altona DIAGNOSTICS

# Instructions for use

# FlexStar®
# SARS-CoV-2
# RT-PCR Detection Mix 1.5

06/2022 EN

## Respiratory

FlexStar

# FlexStar®

# SARS-CoV-2
# RT-PCR Detection Mix 1.5

**For research use only!**

**(RUO)**

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# Content

## 1. Application

The FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific RNA.

**For research use only (RUO)! Not for use in diagnostic procedures.**

## 2. Product content

The FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 contains the following components:

**Table 1:** Kit components

| Lid color | Component | Number of tubes | Nominal volume [µl/tube] |
|---|---|---|---|
| Blue | Detection Mix[1)] | 8 | 240 |
| Red | PC[2)] | 2 | 250 |
| White | NTC[3)] | 2 | 250 |

[1)] Contains biological material of animal origin

[2)] Positive Control [lineage B-βCoV (E gene) and SARS-CoV-2 (S gene) specific RNA and Sampling Control]

[3)] No Template Control (negative control)

## 3. Storage

- The FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 is shipped on dry ice. The product components should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics technical support for assistance (see chapter 9. Technical assistance).
- All components should be stored at -25 °C to -15 °C upon arrival.
- Repeated thawing and freezing of the Detection Mix component should be avoided, as this might affect the performance of the product.
- Repeated thawing and freezing of the Positive Control (PC) and No Template Control (NTC) (more than 4 times) should be avoided, as this might affect the performance of the product.
- Storage at room temperature (max. +30 °C) should not exceed a period of 2 hours.
- Protect the Detection Mix component from light.

## 4. Product description

The FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 is a reagent system. Used in combination with the FlexStar® (RT-)PCR Amplification Mix 1.5 it allows the qualitative detection of lineage B of betacoronaviruses (E gene) and SARS-CoV-2 (S gene + RdRp gene) specific RNA.

The FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 is based on real-time RT-PCR technology, utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of lineage B-βCoV (E gene) and SARS-CoV-2 (S gene + RdRp gene) specific target sequences and fluorescently labeled target specific probes for the detection of the amplified cDNA.

In addition to the lineage B-βCoV (E gene) and SARS-CoV-2 (S gene + RdRp gene) RNA specific amplification and detection systems, the FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 includes oligonucleotides for the amplification and detection of the sampling control (SC) and the internal control (IC, AltoStar® Internal Control 1.5). The SC (human β-actin gene) allows to monitor the quality of sampling. The IC is automatically added at the beginning of the nucleic acid purification procedure on the AltoStar® Automation System AM16 (in the following summarized as AltoStar® AM16). For details refer to the instructions for use of the AltoStar® Internal Control 1.5.

Probes specific for lineage B-βCoV (E gene) RNA are labeled with the fluorophore FAM™ and probes specific for SARS-CoV-2 (S gene + RdRp gene) RNA are labeled with the fluorophore ROX™. The probe for the SC is labeled with the fluorophore Cy5. The probe specific for the IC is labeled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of lineage B-βCoV (E gene), SARS-CoV-2 (S gene + RdRp gene), SC and the IC in the corresponding detection channels of the real-time PCR instrument.

## 4.1 Components

The FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 contains enough reagents for 384 reactions. The product consists of the following components:

- Detection Mix[1)]
- PC[2)]
- NTC[3)]

[1)] Contains biological material of animal origin

[2)] Positive Control [lineage B-βCoV (E gene) and SARS-CoV-2 (S gene) specific RNA and Sampling Control]

[3)] No Template Control (negative control)

Except for the DNA polymerase and the reverse transcriptase, which are included in the FlexStar® (RT-)PCR Amplification Mix 1.5, the Detection Mix component contains all reagents (PCR buffer, magnesium salt, primers and probes) to allow detection and differentiation of lineage B-βCoV (E gene) and SARS-CoV-2 (S gene + RdRp gene) specific RNA and detection of SC, as well as of IC specific RNA.

The PC contains lineage B-βCoV (E gene) and SARS-CoV-2 (S gene) specific RNA as well as SC. It is used to verify the functionality of the lineage B-βCoV, SARS-CoV-2 and SC specific amplification and detection systems.

The NTC contains neither lineage B-βCoV (E gene), SARS-CoV-2 (S gene + RdRp gene) specific RNA, nor SC but does contain the IC template. The NTC is used as negative control for the lineage B-βCoV (E gene), the SARS-CoV-2 (S gene + RdRp gene) RNA and the SC specific real-time PCR and indicates possible contamination of the Detection Mix component.

## 4.2 Real-time PCR instruments

The FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 can be used with the following real-time PCR instruments:

- CFX96™ Deep Well Dx System (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)
- QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)

**NOTE**

Ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer's instructions and recommendations.

# 5. Material required but not provided

The following additional instruments and consumables are required for use of the FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 but not provided with this product:

- Appropriate real-time PCR instrument (see chapter 4.2 Real-time PCR instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 6.1 Sample preparation)
- Vortex mixer
- Centrifuge (e.g. desktop centrifuge) for centrifugation of kit reagents
- Centrifuge for centrifugation of PCR plates
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

Reagents required but not included in the FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5:

- FlexStar® (RT-)PCR Amplification Mix 1.5 (Order No. FS0011505)
- AltoStar® Internal Control 1.5 (Order No. IC15-06)

# 6. Procedure

## 6.1 Sample preparation

Extracted RNA is the starting material for the FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5. The quality of the extracted RNA has a profound impact on the performance of the product.

For additional information and technical support regarding pre-treatment and sample preparation, contact altona Diagnostics technical support (see chapter 9. Technical assistance).

## 6.2 Master mix setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 is configured for use with the FlexStar® (RT-)PCR Amplification Mix 1.5 and the AltoStar® Internal Control 1.5, which allows to control the sample preparation procedure (nucleic acid extraction) and the subsequent RT-PCR.

- ► The IC is automatically added at the beginning of the nucleic acid purification procedure on the AltoStar® AM16.
- ► When using other nucleic acid extraction methods, the IC has to be added during the lysis step either manually or automatically by the respective instrument.
- ► No matter which method/system is used for nucleic acid extraction, never add the IC directly to the sample. The IC should always be added to the sample/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 50 % of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 30 µl of IC per sample must be added into the sample/lysis buffer mixture.
- ► Set up the master mix according to the following pipetting scheme:

**Table 2:** Pipetting scheme (master mix setup)

| Number of reactions (rxns) | 1 | 12 |
|---|---|---|
| Detection Mix | 5 µl | 60 µl |
| Amplification Mix | 15 µl | 180 µl |
| **Volume master mix** | **20 µl** | **240 µl** |

**NOTE**

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

## 6.3 Reaction setup

- ► Pipette 20 µl of the master mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (PC or NTC).

**Table 3:** Pipetting scheme (reaction setup)

| Reaction setup | |
|---|---|
| Master mix | 20 µl |
| Sample or control | 10 µl |
| **Total volume** | **30 µl** |

- ► Make sure that at least 1 PC and 1 NTC is used per run.
- ► Thoroughly mix the samples and controls with the master mix by pipetting up and down.
- ► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor and the reaction tubes in an appropriate centrifuge for 30 seconds at approximately 1,000 x g (~ 3,000 rpm).
- ► The NTC does already contain the IC template in the correct concentration.

# 7. Programming the real-time PCR instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, refer to the user manual of the respective instrument.

For detailed programming instructions regarding the use of the FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 on specific real-time PCR instruments, contact altona Diagnostics technical support (see chapter 9. Technical assistance).

## 7.1 Settings

► Define the following settings:

**Table 4:** Run settings

| Settings | |
|---|---|
| Reaction volume | 30 µl |
| Ramp rate | Default |
| Passive reference | None |

## 7.2 Fluorescence detectors (dyes)

► Define the fluorescence detectors (dyes):

**Table 5:** Fluorescence detectors

| Target | Detector name | Reporter | Quencher |
|---|---|---|---|
| Lineage B-βCoV specific RNA | E gene | FAM™ | (None) |
| SARS-CoV-2 specific RNA | S gene + RdRp | ROX™ | (None) |
| Sampling Control | Sampling Control | Cy5 | (None) |
| Internal Control | Internal Control | JOE™ | (None) |

### 7.3 Temperature profile and dye acquisition

► Define the temperature profile and dye acquisition:

**Table 6:** Temperature profile and dye acquisition

| | Stage | Cycle repeats | Acquisition | Temperature [°C] | Time [min:s] |
|---|---|---|---|---|---|
| Reverse transcription | Hold | 1 | - | 52 | 05:00 |
| Denaturation | Hold | 1 | - | 95 | 00:05 |
| Amplification | Cycling | 45 | - | 95 | 00:05 |
| | | | Yes | 58 | 00:25 |

## 8. Data analysis

For basic information regarding data analysis on specific real-time PCR instruments, refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the FlexStar® SARS-CoV-2 RT-PCR Detection Mix 1.5 on different real-time PCR instruments, contact altona Diagnostics technical support (see chapter 9. Technical assistance).

## 8.1 Interpretation of results

### 8.1.1 Qualitative analysis

**Table 7:** Result interpretation

| Detection channel | | | | Result interpretation |
|---|---|---|---|---|
| FAM™ (E gene) | ROX™ (S gene + RdRp) | Cy5 (SC) | JOE™ (IC) | |
| + | + | +/-* | +/-* | Lineage B-βCoV and SARS-CoV-2 specific RNA detected. |
| + | - | +/-* | +/-* | Only lineage B-βCoV specific RNA detected.** |
| - | + | +/-* | +/-* | Only SARS-CoV-2 specific RNA detected.** |
| - | - | + | + | Neither lineage B-βCoV nor SARS-CoV-2 RNA detected. The sample does not contain detectable amounts of lineage B-βCoV or SARS-CoV-2 specific RNA. |
| - | - | - | + | Collect and test a new sample. |
| - | - | + | - | RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample. |
| - | - | - | - | RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample. |

* Detection of the IC and SC in the JOE™ and Cy5 detection channel is not required for positive results in the ROX™ and/or the FAM™ detection channel. A high lineage B-βCoV (E gene) and/or SARS-CoV-2 (S gene + RdRp gene) RNA load in the sample can lead to reduced or absent SC and/or IC signals.

** Detection in only one of the two respective detection channels for lineage B-βCoV (E gene) and SARS-CoV-2 (S gene + RdRp) might be due to low viral RNA concentration close to the limit of detection or due to mutation of one of the two target sequences.

## 9. Technical assistance

For customer support, contact altona Diagnostics technical support:

**e-mail:** **support@altona-diagnostics.com**

**phone:** **+49-(0)40-5480676-0**

## 10. Trademarks and disclaimers

AltoStar®, FlexStar® (altona Diagnostics); QuantStudio™ (Applied Biosystems); CFX96™ (Bio-Rad); UTM® (Copan); JOE™ (Life Technologies); Rotor-Gene® (QIAGEN); LightCycler® (Roche); FAM™, ROX™ (Thermo Fisher Scientific).

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**For research use only (RUO)! Not for use in diagnostic procedures.**

## 11. Explanation of symbols

| Symbol | Explanation |
|---|---|
| RUO | Research use only |
| LOT | Batch code |
| CONT | Content |
| CAP | Cap color |
| REF | Catalogue number |
| NUM | Number |
| COMP | Component |
|  | Consult instructions for use |
|  | Contains sufficient for "n" tests/reactions (rxns) |
|  | Temperature limit |
|  | Use-by date |
|  | Manufacturer |
| MAT | Material number |
|  | Version |
|  | Note: Information is given to the user that is useful but not essential to the task at hand. |

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