DB-1211

COVID-19 Multiplex RT-PCR Kit

User Manual

Manual Version: DB-1211-001-200626

Revision: 1.6 EN

Last update: 12th August 2020

CE IVD

REF DB-1211-50rxns (sample kit) containing reagents sufficient for 50 reactions.

REF DB-1211-100rxns containing reagents sufficient for 100 reactions.

REF DB-1211-1000rxns containing reagents sufficient for 1000 reactions.

Contents

1 Preface

1.1 Purpose and Kit Usage

COVID-19 Multiplex RT-PCR Kit (Cat No DB-1211) is used for one step RT-PCR detection of SARS-CoV-2 virus (Wuhan coronavirus 2019, COVID-19) from extracted RNA. The kit specifically detects viral **Spike** and **EndoRNAse** genes in a single multiplexed RT-PCR reaction, along with **artificial RNA isolation control.** This control is added before the RNA isolation step and thus controls not only the efficacy of RT-PCR reaction but also of the RNA isolation process.

RNA for detection of COVID-19 using this kit should be isolated from the biological sample (e.g. from nasopharyngeal swab in universal transport media or PBS or from saliva) with kits designed for such purpose. Both spin column-based and magnetic particles-based kits can be used. The RNA must be eluted into water or buffer not inhibiting the RT-PCR reaction. The DIANA RNA isolation kit (Cat No DB-1205 or DB-1206) is recommended RNA isolation kit to be used to achieve optimal results of COVID-19 detection from universal transport media or PBS with nasopharyngeal swabs.

This kit contains artificial RNA isolation control and primers and probe to detect this control. We highly recommend adding this control to each sample prior RNA isolation to track isolation yield of each sample (it should be added to the lysis/binding buffer of the RNA isolation kit, which is then mixed with the sample from which RNA is extracted). If used as recommended, it controls not only the efficacy of RT-PCR reaction itself (revealing potential inhibition) but also the efficacy of the RNA isolation process, which is the key prerequisite for correct diagnostics. Less preferred option is to add the isolation control directly to the RT-PCR mix – in such case it could reveal only RT-PCR inhibition.

1.2 Performance characteristics

Sensitivity and specificity

The primers and probes used in this kit were developed by DIANA Biotechnologies and were optimized for the multiplexed detection. They target sequences in COVID-19 genome, which were not mutated in any of the european sequences available at NCBI database (238 sequences as of 17th May 2020). The primers were designed to be specific for COVID-19, their sequence is not conserved in SARS, MERS, or any other human coronavirus. The primers do not show any nonspecific amplification of human mRNA or human genome in *in silico* PCR analysis.

The kit was validated for RNA isolated from human samples, in total 18 positive samples and 59 negative samples were analyzed, and the results compared to four other CE IVD COVID-19 kits from three different vendors. The kit DB-1211 achieved 100% sensitivity and 100% selectivity. It detects less than 10 copies of the COVID-19 RNA genome, with linear range of up to 100 million copies, as determined by detecting a dilution series of the viral RNA of known concentration.

Accuracy

Variability between both genes (detected in FAM and HEX channels) has been quantified as mean standard deviation between C_t values in both channels for all positive clinical samples with $C_t < 35$ (17 out of 18 samples). The determined SD was < 0.20 cycles while the value of R^2 of the linear regression between the two genes was 0.998 for these samples.

The mean values of C_t determined for both genes for all 18 positive samples were compared to C_t values determined with a reference CE IVD kit targeting E-gene. The value of R^2 of the linear regression between the results from both kits was 0.984 with no outliers.

Reproducibility

Intra and interassay variability was determined for 40 replicates at three concentrations of viral RNA (100,000, 1,000 and 100 copies). Average intrassay variability (standard deviation between C_t of replicates on the same plate) was for both genes for these viral RNA concentrations < 0.1, < 0.2 and < 0.5 cycles, respectively, while interassay variability (standard deviation between C_t of replicates on different plates) were < 0.1, < 0.3 and < 0.6 cycles, respectively.

Suitable instruments

This kit has been validated on several RT-PCR cyclers: Roche LightCycler 480 II, Agilent AriaMX and BioRad CFX96. It can be used also with other instruments capable of triplex detection in FAM, HEX and Cy5 channels, however it is the sole responsibility of the user to perform the correct instrument setup and validate the kit performance with appropriate positive and negative controls. Please refer to the manufacturer instructions of your real-time PCR cycler on how to setup the protocol and the triplex detection in FAM, HEX and Cy5 channels.

1.3 Safety Notice

The kit is designed for professional use only. Follow the general principles of chemical safety. Wear protective equipment (gloves, goggles) and avoid contact with chemicals and refrain from eating or drinking in laboratory areas.

⚠ **Kit components contain 0.02% sodium azide, which is toxic** and upon contact with acids produces toxic gas. The material safety data sheet (MSDS) will be provided on request.

⚠ If you work with biological samples, pay attention to the safety rules for working with infectious biological material, use appropriate protective equipment (e.g. shield, respirator) and work with samples only in designated biohazard boxes or designated areas. Work with infectious samples only in BSL2 + or BSL3 laboratories. Dispose the potentially infectious waste in accordance with applicable legislation.

⚠ Continuously check that the work area is free of spilled solutions, chemicals, and / or biological samples. In the event of a spill, decontaminate the work area immediately. In case of skin or eye contact with reagents, rinse the affected area under running water immediately.

2 List of Materials

2.1 Required laboratory equipment

- Real-time PCR cycler with software capable of multiplex detection in FAM, HEX and Cy5 channels - **follow the operator's manual for your cycler**
- Calibrated handheld pipettes / multichannel pipettes
- Gloves and other protective equipment
- Recommended: benchtop vortex and centrifuge

2.2 Required material not included in the kit

- Disposable pipette tips (filter tips recommended)
- Disposable tubes for mixing of the components
- PCR plate and adhesive optical foil to seal the plate
- Kits or reagents for the RNA isolation (e.g. DB-1205 or DB-1206)

2.3 Kit Components

Components	Volume (µL)			Storage temperature	Vial cap number and color
	50rxns	100rxns	1000rxns		
Enhancer mix (4x)	250	500	5000	-20°C [2,3]	1 [5]
Primer mix (4x)	250	500	5000	-20°C [1,2,3]	2 [5]
Enzyme mix (4x)	250	500	5000	-20°C [2,3]	3 [5]
Positive control	25	50	2x 500	-20°C [2,3]	4
Isolation control [4]	75	150	3x 500	-20°C [2,3]	5

Table 1: Kit components

[1] – **Store at dark compartment** (contains fluorophores, which are photosensitive).

[2] – **Store frozen at -20°C or below**; it can be stored also at -40°C or -80°C. Do not use this kit if any of the components is not frozen upon arrival.

[3] – Restrict number of freeze/thaw cycles, aliquot controls after first thawing.

[4] – Add into the lysis/binding buffer prior RNA extraction or directly to RT-PCR master mix.

[5] – Coloured caps used only in 50rxns and 100rxns size (some lots may have transparent caps). Transparent caps are used in 1000rxns size.

Enhancer Mix (4x)

Contains proprietary additives increasing efficiency of RT-PCR. Provided as 4x concentrate.

Primer Mix (4x)

Contains primer pairs and fluorescently labelled probes for detection of viral **EndoRNAse in FAM channel**, viral **Spike in HEX channel** and artificial **RNA Isolation control in Cy5 channel**. Provided as 4x concentrate.

Enzyme Mix (4x)

Contains thermostable Reverse Transcriptase, hot-start Taq DNA Polymerase, $MgCl_2$, dNTPs, buffer, and proprietary additives. Provided as 4x concentrate.

Positive control

Contains template viral RNA at concentration of approximately 2,000 copies per µL. Opening this vial may cause contamination of the workspace, thus always spin down this vial before opening!

Isolation control

Contains artificial RNA which is added to each sample prior RNA isolation to control efficacy of the RNA extraction and to discover possible inhibition of RT-PCR reaction.

2.4. Stability of the kit components and master mix

The kit components 1, 2 and 3 should be kept at -20°C or below for long-term storage (see package for shelf life). Avoid repeated freeze/thaw cycles, a maximum of four cycles is possible. If you intend to use the same components multiple times, prepare aliquots during first handling of them.

After thawing of these kit components, use them as soon as possible. They are stable for up to 8 hours at 25°C if not combined together. However, it is recommended to use the kit components as soon as possible after thawing, or to keep them on ice (for maximum of 8 hours). Primer mix should be kept in dark container for long-term storage, but all components can be kept on daylight for up to 8 hours if not in direct sunlight.

The RT-PCR master mix (combined components 1, 2 and 3, see section 3.5 for details) is also stable for up to 8 hours when kept on ice, it should not be kept on ambient temperature for longer than 30 minutes. It can be kept on daylight for up to 8 hours if not in direct sunlight. It can be frozen once. You can thus prepare such mastermix for several plates in advance or prepare ready to use PCR plates, but you should aliquot and freeze the mix immediately after you combine and mix the components, flash freezing in liquid nitrogen or putting directly into -80°C is necessary, the mix can be then stored at -80°C for up to one month.

The kit components 4 and 5 contain RNA and they should be thawed only for shortest possible time and should be kept on ice during work, even though they are stable for up to 8 hours at room temperature. If you need to use them multiple times, prepare aliquots during their first handling. Optimal temperature for long-term storage is -80°C, while they can be stored at -20°C together with the rest of the kit.

3 Instructions for use

3.1 General precautions

- Do not use kit components, which are not frozen or damaged upon receipt. Keep the kit components for an eventual claim and contact the manufacturer.
- Inappropriate handling with kit components and deviations from this user guide may adversely affect the results.
- Use version of this user guide (see page header), which is referenced on the package.
- Do not use kit components after kit expiry date, which is indicated on the package.
- Do not mix kit components from different lots of the kit (indicated on the package).

3.2 Avoiding contamination (false positives)

Good laboratory practice to avoid sample cross-contamination should be followed, including the use of disposable filtered pipetting tips, and using a clean tip for each step.

To minimize risk of accidental contaminations of the reactions with template RNA/DNA, handling of clinical samples, positive controls or amplified PCR products (template DNA) should be spatially separated from handling of the stock kit components 1, 2 and 3 to avoid their contamination. The best practice is to prepare the RT-PCR master mix from components 1, 2 and 3 and to transfer this mix into the PCR plate at designated location (e.g. PCR box), in which RNA or DNA templates are never handled. This space should have also assigned equipment (e.g. pipettes, tips, and vials) not used for other purposes (i.e. never used for handling or RNA/DNA templates). The PCR plate with master mix is then transferred to other location (e.g. other PCR box), where the samples or positive controls are added.

Some other general guidelines to avoid accidental contaminations:

- Never open or otherwise handle vials with samples, positive controls, or amplified PCR products in the place where the RT-PCR master mix is prepared.
- Spin down every vial with template RNA/DNA before opening it.
- Leave reagents containers open only for the time necessary to prepare PCR reactions.
- Use ultra-pure or PCR-grade water (or buffers prepared from them) to dilute sample.
- Close all reagent vials before manipulating a positive control.

To control false-positive and false-negative results, we highly recommend running positive and negative controls in each RT-PCR run (on each plate). The negative control can be set up in two ways. The best way is to run the RNA isolation with known negative sample or with a blank sample medium and add the same amount of eluate to the RT-PCR mix as for the actual samples. Such negative control will monitor contaminants at any step of the process. Less robust negative control can be set up by directly adding the clean elution solution to the RT-PCR mix as if it was a sample, but this will monitor only the contamination of elution buffer or of RT-PCR mix. To run the positive control, this kit contains separate vial with the COVID-19 RNA template (Vial No 4).

3.3 Before you start

Components are stored frozen, therefore before each use:

- Thaw the components on ice or at ambient temperature (place on ice if you do not use the components immediately upon thawing).
- Spin down each vial to collect all liquid at the vial bottom before opening.
- Before use, mix the reagents in the vials by vortexing or pipetting. The pipette should be set up for mixing for at least ½ of the volume of the reagent, multiple aspirates and dispenses are needed for proper mixing. This is especially important before aliquoting to ensure the same composition in each aliquot. If vortexing, briefly spin the vial before opening.

3.4 Adding isolation control

RNA Isolation control should be added into the lysis/binding buffer in the RNA extraction kit prior mixing with the sample and subsequent RNA isolation. Add **1 µL of Isolation control from vial No 5 (red cap 🔴)** per one sample to the lysis/binding buffer. Such control is highly recommended, as it reveals efficacy of RNA isolation and possible inhibition of RT-PCR at the same time.

Alternatively, if adding the isolation control to the sample prior RNA isolation is not possible, then add **0.1 µL of Isolation control from vial No 5 (red cap 🔴)** per one sample directly to the RT-PCR mix (see optional step 5 in section 3.5 for more details).

3.5 Preparation of the RT-PCR master mix

Preparation of RT-PCR mix for one reaction is described below. If you are preparing RT-PCR mix for multiple reactions, multiply the volumes by the number of the reaction (and account for some pipetting reserve) – see also Table 2.

1. Thaw and mix each component (see section 3.3. for more details)
2. Into a clean RNase/DNase free vial, pipette **5 µL of Enhancer mix (4x) from kit vial No 1 (green or transparent cap)**,
3. add **5 µL of Primer mix (4x) from kit vial No 2 (blue or transparent cap)** to the same vial and mix by pipetting several times up and down,
4. add **5 µL of Enzyme mix (4x) from kit vial No 3 (black or transparent cap)**, to the same vial and mix by pipetting several times up and down until the mixture is homogeneous (you can also briefly vortex and spin down),
5. ***Optional**: if you do not add the RNA isolation control into the sample prior RNA isolation, **add 0.1 µL of Isolation control kit vial No 5 (red cap)** and mix by pipetting up and down (use pipette with at least ½ volume of the liquid in the vial) or by vortexing.*
6. Transfer **15 µL of the mixture (RT-PCR master mix)** mix into 96-well plate or into micro tube (depending on your cycler) for subsequent addition of sample and RT-PCR analysis. Cover the plate with lid and place on ice if you cannot run RT-PCR immediately (more details on stability of the kit components and of the RT-PCR master mix in section 2.4.)

Kit component	Vial # and cap color	µL per one reaction	µL per 100 reactions
Enhancer mix (4x)	1	5	500
Primer mix (4x)	2	5	500
Enzyme mix (4x)	3	5	500
Isolation control (Optional*)	5	0.1	10
Total volume of RT-PCR master mix		15	1500

Table 2: Preparation of RT-PCR master mix
Order of addition is the same as tube numbering, it is important to add components in this order.
* the volume of Isolation control is neglected; it is added to the RT-PCR mix only if it was not already added during the RNA isolation (see Section 3.4. for more details).

3.6 Adding sample to the RT-PCR reaction

Add 5 µL of the sample into each well (or micro-tube), containing 15 µl of the RT-PCR master mix.

Run at least one positive control and one negative control on each plate. For positive control, add **5 µL of Positive control from kit vial No 4 (yellow cap)**. For negative control, either add **5 µL of eluted RNA from known negative sample** or add **5 µL of the ultra-pure water** or of the elution buffer from your RNA extraction kit. The total reaction volume is always 20 µL.

After adding samples to the 96-well plate, seal the plate with optical foil and start RT-PCR run as described in section 3.7. as soon as possible (ideally within 15 minutes).

3.7 RT-PCR protocol

This kit has been validated for: **Roche LightCycler 480 II**, **Agilent AriaMX** Real-Time PCR System and **BioRad CFX96** real-time PCR. It can be used also with other instruments capable of triplex detection in FAM, HEX and Cy5 channels, however it is the sole responsibility of the user to perform the correct instrument setup and validate the kit performance with appropriate positive and negative controls. Please refer to the manufacturer instructions of your real-time PCR cycler on how to setup the protocol and the triplex detection in FAM, HEX and Cy5 channels.

Roche LightCycler 480 II setup

Use following setting for the triplex detection:

Channel	Excitation filter (nm)	Emmision filter (nm)	Quant factor	Max integration time (sec)
FAM	465	510	10	1
HEX	533	580	10	1
Cy5	618	660	10	3

Table 3: Settings for triplex detection on Roche LightCycler 480 II

The program consists of four steps:

1. Reverse transcription of the viral RNA (RT step)
2. Denaturation: Taq polymerase activation
3. Cycling: PCR amplification (45 cycles)
4. Cooling of the plate

For the target temperature and timing of each step please refer to the table below.

Variable	RT step	Denature	Cycling			Cooling
Analysis mode	None	None	Quantification			None
Cycles	1	1	45			1
Target (°C)	50	95	95	60	72	40
Hold (hh:mm:ss)	00:10:00	00:02:00	00:00:05	00:00:15	00:00:15	00:00:30
Ramp rate (°C/s; 96)	4.4	4.4	4.4	2.2	4.4	2.2
Acquisition mode	None	None	None	Single	None	None

Table 4: Protocol for RT-PCR detection on Roche LightCycler 480 II. Approximate runtime of this protocol on Roche LightCycler 480 II is 70 minutes.

Agilent AriaMX setup

Identical protocol as described above for **Roche LightCycler 480 II** was validated also for **Agilent AriaMX Real-Time PCR System.** Use default ramp rates and default setup of FAM, HEX and Cy5 channels. Runtime on AriaMX is approximately 75 minutes.

BioRad CFX96 setup

Identical protocol as described above for **Roche LightCycler 480 II** was validated also for **BioRad CFX96.** Use default ramp rates and default setup of FAM, HEX and Cy5 channels. Runtime on BioRad CFX96 is approximately 80 minutes.

3.8 Data Analysis

Determination of the cycle threshold value (C_t)

Perform data analysis as described in the operator's manual for your real-time PCR system. For LightCycler 480 II, we recommend using the default color compensation saved in the database for FAM and HEX* channels and using the second derivative maximum method for determination of C_t values. It is possible to use also other instruments and other methods of calculation of C_t values, these values can, however, significantly differ between the methods and instruments. If you use the Fit Points method** and set the fluorescence threshold near the background fluorescence, then you can obtain C_t values lowered by up to 3 cycles with Roche LC480 II. C_t values obtained in this way with Biorad CFX96 or Agilent AriaMX can be also lowered by up to 3 to 4 cycles. However, if you set up the threshold higher, you can obtain cycle values even much higher than with Roche LC480 II and second derivative method (by up to 3 to 4 cycles).

Moreover, any of these methods can lead to incorrect interpretation of detected fluorescence curves and therefore you should always perform visual check of the fluorescence curves, whether the C_t values were assigned correctly. Any curve with steep and consistent rise of fluorescence should be assigned as positive (typical shape of fluorescence vs cycles is shown on page 14), while other curves must be assigned as negative. Examples of false results are falsely negative wells in the second derivative method due to the nonstandard shape of the fluorescence curve (e.g. nick due to a bubble). On the other hand, the fit point method can give false positive result due to the same reason.

All C_t values shown later in text for the controls and in Table 5 correspond to the Roche LightCycler 480 II and second derivative method. If you use another method and/or instrument and to make precise interpretation via Table 5, you should adjust your C_t values by the difference in the C_t value you determined for the positive control (prepared as described in section 3.6, Positive control in vial No 4) and of the reference value C_t = 29 (in both FAM and HEX channels). For example, if your Ct value for positive control would be 26th cycle, then to exactly interpret your results according to the Table 5, either add 3 cycles to your C_t values for your clinical samples, or subtract three cycles from threshold C_t values in the Table 5.

** Use of color compensation is not necessary, however, without the compensation, weak signal in HEX channel can be observed even in the absence of amplification in HEX channel in the case of simultaneous amplification in FAM channel. Do not use the compensation for Cy5 channel.*
*** Also called Threshold Crossing or Cycle Threshold method, where C_t is calculated as a cycle number where the fluorescence grows above the background level to cross a predetermined fluorescence threshold value.*

Inspecting controls

Positive control must show amplification in all three channels: FAM (cycle range 27-31), HEX (cycle range 27-31) and Cy5 (< 35 cycles in case you added the isolation control). Failure to see amplification in any of the channels show insufficient PCR amplification and the results from such run are not valid and must be repeated. The C_t values shown here correspond to the second derivative method, where the reference C_t value for both FAM and HEX channels is 29th cycle. By using fit points method, the C_t values can be by up to 3 to 4 cycles lower.

Negative control must show amplification only in Cy5 channel (< 35 cycles in case you added the isolation control), while there must be no amplification in FAM or HEX channels. Amplification in FAM or HEX channels shows contamination of the reagents with template sequence which can cause false positive results.

Interpreting the sample results

Determine C_t values for each sample in all channels and interpret the results according to the Table 5 and to the results of the positive control. In case your C_t values for the positive control are in the range 27 to 31 cycles, you can apply Table 5 threshold cycles without further adjustments. In opposite case, you should adjust your C_t values as described in section Determination of the cycle threshold value (C_t) above before using Table 5 for their interpretation.

FAM	HEX	Cy5	Interpretation
+ [3]	+ [3]	+ [1] / - [2]	**COVID-19 positive**
-	-	+ [1]	**Not detectable (COVID-19 negative)**
-	-	-	**Unreliable result:** Either poor yield of RNA isolation or RT-PCR is inhibited, **repeated RNA isolation recommended**.
C_t > 30 [3]	-	+ [1]	**Weakly COVID-19 positive: repeated RT-PCR recommended** (repeated positive signal in at least one channel means COVID-19 positive sample)
-	C_t > 30 [3]	+ [1]	
C_t > 30 [3]	-	-	**Weakly COVID-19 positive:** either poor yield of RNA isolation or RT-PCR is inhibited, **repeated RNA isolation recommended** (repeated positive signal RT-PCR in at least one channel means COVID-19 positive sample)
-	C_t > 30 [3]	-	
C_t < 30 [3]	-	+ [1] / - [2]	**Unreliable result:** could indicate contamination with amplification product or mutation in one of the genes, repeated RNA isolation recommended, reanalysis with other RT-PCR kit targeting other genes recommended
-	C_t < 30 [3]	+ [1] / - [2]	

Table 5: Interpretation of the results.
+ means C_t below 40 cycles [3] / - means C_t above 40 cycles [3] or no signal detected
Viral gene EndoRNAse is detected in FAM, viral gene Spike in HEX and Isolation control in Cy5.
[1] In case RNA isolation control was added to the sample prior RNA extraction in the amount as instructed and standard RNA isolation protocol was followed (approx. 1/10 of the elution used for the RT-PCR, e.g. 5 out of 50 µL), the Cy5 C_t value should be lower or around 35th cycle.
[2] Very high viral concentrations may cause impaired amplification of isolation control and thus shift in cycle value or even absence of Cy5 detection (see Figure 1 for more details). Absence of Cy5 signal does not change the interpretation of positive signal in FAM and HEX channels.
[3] The cycle threshold values set forth in this table are based on second derivative method with Roche LC 480 II instrument. Actual observed values can differ with the use of different instrument and/or methods (see above).

Typical data

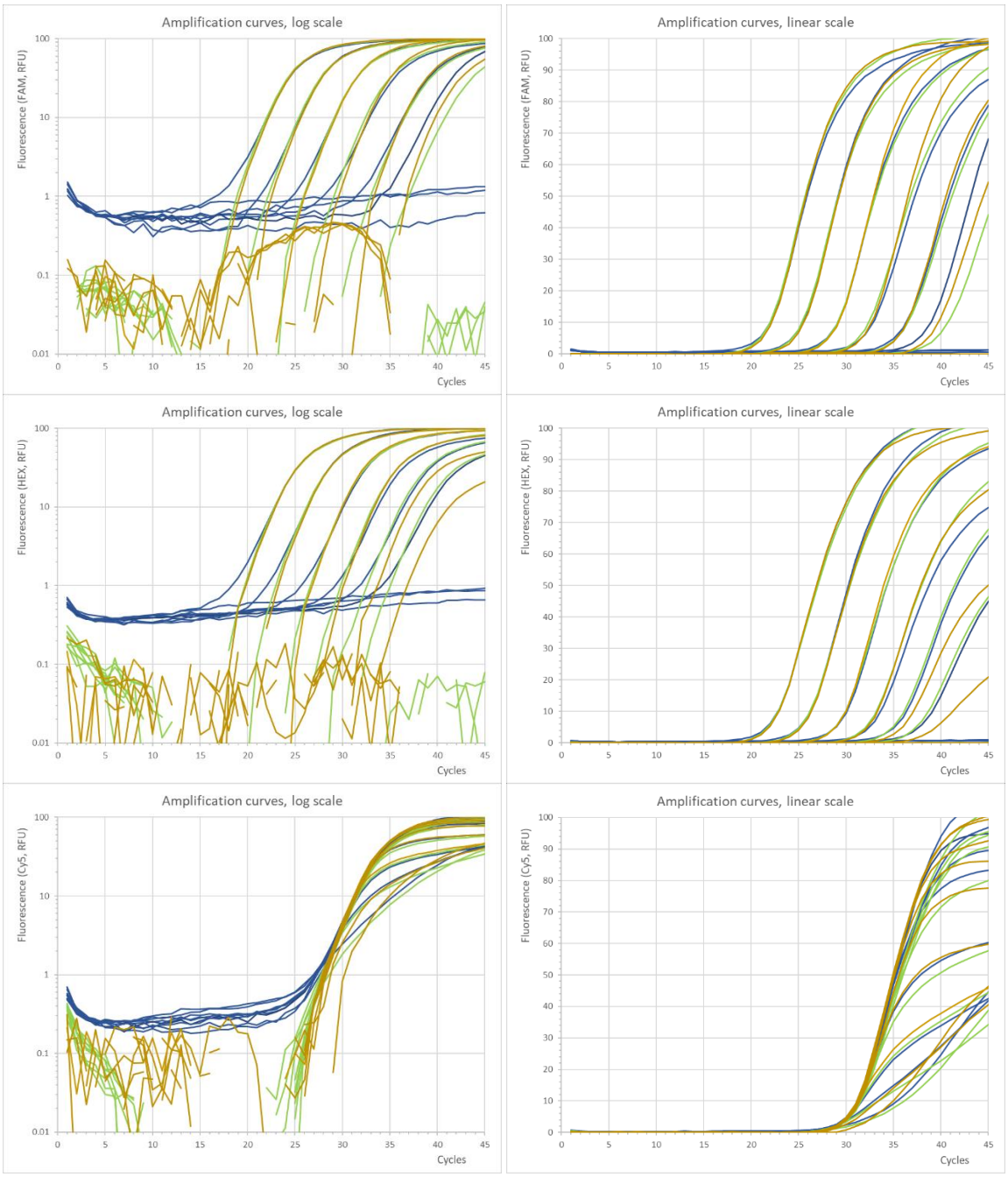

Figure 1: Data from ten-fold dilution series of COVID-19 RNA in the range from 1,000,000 to 10 copies per well (and three negative controls) on Roche LC480 II (blue lines), Agilent AriaMX (green lines) and BioRad CFX96 (brown lines).
Charts show amplification in FAM (viral gene EndoRNAse) in first panel, amplification in HEX (viral gene Spike) in second panel and amplification in Cy5 (RNA isolation control) in last panel. Charts on left shows fluorescence in log scale while charts on right shows fluorescence in linear scale.

Relative fluorescence units were normalized that maximum for each instrument was set to 100. Actual measured values were on LC480 II ~30 RFU for both FAM and HEX and ~8 RFU for Cy5; on CFX96 ~5,000 RFU for both FAM and HEX and ~1,500 RFU for Cy5; on AriaMX ~10,000 RFU for both FAM and HEX and ~10,000 RFU for Cy5.
Amplification of viral RNA in FAM and HEX channels is linear in the full range, Cy5 amplification is lowered at high viral titers, but is detected for all titers (curves with lower maximum fluorescence shown in lowest right chart correspond to wells with highest viral RNA concentrations).

Materials used: FrameStar 96 Well Semi-Skirted PCR plate (4ti-0951, white wells) and LightCycler 480 Sealing Foil (04729757001) for **LC480 II**; Hard-Shell 96 well PCR plate-low-profile-skirted-clear-well (HSP9601) and LightCycler 480 Sealing Foil (04729757001) for **CFX96**, FrameStar 96 Fully-Skirted (F-0961, white wells) and LightCycler 480 Sealing Foil (04729757001) for **AriaMX**.

4 Legal notice

To the fullest extent permitted by the laws of your jurisdiction, the DIANA Biotechnologies, s.r.o. is excluded of any liability for any direct or indirect damages associated with and / or incurred in relation to this document and its use, as well as for any direct and indirect damages associated with and / or incurred in relation to the COVID-19 Multiplex RT-PCR Kit and its use.

5 List of compatible kits

REF DB-1205 DIANA Manual RNA Isolation Kit

REF DB-1206 DIANA Automated RNA Isolation Kit

6 One-page summary protocol

6.1. Kit components

Components	Volume (µL)			Storage temperature	Vial label
	50rxns	100rxns	1000rxns		
Enhancer mix (4x)	250	500	5000	-20°C	1
Primer mix (4x)	250	500	5000	-20°C	2
Enzyme mix (4x)	250	500	5000	-20°C	3
Positive control	25	50	2x 500	-20°C	4
Isolation control	75	150	3x 500	-20°C	5

6.2 RT-PCR preparation

- Spin each vial before opening, mix each component upon thawing.
- In the following order, combine 5 µL of Enhancer mix (Vial No 1) with 5 µL of Primer mix (Vial No 2) and with 5 µL of Enzyme mix (Vial No 3). Mix after addition of each component.
- Transfer 15 µL of this RT-PCR master mix to 96-well plate, add 5 µL of the sample, seal the plate with optical foil and run RT-PCR as soon as possible. For positive control, add 5 µL of the positive control (Vial No 4) instead of the sample.

Table below summarizes volume of kit components per one or per 100 reactions:

Kit component	Vial label	µL per one reaction	µL per 100 reactions
Enhancer mix (4x)	1	5	500
Primer mix (4x)	2	5	500
Enzyme mix (4x)	3	5	500
Isolation control (Optional*)	5	0.1	10
Total volume of RT-PCR master mix		15	1500

* Isolation control (Vial No 5) should be added directly into the PCR mix only if it was not added to samples prior RNA isolation (see section 3.4 for more details).

6.3. RT-PCR protocol

Table below summarizes the protocol setup for **Roche LightCycler 480 II instrument**:

Variable	RT step	Denature	Cycling			Cooling
Analysis mode	None	None	Quantification			None
Cycles	1	1	45			1
Target (°C)	50	95	95	60	72	40
Hold (hh:mm:ss)	00:10:00	00:02:00	00:00:05	00:00:15	00:00:15	00:00:30
Ramp rate (°C/s; 96)	4.4	4.4	4.4	2.2	4.4	2.2
Acquisition mode	None	None	None	Single	None	None

Acquisition must be set for **triplex detection in FAM, HEX and Cy5 channels**. Refer to section 3.7 and to operator's manual of your PCR cycler how to set up such triplex detection.

7 Used graphical symbols

	Manufacturer / Výrobce
	Date of Manufacture / Datum výroby
	Caution / Pozor
	Lot Number / Kód dávky
	Operator´s manual, operating instructions / Čtěte návod k použití
	Catalogue Number / Katalogové číslo
	Lower limit of temperature / Dolní mez teploty
	Temperature limit / Limit teploty
	Upper limit of temperature / Horní mez teploty
	Do not use if package is damaged / Nepoužívat jestliže je balení poškozeno
	Use by date / Použít do data
	Do not reuse / Nepoužívat opětovně
	Keep away from sunlight / Chraňte před slunečním zářením