ROBOSCREEN DIAGNOSTICS

Instructions for Use

Rapid INSTANT Virus RNA/DNA Kit - FX

Rev. 1 _ 02 / 2024

CE

IVD

Order No.:

847-0259200906	2x 96 reactions for 400 µl sample volume

Manufacturer:

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IFU Rapid INSTANT Virus RNA/DNA Kit-FX Rev.1 02 / 2024

Content

1 Introduction

1.1 Intended use

The Rapid INSTANT Virus RNA/DNA Kit - FX is intended for automated isolation of viral RNA and DNA from 400 µl of cell-free human body fluids and is optimized for a minimal processing time.

The extraction procedure is based on the magnetic particle binding technology and is optimized for rapid preparation of highly pure viral RNA and DNA from human plasma and serum, cerebrospinal fluid and swab- and stool-supernatants.

Rapid INSTANT Virus RNA/DNA Kit – FX is configured for exclusive use in combination with the CyBio FeliX Basic Unit equipped with the CyBio FeliX Extraction Set, thereby allowing simultaneous processing of up to 96 samples.

The kit is intended to be applied by professional users in a laboratory environment.

1.2 Technical assistance

If you have any questions or problems regarding any aspects of the Rapid INSTANT Virus RNA/DNA Kit - FX, please do not hesitate to contact our technical support team which consists of experts with long-time experience in the field of molecular diagnostics. For technical assistance please contact us at the manufacturer site as shown inside the cover of the instructions for use.

CONSULT INSTRUCTION FOR USE

This instruction for use must be read carefully prior to use. Given instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.

1.3 Symbols and Abbreviations

For easy reference and orientation, the IFU uses the following warning and information symbols:

Symbol	Information
	**REF** Catalogue number.
	**Content** Contains number of IVD-determinations as indicated.
	**Storage temperature** Store at temperatures between upper and lower limits as indicated.
	**Consult instructions for use** This information must be observed to avoid improper use of the kit.
	**Used by** Expiry date. The product is to be used by the indicated date.
	**Lot number** The lot number of the kit.
	**CE-IVD symbol** In vitro diagnostic medical device.
	**Manufactured by** Contact information of the manufacturer.
	**For single use only** Single use only. Do not use the product twice.
	**Note / Attention** Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following abbreviations are used:

DEPC	diethylpyrocarbonate
DNA	desoxyribonucleic acid
EDTA	ethylenediaminetetraacetate
IFU	instructions for use
NaOH	sodium hydroxide
PCR	polymerase chain reaction
RNA	ribonucleic acid

2 Safety precautions

NOTE

Read this chapter carefully to guarantee your own safety and a trouble-free operation.

Follow all safety instructions explained in the IFU, as well as all given messages and information.

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany

Phone: +49 (0)761 19 240

2.1 Warning and precautions

For more information on GHS classification please request the Safety Data Sheet (SDS) at the manufacturer site as shown inside cover page of the IFU.

FOR SINGLE USE ONLY!

This kit is made for single use only!

ATTENTION

The kit is intended to be applied by professional users in a laboratory environment!

- Pay Attention while handling the materials and reagents contained in the kit. Always wear gloves while handling the reagents and avoid any skin contact! In case of contact, flush eyes, or skin with a large amount of water immediately.
- Do not swallow components of the kit!

- Human body fluids like plasma and serum samples must be considered as potentially infectious. Thus, always wear lab coat and gloves.
- Discard sample and assay waste according to your in-house safety regulations. Please observe the federal, state, and local safety and environmental regulations.
- If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles to avoid any injuries.
- Follow the usual precautions for applications using extracted nucleic acids.
- Do not add bleach or acidic components to the waste after sample preparation!
- Always use clean and nuclease-free equipment. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

2.2 Safety recommendations on handling RNA

RNA is far less stable than DNA and is very sensitive to degradation by RNases. To achieve satisfactory results in RNA extraction from sample material, contamination with RNases must be avoided by application of the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Reduce preparation time as much as possible.
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.

- To ensure absence of RNase-activity, glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240° C for at least four hours before use. This also destroys nucleic acids possibly being present on the surface of the glassware. Glassware can also be cleaned by immersing in 0.1% DEPC for 12 hours at 37° C followed by autoclaving or heating to 100° C for 15 minutes to remove residual DEPC. Autoclaving alone is not suitable to completely inactivate RNase activity!
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

2.3 Starting material

Rapid INSTANT Virus RNA/DNA Kit - FX is validated for automated purification of viral nucleic acids from the following starting material:

- Fresh or frozen human cell-free body fluids (serum, plasma, cerebrospinal fluid and stool).
- Swab collected specimen taken from epithelial surfaces
- Stabilizers: EDTA or citrate
- 400 µl sample volume

NOTE
Do not use heparinized starting material.

NOTE
Avoid repeated freezing and thawing of starting material.

2.4 Restrictions

If other than the recommended sample types or volumes are used incorrect results may be obtained.

Extracted nucleic acids should be used for downstream diagnostic applications immediately since Elution Buffer (EB) is not suitable for long-term storage of nucleic acids.

Do not use expired components.

Do not mix components belonging to kits with different lot numbers.

3 GHS Classification

Com-ponent	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases	EUH
PK	Proteinase K 50-100 %		315, 319, 334, 335	280, 260, 308+313, 342+311 305+351+338, 302+352,501	-
LS	Dihydrate (Ethylenedinitrilo) Tetraacetic Acid, Disodium Salt 0,1-1,0 % Guanidinium thiocyanate 40-50 %		302, 332, 314, 412	260, 280, 308+310, 305+351+338, 303+361+353, 273, 405, 501	032
BS	Guanidinium thiocyanate 30-40 % Propan-2-ol 10-25 %		225, 302, 332, 336, 314, 412	210, 260, 280, 308+310, 305+351+338, 303+361+353, 273, 405, 501	032
WS A	Guanidinium chloride 40-50% Propan-2-ol 25-35%		225, 302, 315 ,319, 336	210, 260, 280, 308+310, 305+351+338, 303+361+353, 501	

3.1 Hazard phrases

225	Highly flammable liquid and vapour.
302	Harmful if swallowed.
314	Causes severe skin burns and eye damage.
315	Causes skin irritation.
319	Causes serious eye irritation.
332	Harmful if inhaled.
334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
335	May cause respiratory irritation.
336	May cause drowsiness or dizziness.
412	Harmful to aquatic life with long lasting effects.

3.2 Precaution phrases

210	Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. No smoking.
260	Do not breathe dust/fume/gas/mist/vapors/spray.
280	Wear protective gloves/protective clothing/eye protection/face protection.
308+310	IF exposed or concerned: Immediately call a POISON CENTER/doctor.
308+313	IF exposed or concerned: Call a POISON CENTER/doctor.
342+311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
305+351+338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
302+352	IF ON SKIN: Wash with plenty of water.
303+361+353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
273	Avoid release to the environment.
405	Store locked up.
501	Dispose of contents/container to special waste collection point.

3.3 EU hazard statements

032 Contact with acids liberates very toxic gas.

4 Performance Assessment

Analytical performance validation of Rapid INSTANT Virus RNA/DNA Kit - FX was done in combination with CE-IVD marked quantitative virus detection kits RoboGene HCV RNA Quantification Kit 3.0 (Roboscreen GmbH, order number 847-0207610-032/096/192) and RoboGene HBV DNA Quantification Kit 3.0 (Roboscreen GmbH, order number 847-0207710-032/096/192).

Validation of clinical performance of Rapid INSTANT Virus RNA/DNA Kit – FX was carried out in combination with CE-IVD marked qualitative SARS-CoV-2 tests Fast Track SARS-CoV-2 Kit (Siemens Healthineers, order number 11416284) and LightMix SarbecoV E-gene plus EAV control (TIB MOLBIOL, order number 40-0776-96).

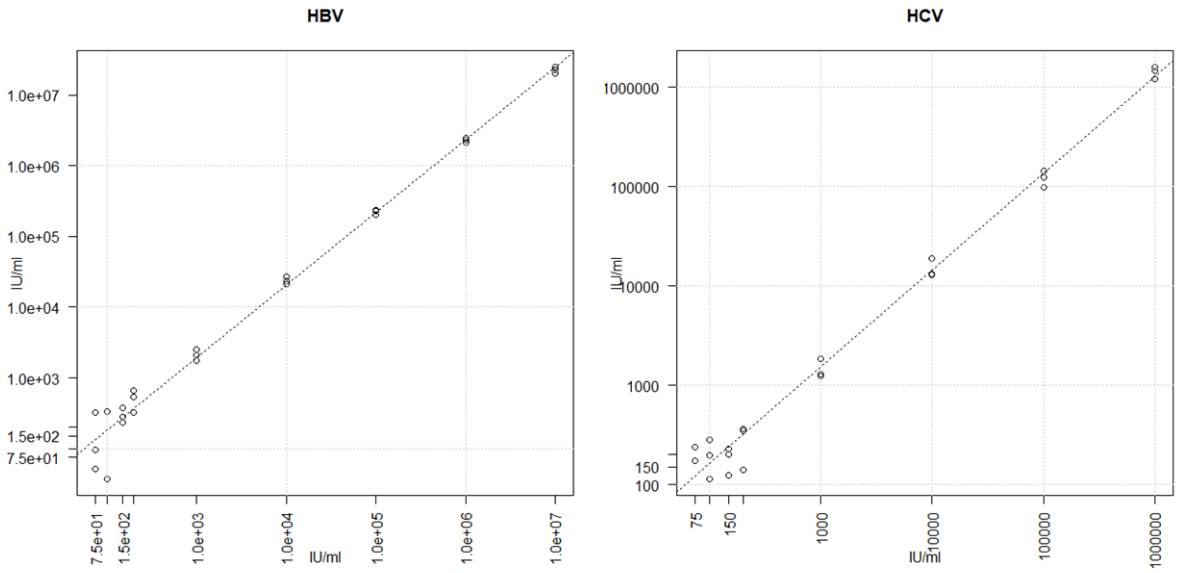
4.1 Linear Range

The linear range of quantification of viral RNA and DNA using the RoboGene Kits mentioned above in combination with Rapid INSTANT Virus RNA/DNA Kit - FX was determined by analyzing a dilution series of native sample material tested positive for HBV ranging from $1x10^7$ to $0.75x10^2$ IU/ml and a dilution series of native sample material tested positive for HCV and covering concentrations between $1x10^6$ and $0.75x10^2$ IU/ml.

Automated nucleic acid purification was performed for each concentration in triplicate and all eluates were quantified in the same analysis using the CFX96 real-time PCR detection system (Bio-Rad). The analysis was carried out according to the CLSI guideline EP06 [1].

[1] CLSI. Evaluation of the Linearity of Quantitative measurement Procedures: A Statistical Approach; Approved Guideline. CLSI Document EP06-A. Wayne, PA: Clinical and Laboratory Standards Institute, 2003.

The analysis revealed a high linearity of the quantitative detection of the extracted nucleic acids described by correlation coefficients of 0,9918 for HCV RNA and 0,9851 for HBV DNA (see Figure 1).



y = 1,0392x + 0,1363
R^2 = 0, 9851

y = 1,0009x + 0,1489
R^2 = 0,9918

Figure 1: Scatterplot of linear range of RNA and DNA quantification using Rapid INSTANT RNA/DNA Kit - FX in combination with RoboGene HBV DNA Quantification kit 3.0 (left) and RoboGene HCV RNA Quantification kit 3.0 (right). Slope, intercept, and correlation (R^2) were calculated for HBV-DNA within concentration range of 0.75x10^2 to 1x10^7 IU/ml and HCV-RNA within 1x10^2 to 1x10^6 IU/ml.

The data shows that the mean deviation for concentration values at the limit of detection (LOD) was approx. ±0.3 log10 IU/ml and above the LOD approx. ±0.2 log10 UI/ml. (see Figure 2).

Therefore, it can be deduced that the value of the lower limit of quantification (LLOQ) corresponds to the value of the LOD.

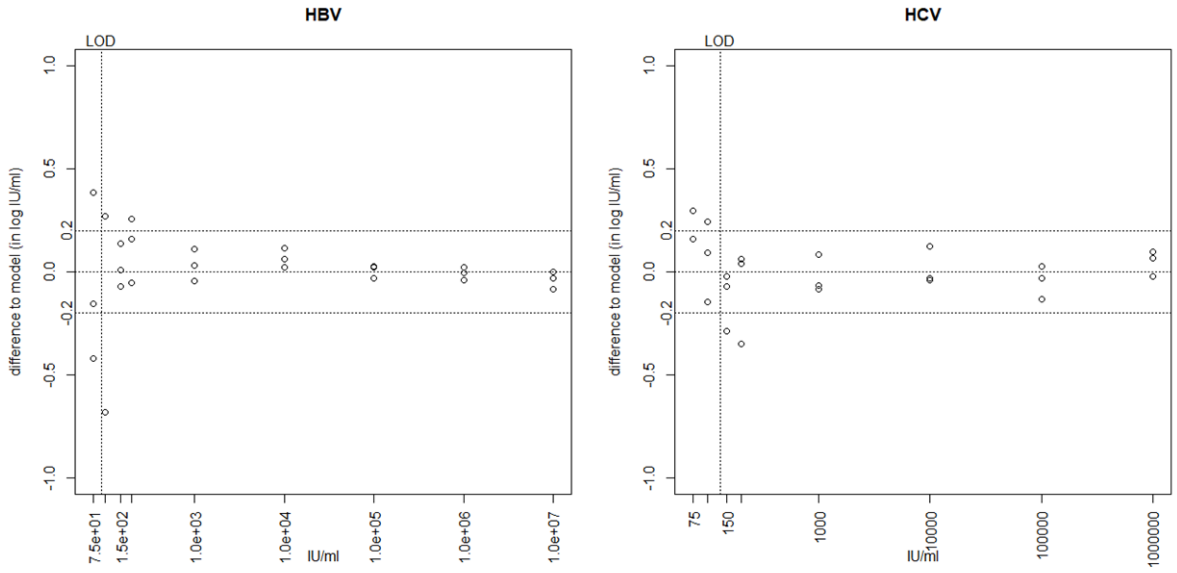


Figure 2: Deviation of RNA and DNA quantifications within the ±0.2 log10 IU/ml acceptance interval of Rapid INSTANT Virus RNA/DNA Kit - FX used in combination with RoboGene HBV DNA Quantification Kit 3.0 (left) and RoboGene HCV RNA Quantification kit 3.0 (right). The LOD of the HBV-DNA- and the HCV-RNA-detection is 91.89 IU/ml and 129.84IU/ml, respectively

4.2 Precision

The precision analysis of nucleic acid extraction with Rapid INSTANT Virus RNA/DNA Kit - FX was carried out in combination with RoboGene HCV RNA Quantification Kit 3.0.

Dilution series consisting of 3 samples tested positive for HCV RNA representing concentrations of 350, 3,500 and 35,000 IU/ml were purified by applying 3 different lots of Rapid INSTANT Virus RNA/DNA Kit – FX. One extraction per lot was carried out. The extracted virus RNA was quantified and the intra- and inter-assay precision were calculated (see Table 1).

Table 1: Intra- & Inter-Assay-Precision for Quantification of HCV-RNA

conc.	**Precision - CV [%]**				
[IU/ml]	**Intra-Assay**	**Inter-Assay**	**Lot 1**	**Lot 2**	**Lot 3**
35,000	11	17	2	13	15
3,500	12	20	11	12	11
350	34	27	22	40	37

4.3 Analytical Sensitivity

For examination of the analytical sensitivity dilution series of the PEI Reference Material HCV RNA (#3443/04, genotype 1) and PEI Reference Material HBV DNA (#3620/05, genotype D) were extracted with Rapid INSTANT Virus RNA/DNA Kit - FX and quantified with RoboGene HCV RNA Quantification Kit 3.0 and RoboGene HBV DNA Quantification Kit 3.0 respectively.

Analytical sensitivity was calculated by PROBIT analysis (95% confidence) of the quantification results of 12 replicates of a series of 14 (HCV) and 12 (HBV) dilution steps of the respective reference material (see Table 2).

Table 2: Determined limits of detection and confidence intervals [IU/ml]

	LOD	**CI - lower**	**CI - upper**
HBV-DNA	91.89	82.02	101.76
HCV-RNA	129.84	117.57	142.11

4.4 Diagnostic Evaluation

The diagnostic evaluation of the Rapid INSTANT Virus RNA/DNA Kit - FX was carried out by analysis of the diagnostic sensitivity. The examination was done externally with assistance of two diagnostic laboratories and under routine conditions.

For the analysis Rapid INSTANT Virus RNA/DNA Kit - FX was combined with the CE-IVD marked Kits for qualitative SARS-CoV-2 diagnostics, Fast Track SARS-CoV-2 Kit (Siemens Healthineers) and LightMix SarbecoV E-gene plus EAV control (TIB MOLBIOL) being applied in the two diagnostic laboratories.

The SARS-CoV-2-tests are validated and routinely applied in combination with INSTANT Virus RNA/DNA Kit – FX for 400 µl sample volume (Roboscreen GmbH, order number 847-0259200904). The validated diagnostic systems are referred as “reference systems”.

The aim of the diagnostic evaluation is the comparison of the performances of the combination of Rapid INSTANT Virus RNA/DNA Kit – FX and two SARS-CoV-2-tests with both of the reference systems.

56 throat and nasal swab samples pre-analyzed with the respective reference system were used as sample material for extraction using Rapid INSTANT Virus RNA/DNA Kit - FX and analysis using the respective qualitative SARS-CoV-2-test.

Results of the analyses are depicted in Figure 3. The obtained Ct-values were compared by means of Deming regression analysis.

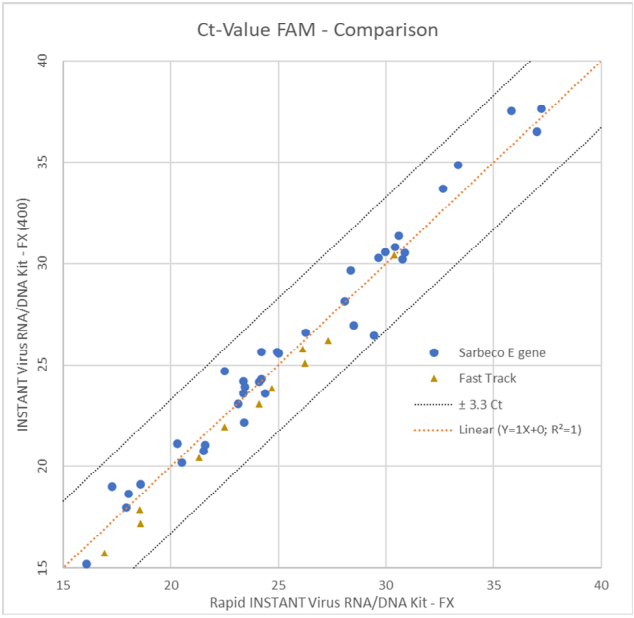


Figure 3: Diagnostic evaluation: Deming Regression scatter plot visualizing data obtained with Rapid INSTANT Virus RNA/DNA Kit - FX in combination with Fast Track SARS-CoV-2 Kit (Siemens Healthineers) and LightMix SarbecoV E-gene plus EAV control (TIB MOLBIOL) and data obtained with both of the mentioned diagnostic reference systems. Indicated is the 3.3 Ct (1 $\log^{10}$) acceptance interval.

Deming regression revealed a high degree of correlation between amplification results obtained with Rapid INSTANT Virus RNA/DNA Kit - FX and the results obtained with the diagnostic reference systems. The correlation coefficients are given in Table 3.

The analysis also shows that all of the 56 individual amplification results (Ct-values) are within the ± 3.3 Ct-acceptance interval. Moreover, all 56 prequalified positive samples are also correctly detected "true positive" by Fast Track SARS-CoV-2 Kit and LightMix SarbecoV E-gene plus EAV control in combination with Rapid INSTANT Virus RNA/DNA Kit – FX.

Table 3: Correlation between the amplification results obtained using Rapid INSTANT Virus RNA/DNA Kit - FX and the results obtained with the diagnostic reference systems

	Fast Track SARS-CoV-2 Kit	**LightMix SarbecoV E-gene plus EAV control**
Correlation (R^2)	0.994	0.969
Out of ± 3.3 Ct range	0/11	0/45

4.5 Robustness

The robustness analysis determines the total failure rate of the diagnostic system for HCV-RNA quantification consisting of Rapid INSTANT Virus RNA/DNA Kit - FX and RoboGene HCV RNA Quantification Kit 3.0.

A total of 112 samples, containing PEI Reference Material HCV RNA (#3443/04, genotype 1) with an HCV-RNA concentration of 350 IU/ml (representing the 3-fold LOD) was analyzed. The analysis was carried out with two separate determination recruiting samples 1-56 and 57-112 respectively. The results are shown in Figure 4 and Table 4.

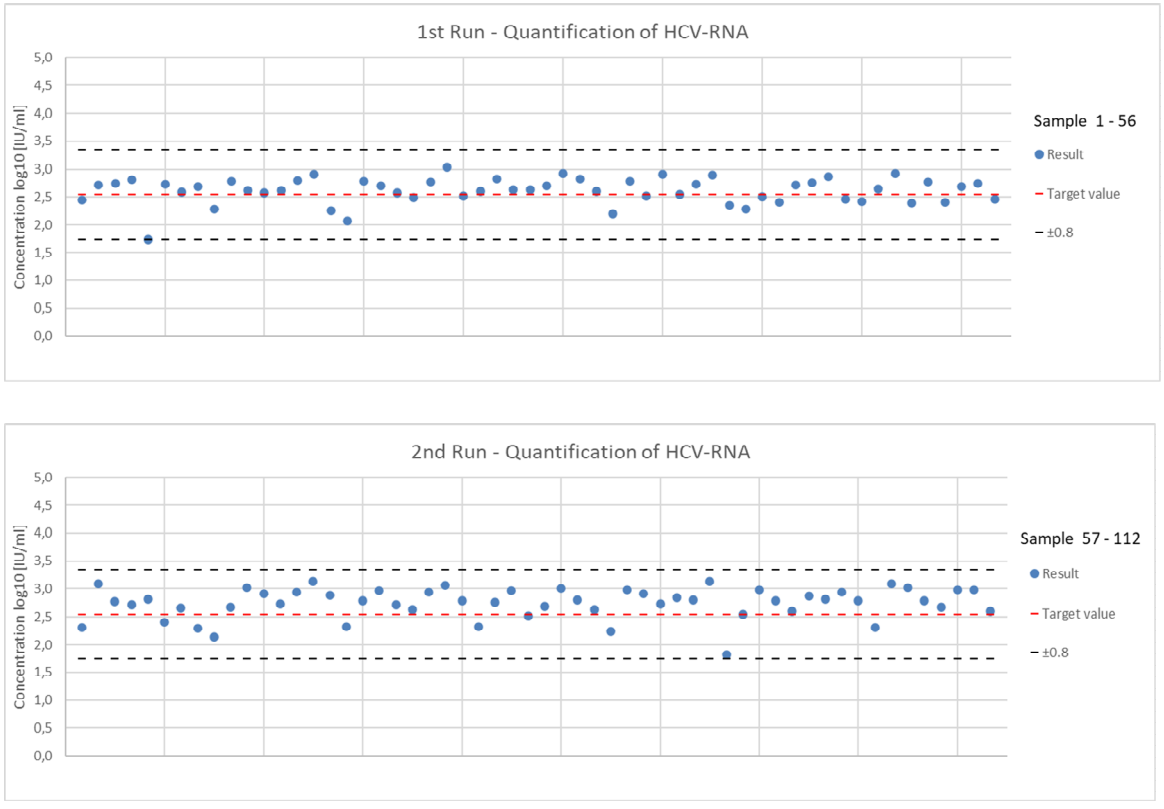


Figure 4: Results of the HCV-RNA quantification. The ±0.8 $\log^{10}$ range is indicated.

Table 4: Results of failure rate study

	Correct results / determinations	Failure rate
HCV-RNA (FAM)	112/112	0%
IC-RNA (YY/Cy5)	112/112	

The analysis shows that all of the 112 extractions of HCV-RNA carried out with Rapid INSTANT Virus RNA/DNA Kit - FX led to correct quantification results.

5 Kit components, storage and stability

5.1 Kit components

Reference number, kit content and number of tests are indicated in the following table.

REF			**847-0259200906**
Description			**Σ 2 x 96**
MAG		Magnetic Particle Suspension	2 x 9 ml
PCR grade H_2O		RNase free water	2 x 10 ml
PK		Proteinase K, lyophilized (For 1.5 ml working solution each)	7 x 30 mg
LS		Lysis Solution	1 x 110 ml
BS		Binding Solution	2 x 100 ml
WS A		Wash Solution A	2 x 70 ml
WS B		Wash Solution B, concentrated	2 x 30 ml
EB		Elution Buffer	2 x 70 ml
IFU		Instructions for use	1

5.2 Storage and Stability

Rapid INSTANT Virus RNA/DNA Kit - FX is delivered at ambient temperature.

Upon arrival, store lyophilized **PK** and **MAG** at 2° C to 10° C.

All other components should be stored at room temperature and remain in the kit box.

Under these conditions, the kit has a shelf life as indicated on the labelling of the kit box.

Aliquot dissolved **PK** and store at -40° C to -15° C. Repeated freezing and thawing will reduce activity dramatically!

Component	Storage conditions
MAG	2° C to 10° C
PCR grade H_2O	2° C to 30° C
PK, lyophilized	2° C to 10 ° C
PK, dissolved	-40° C to -15° C
LS	15° C to 30° C
BS	15° C to 30° C
WS A	15° C to 30° C
WS B	15° C to 30° C
EB	15° C to 30° C

Sealed plates prefilled with WS A, WS B, EB and BS can be stored at 15° C to 30° C in the dark for up to 4 days.

6 Necessary laboratory equipment

6.1 Required Instrumentation and accessories

Component	Manufacturer	Order number
CyBio FeliX Basic Unit	Analytik Jena	OL5015-24-100
Laptop with Application Studio software	Analytik Jena	820-90002-2
CyBio FeliX Extraction Set	Analytik Jena	OL5015-25-120
FX Filter Tips 1000 µl	Roboscreen GmbH	847-FX-TIPS-1000

6.2 Required consumables

Component	Manufacturer	Order number
Prefilling Set 400 – FX	Roboscreen GmbH	847-0259200924
Plate Set 400 Rapid – FX	Roboscreen GmbH	847-0259200936

6.3 Explanation of the CyBio FeliX Extraction Set

Please use accessories only together with supports listed in the table below! Usage of other supports or no support may cause damages of the CyBio FeliX.

Accessories	Support	Abbreviation used in the IFU
96-Channel Magazine (OL3810-13-023)	Support; 97 mm height (OL3317-11-105)	CM96/1000 + S97
Gripper (OL3317-11-800)	Support; 37 mm height (OL3317-11-120)	Gripper + S37
Cover Magazine Head R (OL30-3316-200-11)	Support; 37 mm height (OL3317-11-120)	Cover magazine Head R + S37
8-channel-adapter (OL3317-11-330)	Support; 37 mm height (OL3317-11-120)	8-channel adapter + S37
Tip Transfer Tool (OL3396-25-354)	-	-

ATTENTION

Use of tips and plates not listed in the IFU may cause a heavy damage of the CyBio FeliX and a loss of guarantee.

Also, usage of components/reagents not listed in the IFU may cause severe malfunction of the automated process and loss of samples!

6.4 Recommended Products

Component	Manufacturer	Order number
Carrier RNA (6 tubes - for 400 µl protocol)	Roboscreen GmbH	847-0206201002

6.5 General laboratory equipment required

- Ethanol >96%
- Use only absolute/pure ethanol, but never methylated or denatured alcohol!
- Calibrated pipettes and suitable filtered tips
- Calibrated Multi-Pipette
- 5 and 10 ml Combi-Tip
- Centrifuge
- Vortex mixer
- Sample rack
- Gloves, lab coat
- Tubes, 25 and 50 ml
- Measuring cylinder, 25, 50 and/or 100 ml

7 Test description and principle

7.1 Principle of the Rapid INSTANT Virus RNA/DNA Kit - FX

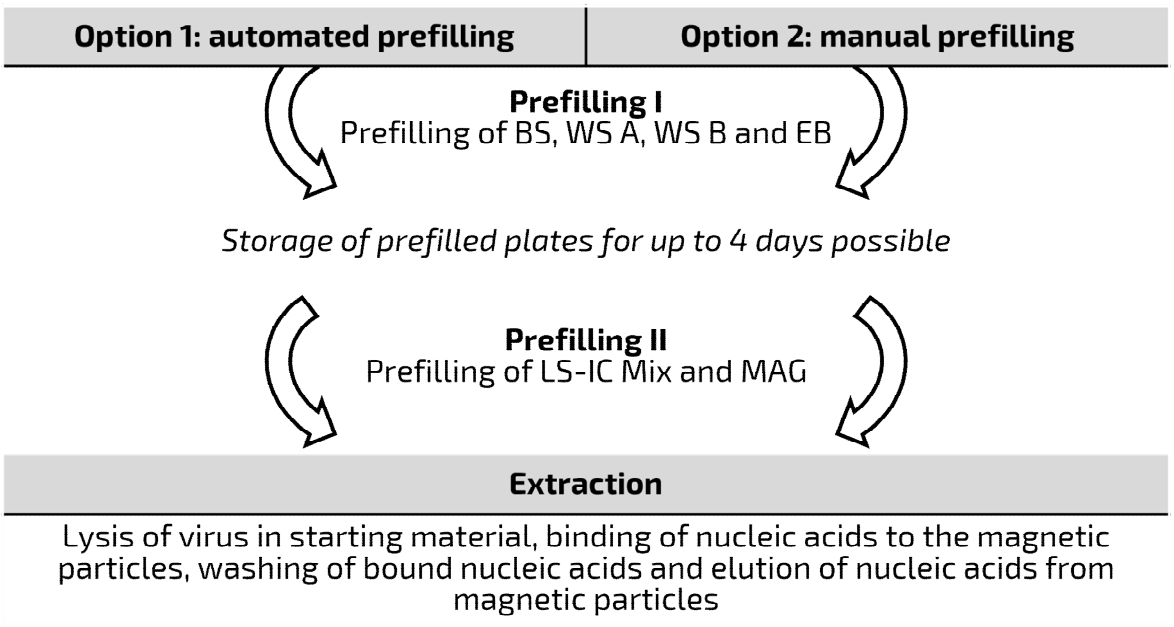
Rapid INSTANT Virus RNA/DNA Kit-FX is a nucleic acid extraction kit based on the magnetic particle technology for reversible binding of nucleic acids to a solid phase. In combination with the Liquid handling station CyBio Felix Basic Unit and the Cybio FeliX Extraction Set up to 96 samples with a volume of 400 µl can be automatically processed at once.

The automated extraction process is subdivided into two steps – prefilling and extraction. Two prefilling options are available:

Option 1: automated prefilling I followed by automated prefilling II (Is recommended for optimal downstream performance)
Option 2: alternatively, prefilling I and II can also be carried out manually

Plates prefilled with BS, WS A, WS B and EB can be stored in the dark for up to 4 days after sealing with sealing foil. This opens the opportunity to produce several sets of prefilled plates in advance and to subsequently run a series of extractions.

After accomplishment of the prefilling the extraction step must be started immediately. This is due to limited stability of MAG and, if LS was combined with an internal control (IC), the LS-IC Mix.



NOTE

For storage cover prefilled plates with sealing foils. Store prefilled plates at 15° C to 30° C in the dark. If more than one prefilling I is done in one day, the 2-well reservoir plates can be reused.

7.2 Duration of the procedures

Process time depends on selected sample number

Times required for process options using	
Prefilling I	5 – 27 min
Prefilling II	3 – 12 min
Extraction	35 min

7.3 Elution Volume

Before extraction the elution volume must be defined.

Adjustable range: **50 – 200 µl**

ATTENTION

The elution volume is essential for the concentration of RNA and DNA in the obtained Eluates! For optimal downstream performance, we recommend an elution volume of 100 µl.

8 Sample material

8.1 Collection and handling of blood samples

- For Plasma collect 5-10 ml blood with standard specimen collection tubes using EDTA or citrate as anticoagulant.
- Store whole blood at 2-25° C not longer than 6 hours, centrifuge for 20 min at 800-1600 x g to separate plasma or serum from blood cells and transfer to sterile tubes.
- Plasma or serum samples may be transported at room temperature; do not exceed 6 hours of time after blood collection.
- For long term storage of samples please use validated protocols. Commonly used protocols recommend storage of plasma and serum samples at -70° C or colder, use of screw-cap tubes and avoidance of repeated freezing and thawing.

8.2 Sample preparation for viral NA extraction from swabs

- For swabs that are stabilized in transport medium after collecting the sample, common transport medium such as AMIS or UTM are suitable.
- In case of using dry swabs please carry out the following preparation instructions before continuing with the protocol.

8.2.1 Preparation for dry swabs

1. Place the swab into a 2.0 ml reaction tube containing 500 to 1000 µl physiological saline (0.9% NaCl) and incubate for 15 minutes at room temperature.
2. Stir with swab to dissolve the sample in the physiological saline.
3. Wring out residual liquid of the swab at the inner wall of the tube and remove the swab.
4. Proceed with 400 µl of the particle-free sample.

8.3 Sample preparation for viral NA extraction from stool

In case of using stool as starting material please carry out the following preparation instructions with the protocol.

1. Collect 1 gram or 1 ml stool, respectively with standard specimen collection tubes.
2. Samples may be transported at room temperature, do not exceed the time 1 day after sample collection. Otherwise ensure transport on ice.
3. Samples may be stored deeply frozen for several months at -20° C to -70° C. Sample stability depends on the chosen storage temperature.

8.3.1 Preparation for stool sample

1. Transfer about 0.1 g of the stool sample into a 1.5 ml reaction tube and add 1000 µl PBS.
2. Resuspend by vortexing the sample for 5 seconds and centrifuge it at max. speed for 3 minutes.
3. Proceed with 400 µl of the particle-free sample.

NOTE

If the stool sample is very solid extend resuspension time and separate the sample into smaller pieces by pipetting up and down. It may be necessary to cut off the pipette tip to increase the opening. If obtained eluates are cloudy, we recommend clarifying the eluates by centrifuging for 3 minutes at maximum speed (20,000 x g).

9 Procedure

9.1 Preparation

9.1.1 Preparation of pipetting plan

It is recommended to create a pipette plan before starting the extraction procedure to keep identity and traceability of samples. To achieve this, note the coordinates of used wells of the 96 well plate for every sample.

Samples are to be added to the sample plate in the order left to right and top to bottom (see section 9.5).

9.1.2 Preparation of WS B

1. Add **45 ml** of **>96% absolute/pure ethanol** to the bottle of the wash solution **WS B**.
2. Close the bottle with the lid and invert the bottle for several times.

9.1.3 Preparation of PK

1. Centrifuge the **PK** tubes briefly at full speed to collect the lyophilized Proteinase K on the bottom of the tube.
2. Add **1.5 ml** of **PCR grade H_2O** to each vial of the **PK**.
3. Close the tubes, mix by vortexing briefly followed by brief centrifugation.

9.1.4 Preparation of Internal Control and Carrier RNA

Rapid INSTANT Virus RNA/DNA Kit - FX allows implementation of Carrier RNA and an internal positive control (IC).

Carrier RNA can prevent RNA degradation and can increase the yield of viral nucleic acid extraction. If carrier RNA is not included in the amplification system or diagnostic kit used in combination with Rapid INSTANT Virus RNA/DNA Kit - FX, we recommend application of carrier RNA (please refer to section 6.4 Recommended products).

Application of IC allows control of accurate execution of the whole diagnostic procedure including nucleic acid extraction and increases reliability of the obtained diagnostic information. For correct concentration of the internal control please refer to the manufacturer's instructions of your diagnostic application.

9.1.5 Preparation of Roboscreen Carrier RNA

If Roboscreen Carrier RNA (please see section 6.4) is used, prepare the Carrier RNA as follows.

1. Centrifuge **Carrier RNA** tubes briefly at full speed to collect the lyophilisate on the bottom.
2. Add **520 µl PCR grade H_2O** per tube, close tube and mix by vortexing briefly followed by brief centrifugation.

9.1.6 Preparation of LS

If **LS** is combined with an **IC**, it is not possible to store the resulting **LS-IC Mix**. For this reason, use the **LS-IC Mix** shortly after preparation for prefilling II and start the extraction procedure.

1. In case of using Roboscreen Carrier RNA please refer to section **10.1 LS-Mix** for the correct ratio of Carrier RNA solution and **lysis buffer (LS)**, mix the solutions depending on your sample numbers as indicated.

2. For correct ratio of **IC and/or Carrier RNA** and **lysis buffer (LS)**, mix the solutions depending on used sample number [n] and volume per reaction [V] of the IC as indicated in the instructions for use of your downstream application.
   Volume of internal control for LS-IC Mix = (n x V) x 1.15
3. Mix solutions gently and avoid foaming. Do not vortex.

9.1.7 Further preparations

Instrument set up

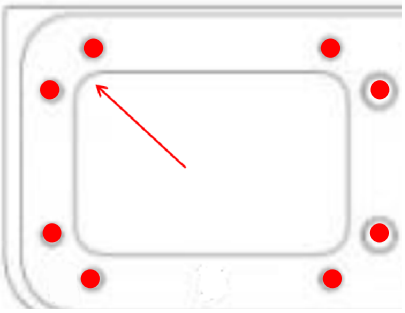
For instrument set up please follow manufacturer's instructions.

Make sure the dense matt of Head R 96/1000 µl is dust-free. For further information, see instruction for use of the pipetting head.

Remove Cover magazine before starting the protocols ("Maintenance" → "Unmount Adapter").

ATTENTION

For correct orientation, the mark ‚A1'of the plates must be orientated towards the upper left corner of the deck positions and the plate labelling must be directed to the front (see arrow).



For correct orientation of the supports and adapters make sure the OL-numbers engraved on the articles point to the front.

Make sure all supports, adapters and plates are correctly placed in the respective Deck position and safely positioned between holders (see red dots).

9.2 Manual Prefilling

Plates can be prefilled manually according to section **10.2 Manual Prefilling**.

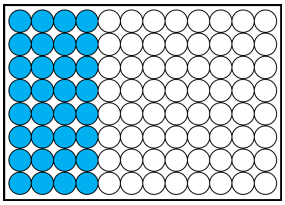
1. Pipette solutions on the bottom of the cavity and keep order of solutions as shown in the tables. Please avoid droplets on the upper vessel wall!
2. Before pipetting **MAG**, make sure to vortex **MAG** vigorously for at least 1 minute. Vortex again after a maximum of 4 pipetting steps and change tip.
3. After Prefilling **MAG** and **LS-IC Mix** (if LS is combined with IC) the extraction part must be carried out immediately as **MAG** and **LS-IC Mix** are not stable for long.

☞ **ATTENTION**

Make sure to vortex **MAG** before use vigorously for at least one minute. If **MAG** is not completely homogenized performance can be dramatically reduced

9.3 Prefilling I

1. Preparation of 2-well reservoir plates R1 and R2. Fill solution/ buffer in the reservoir wells corresponding to **10.3 Automated Prefilling I**.
   a. R1 – BS / EB
   b. R2 – WS A / WS B
2. Fill first four columns of **TR96/1000** (Tip Rack) with new tips (see right). Only use the pipetting tips indicated in section 6.1.



3. Deck modification for prefilling I

    a. Switch on CyBio FeliX and open Application Studio
    b. Click on following buttons in the order described below

        i. Magnetic beads (if available)
        ii. Rapid INSTANT Virus RNA/DNA Kit - FX
        iii. Prefilling I

    c. Open enclosure and set up deck layout according to the scheme presented in the Application Studio.
    d. Close enclosure carefully, check deck layout and confirm with ok.
    e. → The CyBio FeliX now executes prefilling I.
    f. After prefilling I has been carried out open the enclosure and discard reservoir plates and tips.
    g. Then confirm with ok.
    h. If you continue with prefilling II, see Procedure of prefilling II. If you continue with another prefilling I seal prefilled plates with sealing foil and store them as recommended.

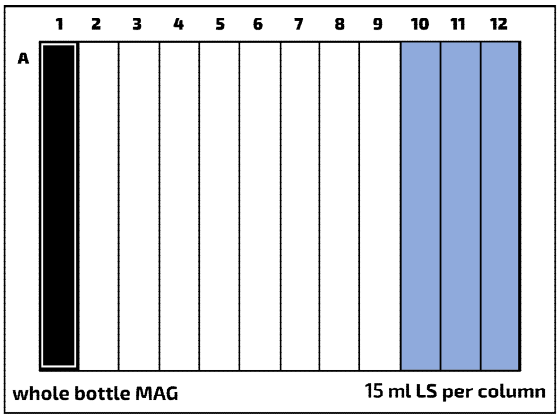
9.4 Prefilling II

1. Vortex **MAG** vigorously for at least 1 minute and immediately fill the homogenized **MAG** suspension into a cavity of 12-well reservoir plate **R3 - MAG / LS - IC** corresponding to section **10.4 Automated Prefilling II** (also see filling scheme below).

ATTENTION

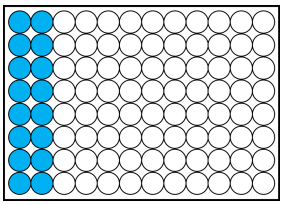
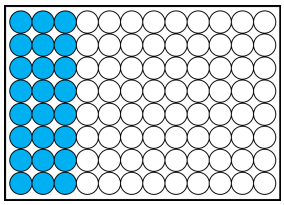
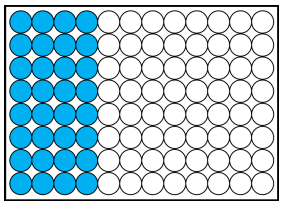
Make sure to vortex **MAG** vigorously before use for at least one minute. If **MAG** is not completely homogenized performance can be dramatically reduced

2. Fill **LS (or LS - IC Mix** when combined with IC) into cavities of 12-well reservoir plate **R3 - MAG / LS - IC** corresponding to section **10.4 Automated Prefilling II** (also see filling scheme below).



Filling scheme of the R3 - MAG / LS – IC Mix.
Depicted is the filling scheme for 96 samples

3. Fill **TR96/1000** (Tip Rack) with new tips. TR96/1000 is filled column-wise depending on the number of samples. The required number of columns is depicted in the following scheme. Only use the pipetting tips indicated in section 6.1.

up to 32 samples	40 to 64 samples	72 to 96 samples
		

4. Pipette **50 µl** of the dissolved **PK** on the bottom of each well of plate **P1 - Samples / LS**, e.g., by use of a Multipette and a 5 ml Combitip
5. Deck modification for prefilling II
    a. Switch on CyBio FeliX and open Application Studio
    b. Click on following buttons in the order described below
        i. Magnetic beads (if available)

ii. Rapid INSTANT Virus RNA/DNA Kit - FX
iii. Prefilling II
iv. Select the number of samples

c. Open enclosure and set up deck layout according to the scheme presented in the Application Studio.
d. Close enclosure carefully, check deck layout and confirm with ok.
e. → The CyBio FeliX now executes prefilling II.
f. After prefilling II has been carried out open the enclosure and discard reservoir plate and tips.
g. When prefilling is accomplished confirm with ok.

6. Continue with extraction part immediately.

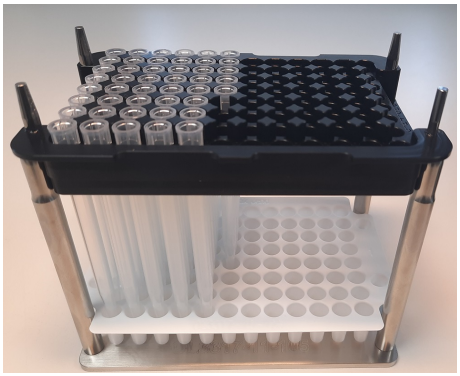
9.5 Extraction

1. Preparation of the sample plate
    a. Mix samples by brief vortexing, followed by brief centrifugation.
    b. Pipette **400 µl** of each **sample** according to your pipetting plan into the wells of plate **P1 - Samples / LS**.
2. Equip **two** sets of **CM96/1000** with **tips** and place a **Protective Plate** as shown in the scheme below. Only use the pipetting tips indicated in section 6.1.
   Place pipetting tips and samples in the order left to right and top to bottom according to your pipetting plan (see example below and section 9.1.1).
    a. Place **Protective Plate** on the bottom of the **S97**.
    b. Put **correct number of tips** according to the number of samples to the specified positions in the **CM96/1000**. If necessary, use **Tip Transfer tool** for a larger number of tips.
    c. Place equipped **CM96/1000** onto the protected **S97**.

ATTENTION
To avoid any technical problems, make sure the **CM96/1000** adapter is dry before use.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 01	Sample 09	Sample 17	Sample 25	Sample 33	Sample 41						
B	Sample 02	Sample 10	Sample 18	Sample 26	Sample 34	Sample 42						
C	Sample 03	Sample 11	Sample 19	Sample 27	Sample 35	Sample 43						
D	Sample 04	Sample 12	Sample 20	Sample 28	Sample 36	Sample 44						
E	Sample 05	Sample 13	Sample 21	Sample 29	Sample 37	Sample 45						
F	Sample 06	Sample 14	Sample 22	Sample 30	Sample 38							
G	Sample 07	Sample 15	Sample 23	Sample 31	Sample 39							
H	Sample 08	Sample 16	Sample 24	Sample 32	Sample 40							

Filling scheme of the sample plate **P1 - Samples / LS.** Depicted is the filling scheme for 45 samples



Filling scheme of **CM96/1000** placed onto **S97** and equipped with a **Protective Plate**.

Depicted is the filling scheme for 45 samples

3. Deck modification for extraction

   a. Switch on CyBio FeliX and open the Application Studio
   b. Click on following buttons in the order described below

      i. Magnetic beads (if available)
      ii. Rapid INSTANT Virus RNA/DNA Kit - FX
      iii. Extraction

   c. Choose the required elution volume. The elution volume can be set in the range of 50 µl to 200 µl (see section 7.3).
   d. Open enclosure and set up deck layout according to the scheme presented in the Application Studio.
   e. Close enclosure carefully, check deck layout and confirm with ok.

   → The CyBio FeliX now executes the Extraction.

4. After extraction has been carried out open the enclosure.
5. Take out the micro plate **P7 - Eluates**.
6. Cover plate **P7 - Eluates** with sealing foil to avoid contamination, store on ice and continue with your downstream process immediately.
7. Discard all plates, tips and protective plates.
8. Finish the extraction program by confirming with ok and return to the start screen of the Application Studio.

9.6 Postprocessing

1. After completing your daily routine place the Cover magazine equipped with S37 at deck position 12. Let the Head mount the cover magazine by activating “Maintenance” → “mount adapter”.
2. The following components must be thoroughly disinfected by an immersion bath with instrument disinfectant. Rinse components afterwards thoroughly with water.
   - CM96/1000 + S97
   - TR96/1000
3. Clean CyBio FeliX decks with instrument disinfectant wipes.
4. Contaminations on adapters should be cleaned with dust-free wipes and ethanol. Be careful not to damage electronic parts!
5. Adapters should be stored dust-free, e.g. in bags.

10 Volumes for preparation and prefilling

10.1 LS-Mix

Solution	LS	Carrier RNA
Sample number	**Volume [ml]**	**Volume [µl]**
8	6	150
16	9	225
24	12	300
32	15	375
40	21	525
48	24	600
56	27	675
64	30	750
72	36	900
80	39	975
88	42	1050
96	45	1125

NOTE

For preparation of LS-Mix with other than the Roboscreen Carrier RNA refer to manufacturer's instruction.

10.2 Manual Prefilling

Plate	Solution / Buffer	Volume per well [µl]
P1 – Samples / LS	PK	50
	LS / LS-Mix	400
P2 – Process	BS	450
	MAG	50
P3 – BS	BS	450
P4 – WS A	WS A	600
P5 – WS B	WS B	600
P6 – EB	EB	600

10.3 Automated Prefilling I

Reservoir	**R1 - BS/EB**		**R2 – WS A/WS B**	
Cavity	**left**	**right**	**left**	**right**
Solution / Buffer	**BS**	**EB**	**WS A**	**WS B**
Sample number	**Volume [ml]**			
8	13	11	11	11
16	20	16	16	16
24	28	20	20	20
32	35	25	25	25
40	42	30	30	30
48	49	35	35	35
56	56	40	40	40
64	64	44	44	44
72	71	49	49	49
80	78	54	54	54
88	85	59	59	59
96	whole bottle	whole bottle	whole bottle	whole bottle

10.4 Automated Prefilling II

Reservoir	**R3 – MAG / LS / LS-Mix**			
Cavity	**1**	**10**	**11**	**12**
Solution / Buffer	**MAG**	**LS / LS-Mix**	**LS / LS-Mix**	**LS / LS-Mix**
Sample number	**Volume [ml]**			
8	2.4	-	-	6
16	2.8	-	-	9
24	3.2	-	-	12
32	3.6	-	-	15
40	4.0	-	6	15
48	4.4	-	9	15
56	4.8	-	12	15
64	5.2	-	15	15
72	5.6	6	15	15
80	6.0	9	15	15
88	6.4	12	15	15
96	whole bottle	15	15	15

11 Troubleshooting

Low amount of extracted RNA/DNA

- Kit or single kit components were stored under non-optimal conditions. Store kit components according to section Storage and Stability.
- Reagents were not prepared correctly according to the instruction for use → please refer to section Preparation.
- Ensure to use the required volume of 50 µl Proteinase K.
- Usage of sample material not listed in section Intended use or section Restrictions. Please use the kit only for samples matching the requirements of the kit!
- Use Internal Controls for verification of the extraction procedure.
- Be sure to create a RNase-free working environment. See section 2.2.
- Be sure to process your starting material correctly → please refer to section 2.3.
- Use RNA for downstream diagnostic applications immediately after elution.

Problems with CyBio FeliX → please refer to „Operating Manual CyBio FeliX"	
▪ Head does not pick up a plate/ accessory	Ensure plates/ accessories are placed correctly in the deck deepening
▪ Head collides with plate/ accessory	Ensure plates/ accessories are placed correctly in the deck deepening
▪ Pipette tips drip	Make sure to clean the sealing mat regularly so that an air-tight contact to the pipette tip is ensured
▪ Bubbles in plate cavities	Be sure that plates are filled correctly → Always use the indicated volume of buffers/ reagents → Be sure to avoid evaporation and/or contamination from plates after prefilling/ elution by immediately sealing the plates with foil

If you have any further questions which are not answered, please contact our technical service.

12 Document Revision

Document Revision Documentation		
Rev.1	November 2023	Section 1.1, 2.2, 2.3 editorial changes. Document Revision added