™ and ROX™ are a trademark of Thermo Fisher Scientific or its subsidiaries. SYBR is a registered trademark of Molecular Probes, Inc. (Thermo Fisher Scientific). MGB probes are a trademark of Elitech group. The Kits have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Kits. The user is personally responsible for data that he/she will obtain and/or he/she will supply to third parties using these kits. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the kit can be returned and the user can be refunded. Kits components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change. Therefore, please refer to our website (www.hyris.net) for the most up-to-date information on Hyris Ltd products.

Troubleshooting

1. Results show no amplification, or anomalous amplification curves

Possible causes	Corrective actions
Evaporation of the sample due to inadequate sealing of the plate/strips	Repeat the test using the appropriate materials and tools to seal correctly the plate/strips
Consumables are not appropriate for the method	Repeat the test using consumables recommended by the supplier of the Real-Time PCR instrument
The quality of nucleic acid extracted is low	Repeat the extraction step. Ensure that the method of extraction has been performed correctly. In any doubt, contact us at support@hyris.net .

2. Invalid test results occur in the automatic interpretation of the results

Possible causes	Corrective actions
The Positive Control provided with the assay shows a low or absent amplification. The positive control(s) was not added or was not properly added	Repeat the test adding the Positive Control. If the problem persists, contact us at support@hyris.net .
The internal control provided with the assay shows a low or absent amplification. The internal control(s) was not added or was not properly added	Repeat the test adding the correct amount of internal control as recommended in the section 4, page 7 (Analysis reagent preparation) of this datasheet. If the problem persists, contact us at support@hyris.net .
A FAM signal was observed in the negative control(s) well(s), caused by a possible contamination of the negative control or the Mix(es) with target RNA	Repeat the test by applying appropriate quality procedures to prevent contamination. Correctly seal the cartridge or plate/strips. If the problem persists, contact us at support@hyris.net .

3. Inconclusive test results occur in the automatic interpretation of the results

Possible causes	Corrective actions
The target IC control shows a low or absent amplification.	Repeat the test adding the correct amount of Internal control into the Mix(es). If the problem persists, contact us at support@hyris.net .

Safety information

1. Biohazard

Biological samples have the potential to transmit infectious pathogens. Follow all applicable local, state/provincial and/or national regulations. Wear appropriate protective equipment which includes but is not limited to protective eyewear, face shield, clothing/lab coat (and a disposable lab coat) and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment. Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials.

2. Waste disposal

Characterize the waste generated by the applications, reagents and substrates used in your laboratory; ensure that the instrument waste is stored, transferred, transported and disposed of according to all local/state/provincial and/or national regulations.

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Important Use Information

The Sars-Cov-2 Environmental bKIT (bKTE-SCV2.01), which is part of the Hyris Virus Finder family, is intended to be used by professionals in commercial settings to detect the presence and/or absence of SARS-CoV-2 on non-porous hard surfaces and fomites. The Environmental test is a great way to determine whether your company's disinfection procedures are working as you and your company's disinfection protocol intend. Follow the use and storage instructions very carefully to ensure that the results obtained are as accurate as possible. Contamination of surfaces can occur at any point after testing or surface disinfection, so it is critical to use the Hyris Virus Finder System after each disinfection procedure.

What Can Sars-Cov-2 Environmental bKIT (bKTE-SCV2.01) NOT Do?

The Sars-Cov-2 Environmental bKIT (bKTE-SCV2.01) is NOT intended for use as a human diagnostic. Results obtained from the Sars-Cov-2 Environmental bKIT (bKTE-SCV2.01) cannot be extrapolated from one surface, substance, or location to another surface, substance, or location. The choice of specific surfaces, substances, or locations to be tested, as well as the frequency of testing, is to be determined by the user's protocol. The Hyris Virus Finder System does not make any representations or recommendations concerning which surfaces, substances, or locations should be tested, or how often testing should be performed. The User must follow all applicable local, state/provincial, and/or national regulations regarding Covid-19 and wear appropriate protective equipment while performing the test. If a surface is found to be contaminated, you must follow your company's disinfection protocol regarding additional disinfection.

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