



EN	REF 1N018	Lyophilized mix for qualitative detection of novel Coronavirus SARS-CoV-2 in Real Time RT-PCR	IVD
	STAT-NAT® COVID-19 B	REAGENT: 3 x (8 x 0.025) mL Assay 1 3 x (8 x 0.025) mL Assay 2 3 x (8 x 0.025) mL Assay 3 BUFFER : 1 x 1.5 mL + 2 x 1.0 mL CONTROL: 1 x (1 x 0.1) mL	 = 24 test 
NOTE: This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.			

INTENDED USE

The STAT-NAT® COVID-19 B kit is a lyophilized Real-time RT-PCR qualitative assay based on nucleic acid amplification for the identification of the novel Coronavirus SARS-CoV-2 RNA in human respiratory tract specimens^{1,2}. This assay is an aid in the diagnosis of SARS-CoV-2 infection.

PRINCIPLE

STAT-NAT® COVID-19 B kit is an in-vitro diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

STAT-NAT® COVID-19 B is based on the protocol "Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR" developed by the Charité, Berlin, Germany and published on 17 January 2020³.

STAT-NAT® COVID-19 B kit is based on Real-Time RT-PCR testing and Sentinel STAT-NAT® (STabilized Amplification Technology – Nucleic Acid Testing) technology. The kit consists of three optimized freeze-dried reaction mixtures: 24 screening tests (Assay 1 – targeting a specific region of the E gene), 24 first line confirmatory tests (Assay 2 – targeting specific regions of the RdRP gene) and 24 second line confirmatory tests (Assay 3 – targeting a specific region of the RdRP gene with specific probe). The Positive Control (PC), in a dried form, is included into the kit.

Each different lyophilized master mix is present in an 8-tube PCR strip, minimizing any potential risks arising from pipetting errors and contamination. The STAT-NAT® COVID-19 B reaction mixtures, including specific primers and probes, guarantee the sensitivity and the specificity of the reaction without intermediate manual steps for setting up reaction mixtures. An endogenous Internal Control (IC) (human RNase P) is included in each reaction and it provides indications on the functionality of the system and on the absence of inhibitors of polymerase activity, which could cause false negatives. STAT-NAT® COVID-19 B kit is stable at +15/+30 °C.

REAGENTS

STAT-NAT® COVID-19 B consists of:

- REAGENT: 3 assays x 3 strips x 8 master mix tubes**- Assay 1: 3 strips x 8 master mix tubes**

The kit includes 3 aluminium pouches labeled "8 x Assay 1 Master Mix Tubes" containing a single 8-tube strip (0.2mL PCR tube) with lyophilized Master Mix and a small orange desiccant sachet.

Each PCR tube contains:

- MgCl₂;
- dNTPs (dATP, dCTP, dGTP and dTTP);
- Reverse Transcriptase;
- Hot Start (Taq) Polymerase;
- Specific primers for Assay 1;
- Specific probe for Assay 1;

- Reaction buffer.

Lyophilized Master Mix must be stored at +15/+30 °C. Use only undamaged packages.

- Assay 2: 3 strips x 8 master mix tubes

The kit includes 3 aluminium pouches labeled "8 x Assay 2 Master Mix Tubes", each contains a single 8-tube strip (0.2mL PCR tube) with lyophilized Master Mix and a small orange desiccant sachet.

Each PCR tube contains:

- MgCl₂;
- dNTPs (dATP, dCTP, dGTP and dTTP);
- Reverse Transcriptase;
- Hot Start (Taq) Polymerase;
- Specific primers for Assay 2;
- Specific probe for Assay 2;
- Reaction buffer.

Lyophilized Master Mix must be stored at +15/+30 °C. Use only undamaged packages.

- Assay 3: 3 strips x 8 master mix tubes

The kit includes 3 aluminium pouches labeled "8 x Assay 3 Master Mix Tubes", each contains a single 8-tube strip (0.2mL PCR tube) with lyophilized Master Mix and a small orange desiccant sachet.

Each PCR tube contains:

- MgCl₂;
- dNTPs (dATP, dCTP, dGTP and dTTP);
- Reverse Transcriptase;
- Hot Start (Taq) Polymerase;
- Specific primers for Assay 3;
- Specific probe for Assay 3;
- Reaction buffer.

Lyophilized Master Mix must be stored at +15/+30 °C. Use only undamaged packages.

- CONTROL: 1 x 1 positive control

The kit contains 1 aluminium pouch labelled "Positive Control" containing 1 tube (green cap) of novel Coronavirus SARS-CoV-2 Positive Control (PC) constituted by a dried pellet of synthetic DNA and a small orange desiccant sachet.

Control must be stored at +15/+30 °C. Use only undamaged packages.

After opening, recap STAT-NAT® Positive Control and store it at -20 °C.

- BUFFER: 2 x 1.0 mL + 1 x 1.5 mL

The kit includes:

- 2 STAT-NAT® Reconstitution Buffer tubes (1.0 mL) in a liquid form;
- 1 STAT-NAT® Control Reconstitution Buffer (1.5 mL) in a liquid form.

Buffers must be stored at +15/+30 °C. Use only undamaged packages.

After opening, recap STAT-NAT® Reconstitution Buffer and STAT-NAT® Control Reconstitution Buffer and store them at +15/+30 °C. Open vial stability: stable up to expiry date indicated on the package if stored at +15/+30 °C.

The STAT-NAT® Control Reconstitution Buffer must be used as No Template Control (NTC).

QUALITY CONTROL

Use only the Positive Control provided with the STAT-NAT® COVID-19 B Kit.

The STAT-NAT® COVID-19 B Positive Control provides indications on the functionality of the system. Each PC tube contains a dried pellet of synthetic DNA.

Reconstitute the PC before using following the instructions below.

Positive Control reconstitution:

1. Centrifuge the STAT-NAT® Positive Control tube prior to opening to ensure that DNA is at the bottom of the tube;
2. Reconstitute the STAT-NAT® Positive Control with 100 µL of STAT-NAT® Control Reconstitution Buffer;
3. Cap the tube and vortex for 30 seconds until the dried DNA is resuspended;
4. Centrifuge for few seconds at medium speed to remove any residue from the cap and eliminate bubbles/foam;
5. Wait for at least 15 minutes before use;
6. Vortex the Positive Control for few seconds at medium speed and centrifuge it for few seconds at medium speed;
7. See Reaction set up section for the next steps.

After use, recap the residue of the reconstituted STAT-NAT® Positive Control and store it at -20 °C.

It is necessary to validate each run using:

- NTC (STAT-NAT® Control Reconstitution Buffer tube);
- STAT-NAT® Positive Control.

If required by laboratory's guidelines, include a negative control (NC) in each run. A verified negative sample can be used as NC.

SAMPLE

For initial diagnostic testing for COVID-19, the World Health Organisation (WHO) recommends the collecting and the testing of upper respiratory specimens (nasopharyngeal and oropharyngeal swabs), and lower respiratory (sputum, if possible) samples for those patients with productive coughs. Induction of sputum is not recommended. Collect the samples into sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

Store specimens at 2-8 °C. The specimens should be processed within 48 hours of collection^{4,5}.

INSTRUMENTATION AND MATERIALS REQUIRED BUT NOT PROVIDED

General molecular laboratory equipment: biosafety cabinet (laminar flow hood) for extractions, centrifuge/micro-centrifuge, vortex mixer, variable volume pipettes, sterile disposable plastics.

Extraction Kit: Nucleic acid extraction should be performed by extraction kits available at the market according to protocols for the particular clinical material extraction.

Reagents: RNA template (best results are obtained with high quality RNA).

Personal protective equipment (PPE) as gloves, laboratory coats, safety glasses, facemasks.

Validated Real-Time PCR Thermal Cycler: Bio-Rad CFX96™ DX and Thermo Scientific (ABI) QuantStudio™ 5 DX.

WARNINGS AND PRECAUTIONS

- This assay is exclusively for IVD use;
- Read all the instructions contained in the kit insert before performing the test;
- Comply with the kit expiration date;
- Do not use reagents from other commercial kits;
- Do not mix up reagents from kits with different Lot Number;
- The MSDS are available at www.sentinel diagnostics.com or at your local supplier;
- Keep all Master Mix Tubes (REAGENT) of the kit protected from light and humidity in their aluminium envelopes;
- Keep all positive controls (CONTROL) of the kit protected by humidity in their aluminium envelopes with dedicated small orange desiccant sachet.
- Always use personal protective equipment for the individual protection;
- The product must be handled by staff trained in molecular biology techniques, such as nucleic acids extraction, amplification and detection;
- It is required to keep separated the sample extraction area, the reagent preparation area and the amplification/detection area;
- Results obtained with the STAT-NAT® COVID-19 B assay should be interpreted in conjunction with other clinical and laboratory findings;
- As with other tests, negative results do not rule out the SARS-CoV-2 infection;
- Mutations within the target regions of the SARS-CoV-2 RNA covered by the STAT-NAT® COVID-19 B kit may affect primers and/or probe pairing resulting in the under-estimation of viral nucleic acids detection.

- **CAUTION** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens⁶, Biosafety Level 2⁷ or other appropriate biosafety practices^{8,9} should be used for materials that contain or are suspected of containing infectious agents.

Lyophilized Master Mix Highlights:

- It is strongly recommended to use the entire 8-tube strip in a single session.
- Examine the lyophilized Master Mix before use to verify that the content has a conic, solid and white appearance (Figure 1). Please discard the product that appears with signs of moisture contamination (i.e. change of colour, collapsing product, etc...);
- Make sure lyophilized Master Mix is at the bottom of the test tubes before to open the strip caps (Figure 1).

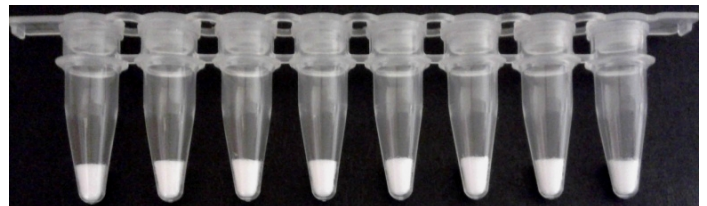


Figure 1. Standard aspect of lyophilized Master Mix

INSTRUCTIONS FOR USE

- Cut the aluminium pouches at the point indicated by the lateral notches.
- Remove the strips and the tubes from the pouches immediately before use.
- Ensure that the pouches are always well sealed and that the desiccant sachets are still inside. Use only undamaged packages.
- Waste the aluminium pouches and their content if the desiccant sachets turn from orange to green.
- All reagents, correctly stored at +15/+30 °C, are stable up to the expiration date indicated on the package.

The STAT-NAT® COVID-19 B kit contains three assays:

- Screening assay (Assay 1);
- First line confirmatory assay (Assay 2);
- Second line confirmation assay (Assay 3).

The Assay 1 is recommended as the screening assays, Assay 2 and 3 as confirmatory assays for the novel Coronavirus SARS-CoV-2.

The three Assays can be run in a unique PCR session from the same RNA extract.

PROCEDURE**Real-Time PCR cycling parameter setting**

1. Turn Real-Time PCR thermal cycler and computer on and open the dedicated software program;
2. Set the detector for the SARS-CoV-2 target probe with reporter "FAM" and quencher "none";
3. Set the detector for the IC of reaction with reporter "HEX/VIC" and quencher "none".
4. Set the Passive Reference field, if requested, select "none";
5. For basic information regarding the setup and programming of the different Real-Time PCR instruments, please refer to the user manual of the respective instrument;
6. Perform Real-Time PCR using the thermal profile shown in **Table A** and **B**.

Table A. Real-Time PCR thermal profile

Segment	Cycle number	Temperature	Time	
1	1	50°C	10 min	
2	1	95°C	2 min	
3	10	95°C	15 sec	Fluorescence detection OFF
		58°C	30 sec	
4	35	95°C	15 sec	Fluorescence detection ON
		58°C	30 sec	

Table B. Real-Time PCR settings

Sample Volume	Ramp Rate
25 µL	Instrument default

Reaction set up

1. Extract RNA from the human respiratory samples (Extraction system is not included in the kit);
2. Prepare the STAT-NAT® Positive Control as indicated in QUALITY CONTROL section;
3. Use STAT-NAT® Control Reconstitution Buffer as No Template Control (NTC);
4. Carry out the first step of the Protocol removing the necessary number of **Assay 1, Assay 2 and Assay 3** Master Mix Tubes from the pouches;
5. Reconstitute the lyophilized Assay 1, Assay 2 and Assay 3 Master Mixes as indicated in **Table C**;

Table C. Reaction volumes

Components	Volume per test tube/reaction
STAT-NAT® Reconstitution Buffer	15 µL
Extracted RNA or PC or NTC or NC	10 µL
Final Reaction volume	25 µL

6. The lyophilized mix will dissolve in few seconds;
 7. Include a negative control (NC) if required ;
 8. Make sure that there are no air bubbles; if so, remove them by aspiration with the tip of a pipette;
 9. Close each Master Mix tube with dedicated cap;
- It is recommended to perform **Assay 1, Assay 2 and Assay 3** in a unique PCR session;

INTERPRETATION OF RESULTS**Data Analysis**

- The analysis of the results is carried out directly using the specific management software.
- Set threshold values as indicated in **Table D** in the additional sheet present in the kit.
- FAM signal indicates the successful amplification of the specific target sequences;
- HEX/VIC signal indicates the successful amplification of the specific sequence for IC.

Validation of the session:

It is necessary to validate each diagnostic run using:

- a NTC: STAT-NAT® Control Reconstitution Buffer;
- STAT-NAT® Positive Control.

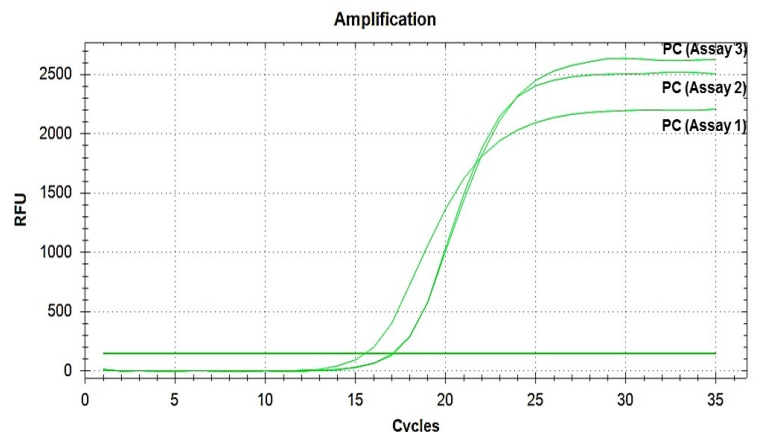
Check the amplification curves for positive controls, negative controls and NTC as indicated in Table E.

Table E. Interpretations of results for PC, NTC and NC

Control	Interpretations	
	FAM	Result
Positive Control	Signal	VALID
Positive Control	No Signal	INVALID
NTC / Negative Controls	No Signal	VALID
NTC / Negative Controls	Signal	INVALID

The session is to be considered INVALID and to be repeated in the event that the Negative Control / NTC provided a positive result.

The expected threshold cycle (Ct) of the PC is 16 ± 3 (figure 2). In case the Ct value is outside of the range, check the troubleshooting section.

**Figure 2.** Example of Positive Control (PC) amplification plot for Assay 1, Assay 2 and Assay 3.

Sample analysis

- FAM signal indicates the successful amplification of the specific target sequences;
- As with other tests, negative results do not rule out SARS-CoV-2 infection.

The **Tables F** shows the validity and interpretation of test run.

Table F. Interpretation of results

Assay 1 FAM (E gene)	Assay 2 FAM (RdRP gene)	Assay 3 FAM (RdRP gene)	IC HEX/VIC (For all assay)	Validity of the test	Detection of SARS-CoV-2 RNA
+	+	+	+	VALID	POSITIVE
+	+	-	+	VALID	POSITIVE
+	-	+	+	VALID	POSITIVE
-	+	+	+	VALID	POSITIVE
-	+	-	+	VALID	LOW POSITIVE [#]
-	-	+	+	VALID	LOW POSITIVE [#]
+	-	-	+	VALID	INDETERMINATE [►]
-	-	-	+	VALID	NEGATIVE [§]
+/-	+/-	+/-	-	INVALID	*

[#] In this case is recommended to repeat the extraction step using the same primary sample or collect a new primary sample and repeat the test. If the same result is obtained, report as positive and follow up the patient with another sample after some days.

[►] Another Coronavirus non-SARS-CoV-2 can be present. In this case is recommended to repeat the extraction step using the same primary sample or collect a new primary sample and repeat the test. If the same result is obtained follow up the patient with another sample after some days or act accordingly local guidelines.

[§] In samples resulting negative for SARS-CoV-2 target, it is not excluded that there is a SARS-CoV-2 RNA concentration lower than the system's sensitivity limit.

* High concentrated samples can inhibit the amplification of the Internal Control.

PERFORMANCES¹⁰**Analytical Sensitivity¹¹**

The Limit of Detection (LoD) was evaluated using a dilution panel of SARS-CoV-2 from 1×10^0 to 1×10^7 copies/reaction. The LoD was calculated on several replicates of samples, the results that show a 95% probability to have a positive result are summarized in **Table G**.

Table G. Limit of Detection

Assay	LoD Results	
	QuantStudio™ 5 DX	Bio-Rad CFX96™ DX
Assay 1	10 copies/reaction	
Assay 2	10 copies/reaction	
Assay 3	10 copies/reaction	

Precision¹²

In this study, the closeness of agreement between measured quantities obtained by replicate measurements on the same analyte under specified conditions was evaluated (**Table H**)

Table H. Precision measurement studies

Assay	Criteria for acceptance	Pass (Y/N)
Assay 1	CV% < 5%	Yes
Assay 2	CV% < 5%	Yes
Assay 3	CV% < 5%	Yes

Cross-Reactivity¹⁰

To evaluate the Cross-Reactivity to several pathogens, an *in silico* approach was used.

All pathogens, except SARS-CoV-2, resulted negative, as indicated in **Table I**.

Table I. Cross-reactivity evaluation

Different Pathogens	Results*
HCoV-HKU1	Negative
HCoV-OC43	Negative
HCoV-NL63	Negative
HCoV-229E	Negative
MERS-CoV	Negative
Influenza A (H1N1/09)	Negative
Influenza A (H3N2)	Negative
Influenza A(H5N1)	Negative
Influenza B	Negative
Rhinovirus/Enterovirus	Negative
Respiratory syncytial virus (A/B)	Negative
Parainfluenza 1 virus	Negative
Parainfluenza 2 virus	Negative
Parainfluenza 3 virus	Negative
Parainfluenza A or -B virus	Negative
Human metapneumovirus	Negative
Adenovirus	Negative
Human bocavirus	Negative
Legionella spp.	Negative
Mycoplasma spp.	Negative

* Data confirmed by the protocol "Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR"³

TROUBLESHOOTING**Problem 1: Weak or no signal in Positive Control:**

1. Real-Time PCR conditions do not comply with the instructions in the kit insert:

- The Positive Control was not added to the reaction. Repeat test;
- Check the Real-Time PCR cycling parameters protocol and select fluorescence channels reported in the kit insert;
- Check the performance of the thermalcycler and carry out the instrument calibration.

2. Primers/probes degradation: the reagent storage conditions do not comply with the instructions in the kit insert:

- Check the kit storage conditions;
- Check the kit expiration date.

3. Wrong selection of channel/filter. Real-Time PCR conditions do not comply with the instructions in the kit insert:

- Check the Real-Time PCR cycling parameters protocol and select fluorescence channels reported in the kit insert.

Problem 2: Weak or no signal in Internal Control.

1. Inhibitory effect of the sample: RNA with a low quality extraction. The result is INVALID:

- Ensure to use a validated RNA extraction method and follow carefully the instructions reported in the kit insert;
- Repeat the test using the same extracted RNA sample. If the result is still negative, repeat the extraction step using the same primary sample. Otherwise, collect a new primary sample and repeat the test.

2. Real-Time PCR conditions do not comply with the instructions in the kit insert:

- Check the Real-Time PCR cycling parameters protocol and select fluorescence channels reported in the kit insert;
- Check the performance of the thermalcycler and carry out the instrument calibration.

3. Primers/probes degradation: the reagent storage conditions do not comply with the instructions in the kit insert:

- Check the kit storage conditions;
- Check the kit expiration date.

4. Wrong selection of channel/filter. Real-Time PCR conditions do not comply with the instructions in the kit insert:

- Check the Real-Time PCR cycling parameters protocol and select fluorescence channels reported in the kit insert.

Problem 3: FAM signal in NTC or in Negative Control.

1. Contamination during the Real-Time PCR preparation procedure: all results are INVALID:

- Clean the workbench and all the instruments;
- Handle Positive Control carefully avoiding contamination;
- Repeat Real-Time PCR using a new set of reagents.

Problem 4: Fluorescence intensity variability or absence of FAM/HEX/VIC signal.

1. Air bubbles trapped in the PCR tubes:

- Remove air bubbles before starting the Real-Time PCR run.

2. Humidity damage for Lyophilized mix: the reagent storage conditions do not comply with the instructions in the kit insert:

- Check the kit expiration date;
- Check the kit storage conditions; ensure that the pouch is always well sealed and that the desiccant sachet is still inside;
- Check if the desiccant sachet turns from orange to green.

3. Inhibitory effect of the sample: RNA with a low quality extraction. The result could be a false negative. The result is INVALID:

- Ensure to use a validated RNA extraction method and follow the instructions reported in the kit insert carefully.

4. The Lyophilized Mix is not well reconstituted:

- Repeat the Real-Time PCR procedure carefully.

Problem 5: No signal at all.

1. Check the performance of the thermalcycler:

- Carry out the instrument calibration.

2. Primers/probes degradation: the reagent storage conditions do not comply with the instructions in the kit insert:

- Check the kit storage conditions;
- Check the kit expiration date.

Problem 6: Error message given by the Real-Time PCR instrument.

Consult the Instrument User Manual or contact the local technical support.

Problem 7: Duplicate samples do not reproduce identical results.

The Ct values of identical samples may differ in individual reactions. Ct variations $> \pm 2$ values suggest pipetting errors or other differences between duplicate samples.

WASTE MANAGEMENT

- The reagents of the kit are not classified as dangerous according to Regulation EC 1272/2008 (CLP). Adopt good working practices, so that the product is not released into the environment. Recover if possible. In so doing, comply with the local and national regulations currently in force.










- Manage and waste all the biological samples as potentially infectious. All the material that come in contact with the biological sample must be treated with 0.5% sodium hypochlorite for at least 30 minutes or sterilized in autoclave at 121 °C for 30 minutes and then wasted.

BIBLIOGRAPHY

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Explanation of symbols			EN
REAGENT / STANDARD / CONTROL / BUFFER			
The terms refers to the: single reagent / standard / control / buffer			
IVD <i>In vitro</i> Diagnostic Medical Device	REF Catalogue number	LOT Batch code	
Cont. Contents of kit	Distributed by Distributed by	 Manufacturer	
 Caution, consult accompanying documents Consult instructions for use	 Temperature limitation		
 Do not reuse	 Do not expose the REAGENT to light	 Use by	
 Date of Manufacture	 Contains sufficient for <n> tests	 Dispose of properly	

STAT-NAT® is a trademark in various jurisdictions which is exclusively licensed to SENTINEL CH. SpA. STAT-NAT® technology is covered by patent No. WO2010133628 A1.

Note: changes in comparison to the previous version are indicated by a vertical bar in the text margin