altona DIAGNOSTICS

Instructions for use

FlexStar®
SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5

11/2023 EN

Respiratory

FlexStar®

FlexStar®

SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5

For research use only!

(RUO)

REF FS0021505

384

11 2023

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INS-FS0021500-EN-S04

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1. Application

The FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 is a reagent system, based on real-time PCR technology, for the qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza virus specific RNA. The SARS-CoV-2 detection is based on the parallel detection of the E gene of lineage B-beta coronavirus (including SARS-CoV-2) and the S gene of SARS-CoV-2.

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

2. Product content

The FlexStar® SARS-CoV-2 Type & FLU 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), SARS-CoV-2 (S gene) and influenza virus specific RNA]

[3)] No Template Control (negative control)

3. Storage

- The FlexStar® SARS-CoV-2 Type & FLU 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 Type & FLU 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 and differentiation of lineage B-beta coronaviruses (lineage B-βCoV, E gene), SARS-CoV-2 (S gene) and influenza (A+B) virus specific RNA.

The FlexStar® SARS-CoV-2 Type & FLU 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), SARS-CoV-2 (S gene) and influenza (A+B) specific target sequences and fluorescently labeled target specific probes for the detection of the amplified cDNA.

In addition to the lineage B-βCoV, SARS-CoV-2 and influenza virus RNA specific amplification and detection systems the FlexStar® SARS-CoV-2 Type & FLU RT-PCR Detection Mix 1.5 includes oligonucleotides for the amplification and detection of the internal control (IC, AltoStar® Internal Control 1.5). 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.

The probe specific for lineage B-βCoV (E gene) RNA is labeled with the fluorophore ROX™, the probe specific for SARS-CoV-2 (S gene) RNA is labeled with the fluorophore Cy5 and probes specific for influenza (A+B) virus RNA are labeled with the fluorophore FAM™, respectively. 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), influenza (A+B) viruses and the IC in the corresponding detection channels of the real-time PCR instrument.

4.1 Components

The FlexStar® SARS-CoV-2 Type & FLU 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), SARS-CoV-2 (S gene) and influenza virus specific RNA]

[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) and detection of influenza (A+B) virus specific RNA, as well as of IC specific RNA.

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

The NTC contains neither lineage B-βCoV, SARS-CoV-2, nor influenza (A+B) virus specific RNA 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) and the influenza (A+B) virus specific real-time PCR and indicates possible contamination of the Detection Mix component.

4.2 Real-time PCR instruments

The FlexStar® SARS-CoV-2 Type & FLU 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)
- 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 Type & FLU 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 Type & FLU RT-PCR Detection Mix 1.5:

- FlexStar® (RT-)PCR Amplification Mix 1.5 (Order No. FS0011503/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 Type & FLU 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 Type & FLU 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 Type & FLU 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

* If applicable

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	ROX™	(None)
SARS-CoV-2 specific RNA	S gene	Cy5	(None)
Influenza (A+B) virus specific RNA	Flu	FAM™	(None)
IC	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 Type & FLU 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
ROX™ (E gene)	Cy5 (S gene)	FAM™ [Influenza (A+B)]	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.**
-	-	+	+/-*	Only influenza (A and/or B) virus RNA detected.
+	-	+	+/-*	Lineage B-βCoV and influenza (A and/or B) virus specific RNA detected.
-	+	+	+/-*	SARS-CoV-2 and influenza (A and/or B) virus specific RNA detected.
+	+	+	+/-*	Lineage B-βCoV, SARS-CoV-2 and influenza (A and/or B) virus specific RNA detected.
-	-	-	+	Neither lineage B-βCoV nor SARS-CoV-2, nor influenza (A and/or B) virus specific RNA detected. The sample does not contain detectable amounts of lineage B-βCoV, SARS-CoV-2 or influenza virus specific RNA.
-	-	-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

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

** Detection in only one of the two respective detection channels for lineage B-βCoV (E gene) and SARS-CoV-2 (S gene) 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); CFX96™ (Bio-Rad); Rotor-Gene® (QIAGEN); LightCycler® (Roche); FAM™, JOE™, ROX™, VIC™ (Thermo Fisher Scientific).

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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

Symbol	Explanation
	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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COV-FlexStar-DM-Respi-RUO-EN-02_10/2022