ROBOSCREEN DIAGNOSTICS

Instructions for Use

INSTANT Virus RNA/DNA Kit - FX

Rev. 4 _ 02 / 2024

CE

IVD

Order No.:

847-0259200902	1x 96 reactions for using 800 µl protocol
847-0259200904	2x 96 reactions for using 400 µl protocol
847-0259200903	4x 96 reactions for using 200 µl protocol

CE

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1 Introduction

1.1 Intended use

The INSTANT Virus RNA/DNA Kit - FX is intended for automated purification of highly pure viral RNA and DNA from cell free human body fluids.

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.

With starting volumes of 200, 400 or 800 µl of human plasma and serum, cerebrospinal fluid and swab- and stool-supernatants a throughput of up to 96 samples is possible in parallel.

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 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

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

- Fresh or frozen cell-free human body fluids (serum, plasma, cerebrospinal fluid and stool).
- Swab collected specimen taken from epithelial surfaces
- Stabilizers: EDTA or citrate
- 200, 400 and 800 µl of 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

Extraction of viral nucleic acids is a very common application in the field of patient sample analysis. Reliable yields of high-quality purified DNA and/or RNA are substantial for further downstream applications to ensure a trustworthy patient diagnosis. To offer a CE-IvD-marked solution for automated extraction of both viral DNA and RNA for high sample throughput laboratories, we combined the highly efficient extraction technology of the INSTANT Virus RNA/DNA Kit – FX with the CyBio FeliX liquid handling station. By applying this extraction method, it is possible to perform automated nucleic acid extraction with up to 96 samples in one run with manageable manual effort. The purified nucleic acids can then be quantitatively detected using various real-time PCR kits.

Performance of the automated nucleic acid extraction using 800 µl protocol via the INSTANT Virus RNA/DNA Kit – FX with the CyBio FeliX in comparison to the manual nucleic acid extraction with the INSTANT Virus RNA/DNA Kit was assessed during validation of CE-IVD marked detection kits RoboGene HCV RNA Quantification Kit 3.0 (article number 847-0207610-032/096/132) and the RoboGene HBV DNA Quantification Kit 3.0 (article number 847-0207710-032/096/132) according to the common technical specifications (CTS) for *in vitro* diagnostic medical devices (2002/364/EC). With either method comparable results were achieved regarding performance specifications, e.g., analytical sensitivity.

The analytical sensitivity of the RoboGene HCV RNA Quantification Kit 3.0 was determined by analyzing dilution series of the PEI Reference Material HCV RNA (#3443/04, genotype 1). Analytical sensitivities for used qPCR devices were determined as summarized in Table 1.

Table 1: Determined device specific limits of detection and confidence intervals of the RoboGene HCV RNA Quantification Kit 3.0

PCR device	**Limit of detection in IU/ml (95% confidence interval, IU/ml)**	
	INSTANT Virus RNA/DNA Kit	**INSTANT Virus RNA/ DNA Kit - FX**
qTOWER^3 (qT)	17.0 (14.8 – 19.2)	11.7 (9.8 – 13.7)
CFX96 (CFX)	13.6 (11.6 – 15.5)	14.7 (12.4 – 17.0)
LightCycler® 480 (LC)	14.1 (11.5 – 16.8)	13.2 (11.2 – 15.1)
7500 Fast (FS)	20.2 (17.1- 23.2)	-
Rotor-Gene® 6000 (RG)	17.4 (14.7 – 20.1)	19.6 (17.2 – 22.0)
Ø	16.6 (15.5 – 17.7)	15.0 (13.9 – 16.1)

The analytical sensitivity of the RoboGene HBV DNA Quantification Kit 3.0 was determined by analyzing dilution series of the PEI Reference Material HBV DNA (#3620/05, genotype D). Analytical sensitivities for used qPCR devices were determined as summarized in Table 2.

Table 2: Determined device specific limits of detection and confidence intervals of the RoboGene HBV DNA Quantification Kit 3.0

PCR device	**Limit of detection in IU/ml (95% confidence interval, IU/ml)**	
	INSTANT Virus RNA/DNA Kit	**INSTANT Virus RNA/ DNA Kit - FX**
qTOWER^3 (qT)	10.0 (6.9 – 13.1)	6.1 (4.9 – 7.2)
CFX96 (CFX)	10.4 (8.4 – 12.4)	6.8 (5.6 – 8.0)
LightCycler® 480 (LC)	8.0 (6.4 – 9.6)	6.7 (5.7 – 7.8)
7500 Fast (FS)	7.3 (5.8- 8.7)	8.3 (7.2 – 9.3)
Rotor-Gene® 3000 (RG)	10.4 (8.4 – 12.4)	6.6 (5.4 – 7.9)
Ø	9.3 (8.4 – 10.2)	7.0 (6.5 – 7.5)

5 Kit components, storage and stability

5.1 Kit components

INSTANT Virus RNA/DNA Kit – FX is available for 200 µl, 400 µl and 800 µl sample volume. Kit content and necessary consumables (see sections 6.2-6.4) are specified for the respective sample volume.

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

REF			**847-0259200902**	**847-0259200904**	**847-0259200903**
Protocol for sample volume of			**800 µl**	**400 µl**	**200 µl**
Description			**Σ 1x 96**	**Σ 2x 96**	**Σ 4x 96**
MAG		Magnetic Particle Suspension	1 x 9 ml	2 x 9 ml	4 x 9 ml
PCR grade H_2O		RNase free water	1 x 10 ml	2 x 10 ml	4 x 10 ml
PK		Proteinase K, lyophilized (For 1.5 ml working solution each)	4 x 30 mg	7 x 30 mg	14 x 30 mg
LS		Lysis Solution	1 x 110 ml	1 x 110 ml	1 x 110 ml
BS		Binding Solution	2 x 100 ml	2 x 100 ml	2 x 100 ml
WS A		Wash Solution A	1 x 110 ml	2 x 110 ml	4 x 110 ml
WS B		Wash Solution B concentrated	1 x 48 ml	2 x 48 ml	4 x 48 ml
EB		Elution Buffer	1 x 70 ml	2 x 70 ml	4 x 70 ml
IFU		Instructions for use	1	1	1

5.2 Storage and Stability

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 for the 800 µl protocol

Component	Manufacturer	Order number
Prefilling Set 800 - FX	Roboscreen GmbH	847-0259200922
Plate Set 800 - FX	Roboscreen GmbH	847-0259200932

6.3 Required consumables for the 400 µl protocol

Component	Manufacturer	Order number
Prefilling Set 400 - FX	Roboscreen GmbH	847-0259200924
Plate Set 400 - FX	Roboscreen GmbH	847-0259200933

6.4 Required consumables for the 200 µl protocol

Component	Manufacturer	Order number
Prefilling Set 200 - FX	Roboscreen GmbH	847-0259200923
Plate Set 200 - FX	Roboscreen GmbH	847-0259200934

6.5 Explanation of 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 in this 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!

6.6 Recommended Products

Component	Manufacturer	Order number
Carrier RNA (1 tube)	Roboscreen GmbH	847-0206201001
Carrier RNA (3 tubes - for 800 µl protocol)	Roboscreen GmbH	847-0206201004
Carrier RNA (12 tubes - for 200 µl protocol)	Roboscreen GmbH	847-0206201003
Carrier RNA (6 tubes - for 400 µl protocol)	Roboscreen GmbH	847-0206201002

6.7 General laboratory equipment required

- >96% ethanol
- 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

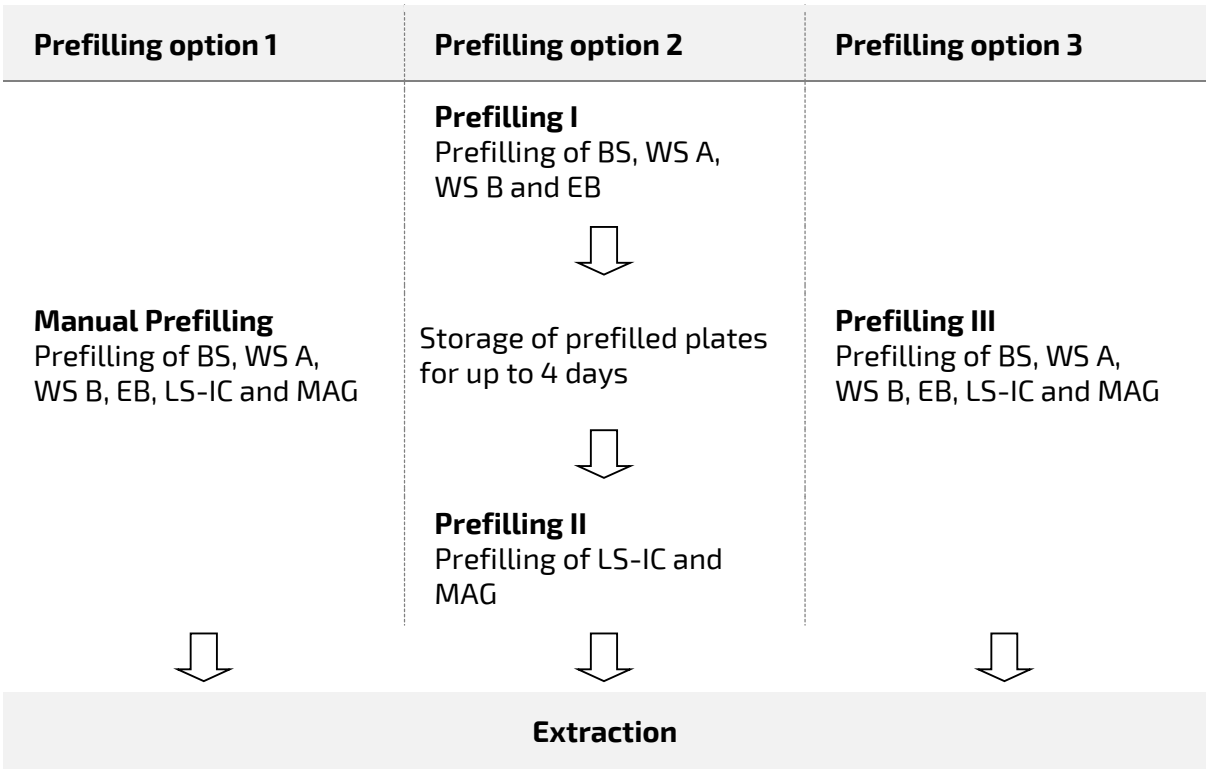
7 Test description and principle

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

The INSTANT Virus RNA/DNA Kit-FX is a nucleic acid extraction kit based on 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 800 µl, 400 µl or 200 µl can be automatically processed at once.

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

Option 1: manual prefilling
Option 2: automated prefilling I + prefilling II
Option 3: automated prefilling III



Lysis of starting material, binding of nucleic acids to the magnetic particles, washing of bound nucleic acids and elution of nucleic acids from the magnetic particles

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

After accomplishment of the prefilling the extraction part must be started immediately. This is due to limited stability of MAG and 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			
	800 µl protocol	**400 µl protocol**	**200 µl protocol**
Prefilling I	8 - 50 min	7 – 44 min	7 - 38 min
Prefilling II	4 - 14 min	4 – 13 min	3 – 11 min
Prefilling III	11 – 62 min	11 – 56 min	10 – 48 min
Extraction	96 min	74 min	65 min

7.3 Elution Volumes

	Elution volume	RoboGene HBV DNA Quantification Kit 3.0	RoboGene HCV RNA Quantification Kit 3.0
200 µl protocol	50 – 200 µl	-	-
400 µl protocol	50 – 200 µl	-	-
800 µl protocol	50 – 200 µl	60 µl	60 µl

ATTENTION

The indicated elution volume is essential for the performance of RoboGene Kits!

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 time 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) depending on the chosen starting volume 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 chosen starting volume of the particle-free sample.

8.3 Sample preparation for viral NA extraction from stool

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, stability depending on the 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 chosen starting volume 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 9.6).

9.1.2 Preparation of WS B

1. Add **72 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.
4. Pool the Proteinase K Solution of all tubes needed in a new vial (optional).

9.1.4 Preparation of Internal Control and Carrier RNA

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 INSTANT Virus RNA/DNA Kit - FX, we recommend application of carrier RNA (please refer to section 6.6 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 the internal control of RoboGene virus kits and Carrier RNA

RoboGene virus kits contain an integrated internal control (IC) as a kit component which already includes Carrier RNA. Any Carrier RNA-free IC can be combined with Roboscreen Carrier RNA. Please follow the subsequent protocol.

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

For IC only:

3. Incubate at 37° C for 5 min and 800-1,000 rpm using a shaking platform, mix by vortexing briefly followed by brief centrifugation.

4. In case of more than one **IC** or **Carrier RNA** tube, pool dissolved internal control vials in one tube, mix by vortexing briefly followed by brief centrifugation.

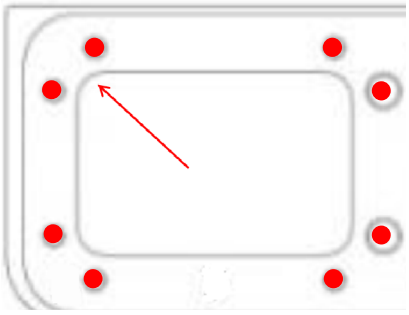
9.1.6 Further preparations

Instrument set up

For instrument set up please follow manufacturer's instructions.

Make sure the dense matt of the 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 / reservoir plates must be on the upper left corner on the deck positions and the label must be directed to the front.	
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 the tables referred to below.

Protocol	Corresponding table
800 µl	Table 1 Manual Prefilling - 800
400 µl	Table 2 Manual Prefilling - 400
200 µl	Table 3 Manual Prefilling - 200

1. Preparation of **LS-IC Mix** → For the correct ratio of **IC/Carrier RNA solution** and **lysis buffer**, mix the solutions depending on your sample numbers and chosen protocol as indicated in **Table 4 LS-IC Mix 800 - 400 - 200**. Mix solutions gently and avoid foaming. Do not vortex.
2. 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!
3. 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.
4. After Prefilling MAG and LS-IC Mix the extraction part must be carried out immediately as MAG and LS-IC Mix are not stable for long.

☞ **ATTENTION**

Make sure to vigorously vortex **MAG** before use 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. →Fill the chambers of the reservoirs referred to the tables below.

Protocol	Reservoir	Corresponding table
800 µl	**R1 – BS/BS** **R2 – WS A / WS B** **R3 - EB**	Table 6 - BS/WS A/WS B/ EB - 800
400 µl	**R1 – BS/ EB** **R2 – WS A / WS B**	Table 8 - BS/WS A/WS B/ EB - 400
200 µl	**R1 – BS/ EB** **R2 – WS A / WS B**	Table 10 - BS/WS A/WS B/ EB - 200

2. Fill first columns of **TR96/1000** with new tips. The required number of columns can be found in **Table 5 Prefilling - Number of Tips for TR96/1000**.
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. INSTANT Virus RNA/DNA Kit – FX200 / 400 / 800
      iii. Prefilling I
      iv. Select the number of samples required
   c. Open enclosure and set up the deck layout according to the scheme presented in the Application Studio.
   d. Close enclosure carefully, check deck layout and confirm with ok. → The CyBioFeliX now executes the prefilling I.
   e. After prefilling I has been carried out open the enclosure and discard the reservoir plates and tips.
   f. Then confirm with ok.
   g. If you continue with Prefilling II, see Procedure **Prefilling II**. If you continue with another Prefilling I seal prefilled plates with sealing foil, store them as recommended.

9.4 Prefilling II

1. Preparation of **LS-IC Mix** → For the correct ratio of **IC/Carrier RNA solution** and **lysis solution (LS)**, mix the solutions depending on your sample number and chosen protocol as indicated in **Table 4 LS-IC Mix - 800 - 400 - 200**. Mix solutions gently and avoid foaming. Do not vortex.
2. Vortex **MAG** for at least 1 minute vigorously directly before filling the solution in the 12-column reservoir (see the following warning).

ATTENTION

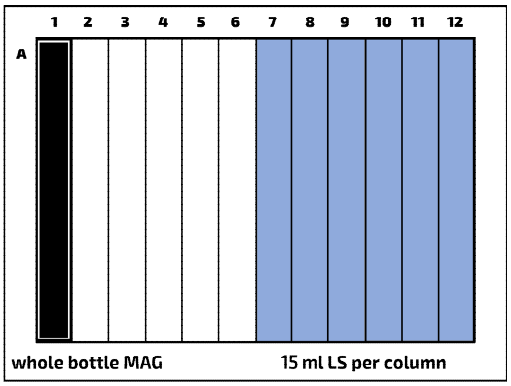
Make sure to vortex **MAG** vigorously before use for at least one minute. If **MAG** is not completely homogenized performance can be dramatically reduced

3. Preparation of the 12-column reservoir plate → Fill the columns of the reservoirs with vortexed **MA**G and **LS-IC-Mix** as given in the respective table.

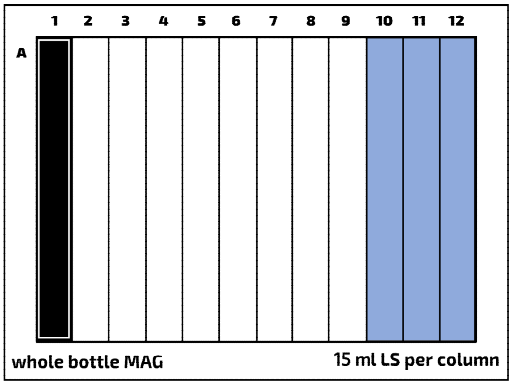
Protocol	Reservoir	Corresponding table
800 µl	**R4 - MAG/LS-IC**	Table 7 - MAG/LS-IC - 800
400 µl	**R3 - MAG/LS-IC**	Table 9 - MAG/LS-IC - 400
200 µl	**R3 - MAG/LS-IC**	Table 11 - MAG/LS-IC - 200

4. Fill first columns of **TR96/1000** with new tips. Depending on your sample number the required number of columns is given in **Table 5 Prefilling - Number of Tips for TR96/1000**.

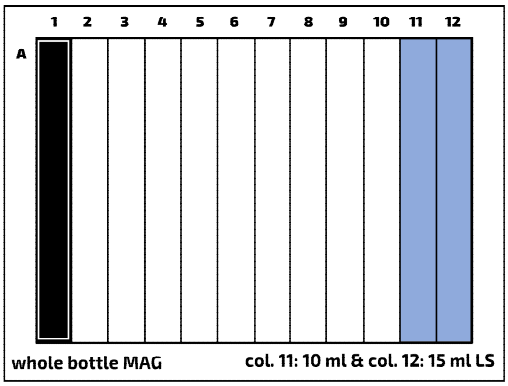
12 – well Reservoir filling schemes



Filling scheme for 800 µl sample volume and 96 samples



Filling scheme for 400 µl sample volume and 96 samples



Filling scheme for 200 µl sample volume and 96 samples

For 400 µl and 200 µl protocol only

5. Add 50 µl of the dissolved PK on the bottom of each well of the plate P1-Samples/LS, e.g., by use of a Multipette and a 5 ml Combitip.

6. 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. INSTANT Virus RNA/DNA Kit - FX200 / 400 / 800
        iii. Prefilling II
        iv. Select the number of samples required
    c. Open enclosure and set up the deck layout according to the scheme presented in the Application Studio.
    d. Close enclosure carefully, check deck layout and confirm with ok. → The CyBioFeliX now executes the prefilling II.
    e. After prefilling II has been carried out open the enclosure and discard the reservoir plate and tips.
    f. When prefilling is done confirm with ok.
7. Continue with extraction part immediately.

9.5 Prefilling III

1. Preparation of 2-well Reservoir Plates.→ Fill the chambers of the reservoirs as given in the respective table.

Protocol	Reservoir	Corresponding table
800 µl	**R1 – BS/BS** **R2 – WS A / WS B** **R3 - EB**	Table 6 - BS/WS A/WS B/ EB - 800
400 µl	**R1 – BS/ EB** **R2 – WS A / WS B**	Table 8 - BS/WS A/WS B/ EB - 400
200 µl	**R1 – BS/ EB** **R2 – WS A / WS B**	Table 10 - BS/WS A/WS B/ EB - 200

2. Preparation of **LS-IC Mix** → For the correct ratio of **IC/Carrier RNA solution** and **lysis solution (LS)**, mix the solutions depending on your sample numbers and chosen protocol as indicated in **Table 4 LS-IC Mix – 800 – 400 - 200**.
3. Mix solutions gently and avoid foaming. Do not vortex.
4. Vortex **MAG** for at least 1 minute vigorously directly before filling the solution in the 12-column reservoir (see the following warning).

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.

5. Preparation of the 12-column reservoir plate → Fill the columns of the reservoirs with vortexed **MAG** and **LS-IC-Mix** as given in the respective table.

Protocol	Plate	Corresponding table
800 µl	**R4 – MAG/LS-IC**	Table 7 - MAG/LS-IC-800
400 µl	**R3 – MAG/LS-IC**	Table 9 - MAG/LS-IC-400
200 µl	**R3 – MAG/LS-IC**	Table 11 - MAG/LS-IC-200

6. Fill first columns of **TR96/1000** with new tips. The required number of columns can be found in **Table 5 - Prefilling - Number of Tips for TR96/1000**.

For 400 µl and 200 µl protocol only

7. Add **50 µl** of the dissolved **PK** on the bottom of each well of the plate P1-Samples/LS, e.g. by use of a Multipette and a 5 ml Combitip.

8. Deck modification for prefilling III
    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. INSTANT Virus RNA/DNA Kit – FX200 / 400 / 800
        iii. Prefilling I
        iv. Select the number of samples required
    c. Open enclosure and set up the deck layout according to the scheme presented in the Application Studio.
    d. Close enclosure carefully, check deck layout and confirm with ok. → The CyBioFeliX now executes the prefilling III.
    e. After Prefilling III has been carried out open the enclosure and discard the reservoirs plates and tips.
    f. Then confirm with ok.
    g. Continue with extraction part immediately.

9.6 Extraction

For 800 µl protocol only

1. Add 50 µl of the dissolved PK on the bottom of each well of the plate P2 - Samples, e.g., by use of a Multipette and a 5 ml Combitip.

2. Preparation of sample plate
   a. Mix samples by brief vortexing, followed by brief centrifugation.
   b. Pipette your samples according to your pipetting plan in the respective well of the plate given in the following table:

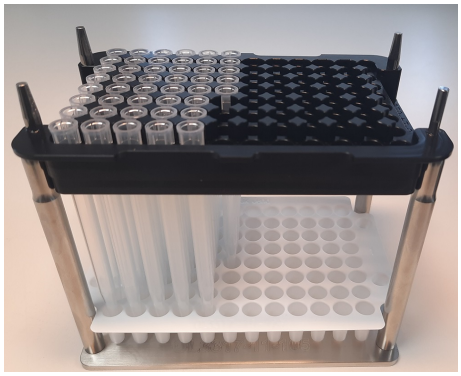
Protocol	Sample Volume	Plate
800 µl	800 µl	P2 - Samples
400 µl	400 µl	P1 - Samples / LS
200 µl	200 µl	P1 - Samples / LS

3. Prepare **two** sets **CM96/1000** with **tips** and a Protective Plate each as shown in the image below. Only use the pipetting tips listed in section 6.1. According to your pipetting plan tips and samples are to be inserted into corresponding positions (see example below).
   a. Place **Protective Plate** on the bottom of the **S97**.
   b. Transfer **the number of tips** according to your number of samples to the specified positions in the **CM96/1000**. If necessary, the **Tip Transfer tool** can be used for a larger number of tips.
   c. Place **CM96/1000** with tips onto **S97** with **Protective Plate**.

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							



Example
According to a pipetting plan for 45 samples a CM96/1000 placed onto S97 and equipped with a Protective Plate is loaded with 45 tips.
Note that samples and tips have to be placed into corresponding positions.

4. Deck modification for extraction
   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. INSTANT Virus RNA/DNA Kit - FX200 / 400 / 800
      iii. Extraction
   c. Choose your required elution volume. It can be set from 50 to 200 µl (see section 7.3).
   d. Open enclosure and set up the 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.

5. After extraction has been carried out open the enclosure.
6. Take out the plate referred to in the table below.

Protocol	Plate
800 µl	P9 - Eluates
400 µl	P8 - Eluates
200 µl	P8 - Eluates

7. Cover with sealing foil to avoid contamination, store on ice and continue with further downstream process immediately.
8. Discard all plates, tips, and protective plates.
9. Finish the extraction program by confirming with ok and return to the start screen of the Application Studio.

9.7 Postprocessing

1. After completing your daily routine place the Cover magazine with S37 on deck position 12. Let the Head lift the cover magazine over the function “Maintenance” → “mount adapter”.
2. The following components must be thoroughly disinfected by an immersion bath in 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 Corresponding tables for prefilling

10.1 Table 1 Manual Prefilling - 800

Plate	Solution / Buffer	Volume per well [µl]
P1 - LS	LS-IC	750
P2 - Samples	PK	50
P3 - Process	BS	530
	MAG	50
P4 - BS	BS	1100
P5 - WS A	WS A	1100
P6 - WS B	WS B	1100
P8 - EB	EB	600

10.2 Table 2 Manual Prefilling - 400

Plate	Solution / Buffer	Volume per well [µl]
P1 - Samples/LS	PK	50
	LS-IC	400
P2 - Process	BS	450
	MAG	50
P3 - BS	BS	450
P4 - WS A	WS A	1100
P5 - WS B	WS B	1100
P7 - EB	EB	600

10.3 Table 3 Manual Prefilling - 200

Plate	Solution / Buffer	Volume per well [µl]
P1 - Samples/LS	PK	50
	LS-IC	200
P2 - Process	BS	450
	MAG	50
P4 - WS A	WS A	1100
P5 - WS B	WS B	1100
P7 - EB	EB	600

10.4 General tables for automated Prefilling I + II / III

Table 4 LS-IC Mix - 800 – 400 - 200

Protocol	**800 µl protocol**		**400 µl protocol**		**200 µl protocol**	
Solution	**LS**	**IC**	**LS**	**IC**	**LS**	**IC**
Sample no.	**Volume [ml]**					
8	7,5	94	6	150	5	250
16	15	188	9	225	7	350
24	23	289	12	300	8	400
32	31	389	15	375	10	500
40	39	489	21	525	12	600
48	47	590	24	600	13	650
56	55	690	27	675	14	700
64	63	790	30	750	15	750
72	71	891	36	900	20	1000
80	79	991	39	975	22	1100
88	87	1091	42	1050	23	1150
96	whole Bottle	1380	45	1125	25	1250

Table 5 Prefilling - Number of Tips for TR96/1000

Fill columns (left to right) of TR96/1000 with number of tips as shown in the table

Protocol	**Prefilling I**	**Prefilling II**	**Prefilling III**
Sample no.	**[Number of columns to be equipped with tips 1000 µl]**		
8/16/24/32	4	2	6
40/48/56/64	4	3	7
72/80/88/96	4	4	8

NOTE

Prepare internal controls/ carrier mixes of other than the RoboGene Quantification Kits according to the manufacturer's instruction.

For automated prefilling (Prefilling II or Prefilling III) pay attention to the required volumes for the 12-well reservoir as listed in **Table 4 LS-IC Mix – 800 – 400 - 200**.

For manual prefilling mix lysis and internal control/carrier for required number of reactions plus one (N+1) as shown in the table.

Sample volume	**800 µl**	**200 µl**	**400 µl**
Volume LS	(N+1) x 800 µl	(N+1) x 200 µl	(N+1) x 400 µl
Volume IC	(N+1) x Vol IC per reaction		

10.5 Tables for automated Prefilling with 800 µl protocol

Table 6 – BS/WS A/WS B/ EB - 800

Reservoir	**R1 - BS/BS**		**R2 – WS A/WS B**		**R3 - EB**	
Cavity	**left**	**right**	**left**	**right**	**left**	**right**
Solution	**BS**	**BS**	**WS A**	**WS B**	**EB**	
Sample no.	**Volume [ml]**					
8	20	-	15	15	11	-
16	32	-	24	24	16	-
24	45	-	33	33	21	-
32	50	15	42	42	26	-
40	54	24	50	50	30	-
48	58	33	59	59	35	-
56	62	42	68	68	40	-
64	66	50	77	77	45	-
72	71	60	86	86	50	-
80	75	70	94	94	54	-
88	80	80	103	103	59	-
96	whole bottle 1	whole bottle 2	whole bottle	whole bottle	whole bottle	-

Table 7 – MAG/LS-IC - 800

Cavity	**1**	**7**	**8**	**9**	**10**	**11**	**12**
Solution	**MAG**	**LS-IC**	**LS-IC**	**LS-IC**	**LS-IC**	**LS-IC**	**LS-IC**
Sample no.	**Volume [ml]**						
8	2,4	-	-	-	-	-	7,5
16	2,8	-	-	-	-	-	15
24	3,2	-	-	-	-	7,5	15
32	3,6	-	-	-	-	15	15
40	4,0	-	-	-	7,5	15	15
48	4,4	-	-	-	15	15	15
56	4,8	-	-	7,5	15	15	15
64	5,2	-	-	15	15	15	15
72	5,6	-	7,5	15	15	15	15
80	6,0	-	15	15	15	15	15
88	6,4	7,5	15	15	15	15	15
96	whole bottle	15	15	15	15	15	15

10.6 Tables for automated Prefilling with 400 µl protocol

Table 8 - BS/WS A/WS B/ EB - 400

Reservoir	**R1 - BS/EB**		**R2 – WS A/WS B**	
Cavity	**left**	**right**	**left**	**right**
Solution	**BS**	**EB**	**WS A**	**WS B**
Sample no.	**Volume [ml]**			
8	13	11	15	15
16	20	16	24	24
24	28	20	32	32
32	35	25	41	41
40	42	30	50	50
48	49	35	59	59
56	56	40	68	68
64	64	44	76	76
72	71	49	85	85
80	78	54	94	94
88	85	59	103	103
96	whole bottle	whole bottle	whole bottle	whole bottle

Table 9 - MAG/LS-IC - 400

Cavity	**1**	**10**	**11**	**12**
Solution	**MAG**	**LS-IC**	**LS-IC**	**LS-IC**
Sample no.	**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

10.7 Tables for automated Prefilling with 200 µl protocol

Table 10 - BS/WS A/WS B/ EB - 200

Reservoir	**R1 - BS/EB**		**R2 – WS A/WS B**	
Cavity	**left**	**right**	**left**	**right**
Solution	**BS**	**EB**	**WS A**	**WS B**
Sample no.	**Volume [ml]**			
8	10	11	15	15
16	13	16	24	24
24	17	20	32	32
32	20	25	41	41
40	24	30	50	50
48	28	35	59	59
56	31	40	68	68
64	35	44	76	76
72	38	49	85	85
80	42	54	94	94
88	46	59	103	103
96	50	whole bottle	whole bottle	whole bottle

Table 11 - MAG/LS-IC - 200

Cavity	**1**	**11**	**12**
Solution	**MAG**	**LS-IC**	**LS-IC**
Sample no.	**Volume [ml]**		
8	2,4	-	5
16	2,8	-	7
24	3,2	-	8
32	3,6	-	10
40	4,0	-	12
48	4,4	-	13
56	4,8	-	14
64	5,2	-	15
72	5,6	5	15
80	6,0	7	15
88	6,4	8	15
96	whole bottle	10	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 General 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 General notes and safety recommendations on handling RNA.
- Be sure to process your starting material correctly → please refer to section Sampling Starting Material.
- 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	February 2024	▪ Section 1.1, 1.2, 2.2, 2,3, 6.5, 7.1, 9.1.4, 9.1.5 and 9.3 editorial changes. ▪ Former section 12 removed ▪ Document Revision added