EN

**REF 1N036**

# STAT-NAT® COVID-19 MULTI

## Lyophilized mix for qualitative detection of novel Coronavirus SARS-CoV-2 in Real Time RT-PCR

REAGENT: 12 x (8 x 0.025) mL

Σ = 96 test

BUFFER : 1 x 1.5 mL + 2 x 1.0 mL
CONTROL: 1 x (1 x 0.1) mL

IVD

CE

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 MULTI kit is a lyophilized Real-time RT-PCR qualitative multiplex 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 MULTI 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 MULTI kit is based on Real-Time RT-PCR testing and Sentinel STAT-NAT® (STabilized Amplification Technology – Nucleic Acid Testing) technology. The kit consists of one optimized freeze-dried reaction mixture (96 reactions total) targeting simultaneously two regions of the specific SARS-CoV-2 RdRP and Orf1b genes. All probes for the specific regions are both labelled with FAM fluorophore, allowing fast and simple results evaluation.
Primers and probes specific for a housekeeping gene (human RNase P), labelled with HEX fluorophore, are present in each reaction and they are used as endogenous Internal Control (IC). This provides indications on the functionality of the system and on the absence of inhibitors of polymerase activity, which could cause false negatives.
The 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 MULTI 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.
The Positive Control (PC), in a dried form, is included in the kit. It is also available separately as an additional kit (STAT-NAT® COVID-19 Positive Control – Sentinel REF. 1N025).
STAT-NAT® COVID-19 MULTI kit is stable at +15/+30 °C.

**REAGENTS**

**Figure 1**. Kit configuration

| REAGENT | CONTROL | BUFFER |
|---|---|---|
| 12 strips x 8 master mix tubes | 1 X 1 COVID-19 Positive Control | 2 X 1 mL Reconstitution buffer<br>1 X 1.5 mL Control Reconstitution Buffer |

STAT-NAT® COVID-19 MULTI consists of:

- **REAGENT: 12 strips x 8 master mix tubes**

The kit includes 12 aluminium pouches labeled “8 x 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:
- $MgCl_2$;
- dNTPs (dATP, dCTP, dGTP and dTTP);
- Reverse Transcriptase;
- Hot Start (Taq) Polymerase;
- Specific primers for RdRP and Orf1b genes;
- Specific probe for RdRP and Orf1b genes;
- 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, red label) in a liquid form;
- 1 STAT-NAT® Control Reconstitution Buffer (1.5 mL, blue label) 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.
In use 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 MULTI kit or STAT-NAT® COVID-19 Positive Control – Sentinel REF. 1N025.
The STAT-NAT® COVID-19 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.. 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[3,4].

**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 on the market according to protocols for the particular clinical material extraction.
**Sample:** 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 mix up reagents for amplification (i.e. buffer) from other commercial kits;
- Do not mix up reagents from kits with different Lot Number;
- The MSDS are available at www.sentineldiagnostics.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 positive control (CONTROL) of the kit protected by humidity in its aluminium envelope 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 MULTI 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 MULTI kit may affect primers and/or probe pairing resulting in the under-estimation of viral nucleic acids detection.
- False negative or invalid results may occur due to interference. The Internal Control is included in STAT-NAT® COVID-19 MULTI to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
- ⚠ **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 Pathogens[5], Biosafety Level 2[6] or other appropriate biosafety practices[7,8] 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. Please discard the product that appears with signs of moisture contamination (i.e. change of colour, collapsing product, etc…).

**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.

**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 **Master Mix Tubes** from the pouches;
5. Reconstitute the lyophilized 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.

**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**.

**Table D.** Threshold values

| Instrument | FAM (Targets) | HEX/VIC (IC) |
|---|---|---|
| ABI QuantStudio™ 5 DX | 5% Positive Control fluorescence | 5% of the highest IC fluorescence among samples |
| Bio-Rad CFX96™ DX | | |

- 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.

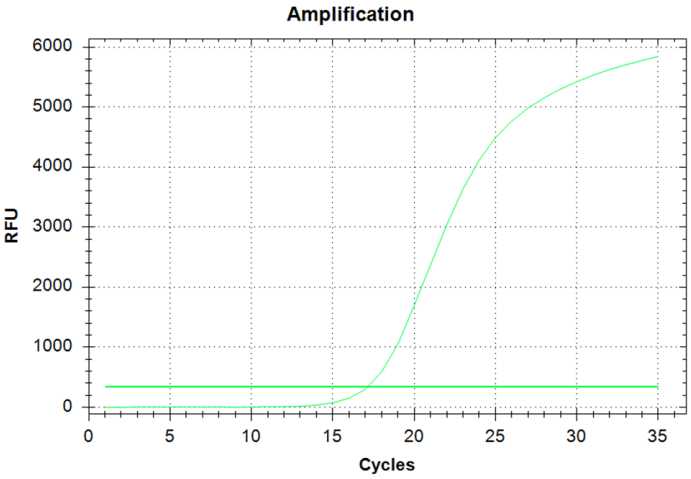
**Table E.** Interpretations of results for PC, NTC and NC

| 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 17.5 ± 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




**Sample analysis**

- FAM signal indicates the successful amplification of the specific target sequences;
- HEX/VIC signal indicates the successful amplification of the endogenous Internal Control (IC) (human RNase P). Internal Control (IC) Ct value > 30 cycles could indicate an error in the sample dispensing or a poor RNA quality. We advise to repeat the amplification or/and the sample extraction.
- 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

| FAM Channel (RdRP and Orf1B genes) | HEX/VIC Channel (IC) | Validity of the test | Detection of SARS-CoV-2 RNA |
|---|---|---|---|
| + | + | VALID | POSITIVE |
| - | + | VALID | NEGATIVE§ |
| + | - | VALID | POSITIVE# |
| - | - | INVALID | * |

§ 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.

* 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.

**PERFORMANCES[9]**

**Analytical Sensitivity[10]**

The Limit of Detection (LoD) was evaluated using a dilution panel of SARS-CoV-2 from $1x10^0$ to $1x10^7$ 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

| LoD Results | |
|---|---|
| QuantStudio™ 5 DX | Bio-Rad CFX96™ DX |
| 10 copies/reaction | |

**Precision[11]**

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

| Criteria for acceptance | Pass (Y/N) |
|---|---|
| CV% < 5% | Yes |

**Cross-Reactivity[9]**

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 |

**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 > ± 2 values suggest pipetting errors or other differences between duplicate samples.

**Problem 8: Ct > 30 in the Internal Control.**
Sample with low quality extracted RNA or errors in sample dispensing in the reaction set up.
– Repeat the test using the same extracted RNA sample. If the IC Ct result is still > 30, repeat the extraction step using the same primary sample. Otherwise, collect a new primary sample and repeat the test.

## 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.

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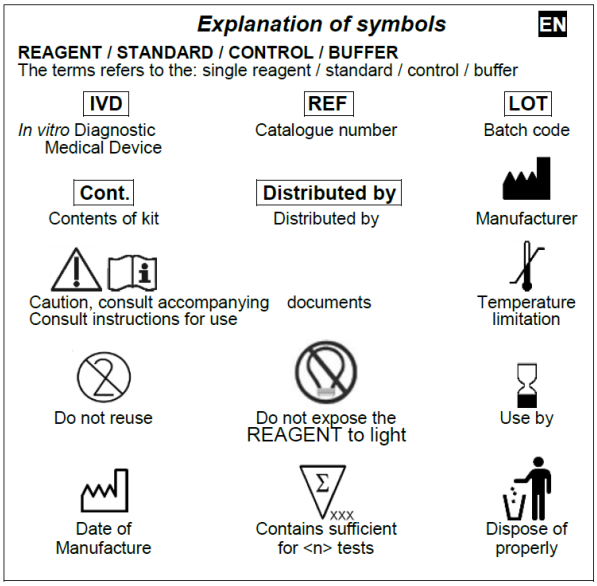


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**Note: changes in comparison to the previous version are indicated by a vertical bar in the text margin**



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