ROBOSCREEN DIAGNOSTICS

Instructions for Use

INSTANT Virus RNA/DNA Kit - FX

Rev. 3 _ 07 / 2021

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 viral nucleic acid from different kinds of starting material on the CyBio FeliX Basic Unit in combination with the CyBio FeliX Extraction Set. With possible starting volumes of 200, 400 or 800 µl per specimen a throughput of up to 96 samples is possible at once. The INSTANT Virus RNA/DNA Kit - FX is optimized for the rapid preparation of highly pure viral RNA and DNA from cell free fluid biological samples, for example: plasma and serum.

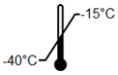
1.2 Technical assistance

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 IFU.

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 as well as the shown methodology:

Symbol	Information
	**REF** Catalogue number
	**Content** Contains sufficient reagents for <N> tests
	**Storage conditions**
	**Consult instructions for use**. This information must be observed to avoid improper use of the kit and the kit components.
	**Use by**
	**Lot number** Lot number of the kit or component
	**IVD symbol** This kit is an *in vitro* diagnostic medical device
	**Manufactured by**
	**For single use only**
	**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 in the IFU:

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 through this chapter carefully prior to guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the IFU, as well as all messages and information, which are shown.

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.

☞ **ATTENTION!**

The kit shall only be handled by educated personnel 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 have to 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. Roboscreen GmbH has not tested the liquid waste generated during using the kit for potential residual infectious components. This case is highly unlikely but cannot be excluded completely. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.
- 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 General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surfaces of the skin or from dusty laboratory equipment.
- 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.
- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240 °C for four hours or more before use. Autoclaving will not inactivate RNase activity completely. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC. The glassware has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C followed by autoclaving or heating to 100 °C for 15 minutes to remove residual DEPC.

- 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 Restrictions

This kit is validated for automated purification of viral nucleic acids from the following starting material:

- Fresh or frozen cell-free biological fluids (e.g. serum, plasma, sputum, swab supernatant, stool)
- Stabilizers: EDTA or citrate

The kit is to be used in conjunction with the CyBio FeliX with possible starting volumes of either 200, 400 or 800 µl per specimen.

If other than the recommended sample types or volumes are used incorrect results may be obtained. The product is to be used only by personnel specially instructed and trained in *in vitro* diagnostics procedures. Do not use expired components or mix with components from different batches.

Depending on downstream process additional restrictions could be valid. Please pay attention to the corresponding IFU!

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. In order 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 below 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 below 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

REF			847-0259200902	847-0259200904	847-0259200903
Protocol for starting 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		Instruction for use	1	1	1

5.2 Storage and Stability

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

Protect from heat and direct sunlight. Under these conditions, the kit has a shelf life as indicated on the kit box.

Upon arrival, store lyophilized **PK** and **MAG** at 2 °C to 10 °C. Aliquot dissolved **Proteinase K** and store at -40 °C to -15 °C. Repeated freezing and thawing will reduce activity dramatically!

All other components should be stored dry at ambient temperature.

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 – 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 consumambles for using 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 consumambles for using 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 using 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 only the accessories with the listed supports! Usage of other supports or of no supports 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)	-	-

To become familiar with all parts participating in automated extraction with the CyBio FeliX –System please look at the Information material that you received from Roboscreen GmbH.

☞ **ATTENTION!**

The usage of other tips and plates may cause a heavy damage of the CyBio FeliX and a loss of guarantee.
Also, the usage of other components/ reagents may cause malfunction of the whole protocol and loss of samples!

APPLICATION NOTE
Please use only the accessories with the recommended supports! Usage of other supports or of no supports may cause damages of the CyBio FeliX.

6.6 Recommended Products

Component	Manufacturer	Order number
Carrier RNA (1 tube)	Roboscreen GmbH	847-0206201001
Carrier RNA (3 tubes - for using 800 µl protocol)	Roboscreen GmbH	847-0206201004
Carrier RNA (12 tubes - for using 200 µl protocol)	Roboscreen GmbH	847-0206201003
Carrier RNA (6 tubes - for using 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 and 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 Detailed overview of the components for the entire process

7.1 Components for 800 µl protocol

Prefilling Set **847-0259200922**	**Plate Set** **847-0259200932**	**INSTANT Virus RNA/DNA Kit - FX** for use with 800 µl protocol **847-0259200902**	**FX- Parts**	**Consumables**
R1 - BS/BS	R4 - MAG/LS-IC	PK (4x)	Magnet Adapter	max. 3x96 tips 1000 µl
R2 - WS A/WS B	P1 - LS	MAG (1x)	Bioshake 3000-T elm 8-Kanal Adapter + S37	
R3 - EB/ -	P2 - Samples	PCR grade H2O (1x)		
Sealing Foil (5x)	P3 - Process	LS (1x)	Gripper + S37	
	P4 - BS	BS (2x)	TR96/1000	
	P5 - WS A	WS A (1x)	CM 96/1000 + S97 (2x)	
	P6 - WS B	WS B (1x)		
	P7 - EB Transfer	EB (1x)		
	P8 - EB			
	P9 - Eluates			
	Protective Plate (2x)			
	Sealing Foil			

7.2 Components for 400 µl protocol

Prefilling Set **847-0259200924**	**Plate Set** **847-0259200933**	**INSTANT Virus RNA/DNA Kit - FX** for use with 400 µl protocol **847-0259200904**	**FX- Parts**	**Consumables**
R1 – BS/EB	R3 – MAG/LS-IC	PK (7x)	Magnet Adapter	max. 3x96 tips 1000 µl
R2 – WS A/WS B	P1 – Samples/LS	MAG (2x)	Bioshake 3000-Telm	
Sealing Foil (5x)	P2 – Process	PCR grade H2O (2x)	8-Kanal Adapter + S37	
	P3 – BS	LS (1x)	Gripper + S37	
	P4 – WS A	BS (2x)	TR96/1000	
	P5 – WS B	WS A (2x)	CM 96/1000 + S97 (2x)	
	P6 – EB Transfer	WS B (2x)		
	P7 – EB	EB (2x)		
	P8 - Eluates			
	Protective Plate (2x)			
	Sealing Foil			

7.3 Components for 200 µl protocol

Prefilling Set **847-0259200923**	**Plate Set** **847-0259200934**	**INSTANT Virus RNA/DNA Kit - FX** for use with 200 µl protocol **847-0259200903**	**FX- Parts**	**Consumables**
R1 – BS/EB	R3 – MAG/LS-IC	PK (14x)	Magnet Adapter	max. 3x96 tips 1000 µl
R2 – WS A/WS B	P1 – Samples/LS	MAG (4x)	ioshake 3000-T elm	
Sealing Foil (5x)	P2 – Process	PCR grade H2O (4x)	8-Kanal Adapter + S37	
	P3 – Waste	LS (1x)	Gripper + S37	
	P4 – WS A	BS (2x)	TR96/1000	
	P5 – WS B	WS A (4x)	CM 96/1000 + S97 (2x)	
	P6 – EB Transfer	WS B (4x)		
	P7 – EB	EB (4x)		
	P8 - Eluates			
	Protective Plate (2x)			
	Sealing Foil			

8 Test description and principle

8.1 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 binding the nucleic acids to magnetic particles. In combination with the Liquid handling station CyBio Felix Basic Unit and the Cybio FeliX Extraction Set sample numbers of up to 96 specimen can be processed automatically at once. Either 200, 400 or 800 µl cell-free biological fluids per specimen are required for purification. A corresponding protocol is available depending on the selected sample volume. The following tables show the process for each protocol.

Order No.:	Kitversion description
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

The procedure for the process of automated nucleic acid purification from either **800 µl, 400 µl or 200 µl cell-free biological fluid** per specimen using CyBio FeliX and the INSTANT Virus RNA/ DNA Kit – FX is shown in the following scheme. The procedure is subdivided into two steps – prefilling and extraction. In the prefilling part the 96 deep well plates required for automated extraction are filled with the extraction solutions. During the extraction part the nucleic acids of your samples get purified.

Process principle – option 1	Process principle – option 2	Process principle - option 3
	Prefilling I Prefilling of BS, WS A, WS B and EB in the corresponding plates	
Manual Prefilling Prefilling of BS, WS A, WS B, EB, LS-IC and MAG in the corresponding plates	Storage of prefilled plates for up to 4 days **Prefilling II** Prefilling of LS-IC and MAG in the corresponding plate	**Prefilling III** Prefilling of BS, WS A, WS B, EB, LS-IC and MAG in the corresponding plates

Extraction

Lyse of the starting material, binding of the nucleic acids to the magnetic particles, washing steps of the samples followed by the elution of the nucleic acids from the magnetic particles

For prefilling three options are available. Next to the manual prefilling, automated options are prefilling I + prefilling II or prefilling III. As the corresponding plates filled with BS, WS A, WS B and EB can be stored in the dark for up to 4 days, prefilling I can be done separately. After Prefilling II or III the extraction part must be carried out immediately as MAG and LS-IC Mix are not stable for long.
For example, several prefilling I can be prepared for the week to ensure a smooth process, so that only the short prefilling II and the extraction part have to be carried out in the course of the day.

☞ **Note**

For storage of the prefilled plates with BS, WS A, WS B and EB cover the plates with the sealing foils. Store the plates in the dark at 15°C to 30°C.

If more than one preflling I is done in one day, the 2-well reservoir plates can be reused.

8.2 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

8.3 Elution Volume

	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**

Indicated elution volume is essential for RoboGene Kit performance!

9 Starting material

9.1 Collection and handling of clinical plasma/serum 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 your in-house regulations and 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.

☞ **NOTE**

If you want to use a RoboGene Quantification Kit for quantification of nucleic acids, EDTA or citrate anticoagulant has to be used; heparin is non-applicable, because of its inhibitory effect on PCR.

9.2 Sample preparation for viral NA extraction from swabs

In case of using dry swabs please carry out the following preparation instructions before continuing with the protocol.

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**.

9.3 Sample collection and 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

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

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, the resuspension can be extended; or try to separate the sample into smaller pieces by pipetting up and down. It may be necessary to cut off the pipette tip in order to be able to pipette more easily. If the eluates obtained are cloudy, we recommend centrifuging them for 3 minutes at maximum speed (20,000 g) to clarify the eluates. This centrifugation does not have a negative effect on the eluates; rather, it improves the results that are obtained with cloudy eluates in subsequent applications.

10 Procedure

10.1 Preparation

10.1.1 Preparation of pipetting plan

It is recommended to create a pipette plan before starting the whole extraction procedure to avoid unwanted interchanges of samples later. Therefore, note the position of every sample on the 96 well sample plate.

10.1.2 Preparation of WS B

1. Fill 72 ml of >96% absolute/pure ethanol in the bottle of the wash solution **WS B**.
2. Close the bottle with the lid and invert for several times.

10.1.3 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 **1500 µl 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).

10.1.4 Preparation of Internal Control and Carrier RNA

The application of carrier-RNA and an internal control for RNA and DNA is strongly recommended when using the INSTANT Virus RNA/DNA Kit -FX in combination with diagnostic amplification systems. Amongst other effects Carrier RNA prevents RNA degradation. If not added it can lead to reduced viral load.

The entrainment of an internal control of RNA and DNA serves as a control of an accurate execution of the whole procedure. For correct concentration of internal control and carrier RNA please refer to manufacturer's instructions of your further downstream process.

10.1.5 Preparation of the internal control of RoboGene Quantification Kits and /or Carrier RNA (Roboscreen)

Roboscreen offers two options. If a RoboGene Kit for quantification of viral nucleic acids is used an internal control (IC) with Carrier RNA in combination is included. If other internal control without Carrier is used Roboscreen's Carrier RNA can be used.

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 vial; close the tube(s), mix by vortexing briefly followed by brief centrifugation.

For IC only:

3. Incubate at 37 °C for 5 min using a shaking platform (800-1,000 rpm), 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.

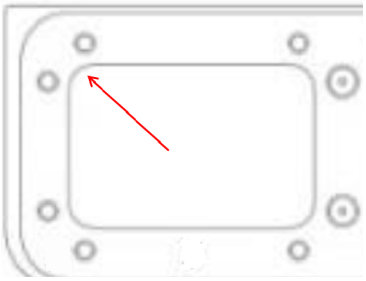
10.1.6 Further preparations

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 has to be on the upper left corner on the deck positions and the label has to 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.	

10.2 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

→ 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 the **Table 4 LS-IC Mix_800_400_200**. Mix solutions gently and avoid foaming. Do not vortex.

→ 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!

→ 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.

→ 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 vortex **MAG** vigorously before use for at least one minute. If **MAG** is not homogenized as described performance can be reduced dramatically.

10.3 Prefilling I

NOTE

The number of samples for the automatic extraction process is to be selected in steps of 8 (column by column). If the number of samples do not correspond to the column scheme, the remaining cavities in this column still will be filled.

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. Therefor, look at the required scheme.
      i. Scheme 1 Deck layout Prefilling I – 800 µl
      ii. Scheme 5 Deck layout Prefilling I – 400 µl
      iii. Scheme 9 Deck layout Prefilling I – 200 µl

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 of Prefilling II. If you continue with another Prefilling I seal prefilled plates with sealing foil, store them as recommended.

10.4 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 numbers and chosen protocol as indicated in the **Table 4 LS-IC Mix-800-400-200**. Mix solutions gently and avoid foaming. Do not vortex.

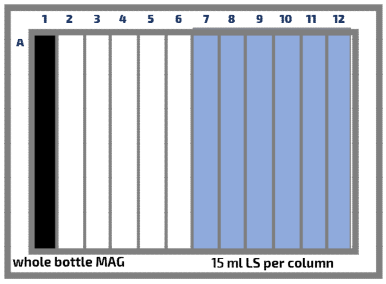
2. Vortex **MAG** for at least 1 minute vigorously directly before filling the solution in the 12-column reservoir (see the following point).

☞ **Attention**

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

3. 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	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



Example of the 12 – well Reservoir for 800 µl protocol for 96 samples

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

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. Therefor, look at the required scheme.
      i. Scheme 2 Deck layout Prefilling II – 800 µl
      ii. Scheme 6 Deck layout Prefilling II – 400 µl
      iii. Scheme 10 Deck layout Prefilling II – 200 µl
   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.

10.5 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 the **Table 4 LS-IC Mix-800-400-200**. Mix solutions gently and avoid foaming. Do not vortex.

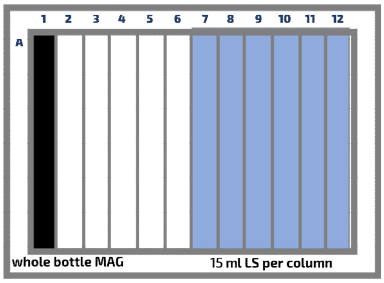
3. Vortex MAG for at least 1 minute vigorously directly before filling the solution in the 12-column reservoir (see the following point)

☞ **Attention**

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

4. 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



Example of the 12 – well Reservoir for 800 µl protocol for 96 samples

5. 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

6. 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

7. 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. Therefor, look at the required scheme.
      i. **Scheme 3 Deck layout Prefilling III – 800 µl**
      ii. **Scheme 7 Deck layout Prefilling III – 400 µl**
      iii. **Scheme 11 Deck layout Prefilling III – 200 µl**
   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.

10.6 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:

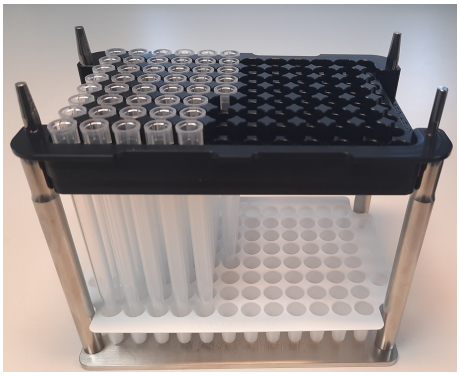
Protocol	Sample Volume	Plate
800 µl	800 µl	P2 – Samples
400 µl	400 µl	P1 – Samples / LS
200 µl	200 µl	P1 – Samples / LS

3. Prepare **two** sets **CM96/1000** with **tip**s and one Protective Plate each as shown in the image below. Only use the pipetting tips mentioned under 6.1. According to the number of samples to be prepared, the tips are to be inserted on the same position according to your pipetting plan (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** is absolutely 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 pipetting plan for 45 samples a prepared CM96/1000 with 45 tips onto S97 with Protective Plate is shown.

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 under point Elution volume).
    d. Open enclosure and set up the deck layout. Therefor, look at the required scheme.
        i. **Scheme 4 Deck layout Extraction – 800 µl**
        ii. **Scheme 8 Deck layout Extraction – 400 µl**
        iii. **Scheme 12 Deck layout Extraction – 200 µl**

e. Close enclosure carefully, check deck layout and confirm with ok. → The CyBioFeliX 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.

10.7 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 have to 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.

11 Corresponding tables for prefilling

11.1 Table 1 Manual Prefilling - 800

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

11.2 Table 2 Manual Prefilling - 400

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

11.3 Table 3 Manual Prefilling - 200

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

11.4 General tables for automated Prefilling I + II / III

11.4.1 Table 4 LS-IC Mix-800-400-200

Protocol	800 µl protocol		400 µl protocol		200 µl protocol	
Solution	Vol. LS [ml]	Vol. IC [µl]	Vol. LS [ml]	Vol. IC [µl]	Vol. LS [ml]	Vol. IC [µl]
Sample						
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

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 mentioned 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.

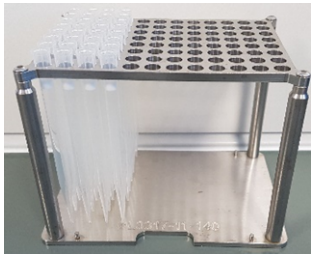
Sample volume	800 µl	200 µl	400 µl
Volume LS	(N+1) x 800 µl	(N+1) x 200 µl	(N+1) x 400 µl
Volume IC	(N+1) x Vol IC per reaction		

11.4.2 Table 5 Prefilling - Number of Tips for TR96/1000

Sample no	Prefilling I	Prefilling II	Prefilling III
	[number of columns to fill 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

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

➔ Only use the pipetting tips mentioned under chapter 6.



Example: TR96/1000 filled with first 4 columns of tips for Prefilling I

11.5 Tables for automated Prefilling with 800 µl protocol

11.5.1 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	Vol. BS	Vol. BS	Vol. WS A	Vol. WS B	Vol. EB	
	[ml]	[ml]	[ml]	[ml]	[ml]	[ml]
Sample no.						
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	-

11.5.2 Table 7 – MAG/LS-IC-800

Cavity	1	7	8	9	10	11	12
Solution	Vol. MAG	Vol. LS-IC	Vol. LS-IC	Vol. LS-IC	Vol. LS-IC	Vol. LS-IC	Vol. LS-IC
	[ml]	[ml]	[ml]	[ml]	[ml]	[ml]	[ml]
Sample no.							
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

11.6 Tables for automated Prefilling with 400 µl protocol

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

reservoir	R1 – BS/EB		R2 – WS A/WS B	
Cavity	left	right	left	right
Solution	Vol. BS [ml]	Vol. EB [ml]	Vol. WS A [ml]	Vol. WS B [ml]
Sample no.				
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

11.6.2 Table 9 – MAG/LS-IC-400

Cavity	1	10	11	12
Solution	Vol. MAG	Vol. LS-IC	Vol. LS-IC	Vol. LS-IC
	[ml]	[ml]	[ml]	[ml]
Sample no.				
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

11.7 Tables for automated Prefilling with 200 µl protocol

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

reservoir	R1 – BS/EB		R2 – WS A/WS B	
Cavity	left	right	left	right
Solution	Vol. BS [ml]	Vol. EB [ml]	Vol. WS A [ml]	Vol. WS B [ml]
Sample no.				
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

11.7.2 Table 11 – MAG/LS-IC-200

Cavity	1	11	12
Solution	Vol. MAG	Vol. LS-IC	Vol. LS-IC
	[ml]	[ml]	[ml]
Sample no.			
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

12 Scheme Deck layouts for Prefilling I, II, III and Extraction

12.1 Prefilling I - Scheme 1 Deck layouts for 800 µl protocol

Prefilling I – 800 µl protocol Decklayout - START			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm		
Pos. 2		R1 – BS / BS	filled with BS
Pos. 3	8-Channel Adapter + S37		
Pos. 4	TR96/1000	4 columns of tips	new tips
Pos. 5		R2 – WS A /WS B	filled with WS A and WS B
Pos. 6		R3 - EB	filled with EB
Pos. 7		P4 – BS	empty
Pos. 8		P5 – WS A	empty
Pos. 9		P6 – WS B	empty
Pos. 10	Magnet Adapter	P8 - EB	empty
Pos. 11		P3 – Process	empty
Pos. 12			

Decklayout - FINISH			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm		
Pos. 2		R1 – BS / BS	empty
Pos. 3	8-Channel Adapter – S37		
Pos. 4	TR96/1000	4 columns of tips	used tips
Pos. 5		R2 – WS A /WS B	empty
Pos. 6		R3 - EB	empty
Pos. 7		P4 – BS	filled with BS
Pos. 8		P5 – WS A	filled with WS A
Pos. 9		P6 – WS B	filled with WS B
Pos. 10	Magnet Adapter	P8 - EB	filled with EB
Pos. 11		P3 – Process	filled with BS
Pos. 12			

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

12.2 Prefilling II - Scheme 2 Deck layouts for 800 µl protocol

Prefilling II – 800 µl protocol Decklayout - START			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P1 - LS-IC	empty
Pos. 2			
Pos. 3	8-Channel Adapter + S37		
Pos. 4	TR96/1000	2 to 4 columns of tips	new tips
Pos. 5			
Pos. 6			
Pos. 7		P4 – BS	filled with BS
Pos. 8		P5 – WS A	filled with WS A
Pos. 9		P6 – WS B	filled with WS B
Pos. 10	Magnet Adapter	P8 - EB	filled with EB
Pos. 11		P3 – Process	filled with BS
Pos. 12		R4 - MAG / LS-IC	filled with MAG and LS-IC

Decklayout - FINISH			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P1 - LS-IC	filled with LS-IC
Pos. 2			
Pos. 3	8-Channel Adapter + S37		
Pos. 4	TR96/1000	2 to 4 columns of tips	used tips
Pos. 5			
Pos. 6			
Pos. 7		P4 – BS	filled with BS
Pos. 8		P5 – WS A	filled with WS A
Pos. 9		P6 – WS B	filled with WS B
Pos. 10	Magnet Adapter	P8 - EB	filled with EB
Pos. 11		P3 – Process	filled with BS and MAG
Pos. 12		R4 - MAG / LS-IC	empty

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

12.3 Prefilling III - Scheme 3 Deck layouts for 800 µl protocol

Prefilling III- 800 Decklayout - START			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P1 - LS-IC	empty
Pos. 2		R1 – BS / BS	filled with BS
Pos. 3	8-Channel Adapter + S37		
Pos. 4	TR96/1000	6 to 8 columns of tips	new tips
Pos. 5		R2 – WS A /WS B	filled with WS A and WS B
Pos. 6		R3 - EB	filled with EB
Pos. 7		P4 – BS	empty
Pos. 8		P5 – WS A	empty
Pos. 9		P6 – WS B	empty
Pos. 10	Magnet Adapter	P8 - EB	empty
Pos. 11		P3 – Process	empty
Pos. 12		R4 - MAG / LS-IC	filled with MAG and LS-IC

Decklayout - FINISH			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P1 - LS-IC	filled with LS-IC
Pos. 2		R1 – BS / BS	empty
Pos. 3	8-Channel Adapter + S37		
Pos. 4	TR96/1000	6 to 8 columns of tips	used tips
Pos. 5		R2 – WS A /WS B	empty
Pos. 6		R3 - EB	empty
Pos. 7		P4 – BS	filled with BS
Pos. 8		P5 – WS A	filled with WS A
Pos. 9		P6 – WS B	filled with WS B
Pos. 10	Magnet Adapter	P8 - EB	filled with EB
Pos. 11		P3 – Process	filled with BS and MAG
Pos. 12		R4 - MAG / LS-IC	empty

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

12.4 Extraction - Scheme 4 Deck layouts for 800 µl protocol

Extraction - 800 Decklayout - START			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P1 - LS-IC	filled with LS-IC
Pos. 2	CM96/1000 + S97	tips with protective plate	new tips
Pos. 3	Gripper + S37		
Pos. 4		P2 - Samples	filled with PK and samples
Pos. 5	CM96/1000 + S97	tips with protective plate	new tips
		top: P7 - EB Transfer	empty
Pos. 6		bottom: P8 - EB	filled with EB
Pos. 7		P4 – BS	filled with BS
Pos. 8		P5 – WS A	filled with WS A
Pos. 9		P6 – WS B	filled with WS B
Pos. 10	Magnet Adapter		
Pos. 11		P3 – Process	filled with BS and MAG
Pos. 12		P9 - Eluates	empty

Decklayout - FINISH			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	empty	
Pos. 2	CM96/1000 + S97	tips with protective plate	used tips
Pos. 3	Gripper + S37		
		top: P1 LS-IC	filled with waste
Pos. 4		bottom: P2 - Samples	filled with waste
Pos. 5	CM96/1000 + S97	tips with protective plate	used tips
		top: P3 - Process	filled with waste
Pos. 6		bottom: P8 - EB	filled with waste
Pos. 7		P4 – BS	filled with waste
Pos. 8		P5 – WS A	filled with waste
Pos. 9		P6 – WS B	filled with waste
Pos. 10	Magnet Adapter	P7 - EB Transfer	filled with waste
Pos. 11		empty	
Pos. 12		P9 - Eluates	filled with eluates

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

12.5 Prefilling I - Scheme 5 Deck layouts for 400 µl protocol

		Prefilling I - 400 Decklayout - START	
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	empty
Pos. 2		R1 – BS / EB	filled with BS and EB
Pos. 3	Gripper + S37		
Pos. 4	TR96/1000	4 columns of tips	new tips
Pos. 5		R2 – WS A /WS B	filled with WS A and WS B
Pos. 6	8-Channel Adapter + S37		
Pos. 7		P3 – BS	empty
Pos. 8		P4 – WS A	empty
Pos. 9		P5 – WS B	empty
Pos. 10	Magnet Adapter		
Pos. 11		P2 – Process	empty
Pos. 12			

		Decklayout - FINISH	
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	filled with EB
Pos. 2		R1 – BS / EB	empty
Pos. 3	Gripper + S37		
Pos. 4	TR96/1000	4 columns of tips	used tips
Pos. 5		R2 – WS A /WS B	empty
Pos. 6	8-Channel Adapter + S37		
Pos. 7		P3 – BS	filled with BS
Pos. 8		P4 – WS A	filled with WS A
Pos. 9		P5 – WS B	filled with WS B
Pos. 10	Magnet Adapter		
Pos. 11		P2 – Process	filled with BS
Pos. 12			

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

12.6 Prefilling II - Scheme 6 Deck layouts for 400 µl protocol

Prefilling II - 400 Decklayout - START			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	filled with EB
Pos. 2			
Pos. 3	Gripper + S37		
Pos. 4	TR96/1000	2 to 4 columns of tips	new tips
Pos. 5			
Pos. 6	8-Channel Adapter + S37		
Pos. 7		P3 - BS	filled with BS
Pos. 8		P4 – WS A	filled with WS A
Pos. 9		P5 – WS B	filled with WS B
Pos. 10	Magnet Adapter	P1 - Samples / LS	filled with PK
Pos. 11		P2 – Process	filled with BS
Pos. 12		R3 - MAG / LS-IC	filled with MAG and LS-IC

Decklayout - FINISH			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	filled with EB
Pos. 2			
Pos. 3	Gripper + S37		
Pos. 4	TR96/1000	2 to 4 columns of tips	used tips
Pos. 5			
Pos. 6	8-Channel Adapter + S37		
Pos. 7		P3 - BS	filled with BS
Pos. 8		P4 – WS A	filled with WS A
Pos. 9		P5 – WS B	filled with WS B
Pos. 10	Magnet Adapter	P1 - Samples / LS	filled with PK and LS-IC
Pos. 11		P2 – Process	filled with BS and MAG
Pos. 12		R3 - MAG / LS-IC	empty

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

12.7 Prefilling III - Scheme 7 Deck layouts for 400 µl protocol

Prefilling III 400 Decklayout - START			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	empty
Pos. 2		R1 – BS / EB	filled with BS and EB
Pos. 3	Gripper + S37		
Pos. 4	TR96/1000	6 to 8 columns of tips	new tips
Pos. 5		R2 – WS A /WS B	filled with WS A and WS B
Pos. 6	8-Channel Adapter + S37		
Pos. 7		P3 - BS	empty
Pos. 8		P4 – WS A	empty
Pos. 9		P5 – WS B	empty
Pos. 10	Magnet Adapter	P1 - Samples / LS	filled with PK
Pos. 11		P2 – Process	empty
Pos. 12		R3 - MAG / LS-IC	filled with MAG and LS-IC

Decklayout - FINISH			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	filled with EB
Pos. 2		R1 – BS / EB	empty
Pos. 3	Gripper +S37		
Pos. 4	TR96/1000	6 to 8 columns of tips	used tips
Pos. 5		R2 – WS A /WS B	empty
Pos. 6	8-Channel Adapter + S37		
Pos. 7		P3 - BS	filled with BS
Pos. 8		P4 – WS A	filled with WS A
Pos. 9		P5 – WS B	filled with WS B
Pos. 10	Magnet Adapter	P1 - Samples / LS	filled with PK and LS-IC
Pos. 11		P2 – Process	filled with BS and MAG
Pos. 12		R3 - MAG / LS-IC	empty

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

12.8 Extraction - Scheme 8 Deck layouts for 400 µl protocol

Extraction - 400			
		Decklayout - START	
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P1 - Samples / LS	filled with PK, LS-IC and samples
Pos. 2	CM96/1000 + S97	tips with protective plate	new tips
Pos. 3	Gripper + S37		
Pos. 4			
Pos. 5	CM96/1000 + S97	tips with protective plate	new tips
Pos. 6		top: P6 - EB Transfer	empty
		bottom: P7 - EB	filled with EB
Pos. 7		P3 - BS	filled with BS
Pos. 8		P4 – WS A	filled with WS A
Pos. 9		P5 – WS B	filled with WS B
Pos. 10	Magnet Adapter		
Pos. 11		P2 – Process	filled with BS and MAG
Pos. 12		P8 - Eluates	empty

		Decklayout - FINISH	
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm		
Pos. 2	CM96/1000 + S97	tips with protective plate	used tips
Pos. 3	Gripper + S37		
Pos. 4		P1 - Samples / LS	filled with waste
Pos. 5	CM96/1000 + S97	tips with protective plate	used tips
Pos. 6		top: P2 - Process	filled with waste
		bottom: P7 - EB	filled with waste
Pos. 7		P3 - BS	filled with waste
Pos. 8		P4 – WS A	filled with waste
Pos. 9		P5 – WS B	filled with waste
Pos. 10	Magnet Adapter	P6 - EB Transfer	filled with waste
Pos. 11			
Pos. 12		P8 - Eluates	filled with eluates

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

12.9 Prefilling I - Scheme 9 Deck layouts for 200 µl protocol

Prefilling I - 200 Decklayout - START			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	empty
Pos. 2		R1 – BS / EB	filled with BS and EB
Pos. 3	Gripper + S37		
Pos. 4	TR96/1000	4 columns of tips	new tips
Pos. 5		R2 – WS A /WS B	filled with WS A and WS B
Pos. 6	8-Channel Adapter + S37		
Pos. 7			
Pos. 8		P4 – WS A	empty
Pos. 9		P5 – WS B	empty
Pos. 10	Magnet Adapter		
Pos. 11		P2 – Process	empty
Pos. 12			

Decklayout - FINISH			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	filled with EB
Pos. 2		R1 – BS / EB	empty
Pos. 3	Gripper + S37		
Pos. 4	TR96/1000	4 columns of tips	used tips
Pos. 5		R2 – WS A /WS B	empty
Pos. 6	8-Channel Adapter – S37		
Pos. 7			
Pos. 8		P4 – WS A	filled with WS A
Pos. 9		P5 – WS B	filled with WS B
Pos. 10	Magnet Adapter		
Pos. 11		P2 – Process	filled with BS
Pos. 12			

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

12.10 Prefilling II - Scheme 10 Deck layouts for 200 µl protocol

Prefilling II - 200 Decklayout - START			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	filled with EB
Pos. 2			
Pos. 3	Gripper + S37		
Pos. 4	TR96/1000	2 to 4 columns of tips	new tips
Pos. 5			
Pos. 6	8-Channel Adapter + S37		
Pos. 7			
Pos. 8		P4 – WS A	filled with WS A
Pos. 9		P5 – WS B	filled with WS B
Pos. 10	Magnet Adapter	P1 - Samples / LS	filled with PK
Pos. 11		P2 – Process	filled with BS
Pos. 12		R3 - MAG / LS-IC	filled with MAG and LS-IC

Decklayout - FINISH			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	filled with EB
Pos. 2			
Pos. 3	Gripper + S37		
Pos. 4	TR96/1000	2 to 4 columns of tips	used tips
Pos. 5			
Pos. 6	8-Channel Adapter + S37		
Pos. 7			
Pos. 8		P4 – WS A	filled with WS A
Pos. 9		P5 – WS B	filled with WS B
Pos. 10	Magnet Adapter	P1 - Samples / LS	filled with PK and LS-IC
Pos. 11		P2 – Process	filled with BS and MAG
Pos. 12		R3 - MAG / LS-IC	empty

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

12.11 Prefilling III - Scheme 11 Deck layouts for 200 µl protocol

Prefilling III - 200 Decklayout - START			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	empty
Pos. 2		R1 – BS / EB	filled with BS and EB
Pos. 3	Gripper + S37		
Pos. 4	TR96/1000	6 to 8 columns of tips	new tips
Pos. 5		R2 – WS A /WS B	filled with WS A and WS B
Pos. 6	8-Channel Adapter + S37		
Pos. 7			
Pos. 8		P4 – WS A	empty
Pos. 9		P5 – WS B	empty
Pos. 10	Magnet Adapter	P1 - Samples / LS	filled with PK
Pos. 11		P2 – Process	empty
Pos. 12		R3 - MAG / LS-IC	filled with MAG and LS-IC

Decklayout - FINISH			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P7 - EB	filled with EB
Pos. 2		R1 – BS / EB	empty
Pos. 3	Gripper + S37		
Pos. 4	TR96/1000	6 to 8 columns of tips	used tips
Pos. 5		R2 – WS A /WS B	empty
Pos. 6	8-Channel Adapter + S37		
Pos. 7			
Pos. 8		P4 – WS A	filled with WS A
Pos. 9		P5 – WS B	filled with WS B
Pos. 10	Magnet Adapter	P1 - Samples / LS	filled with PK and LS-IC
Pos. 11		P2 – Process	filled with BS and MAG
Pos. 12		R3 - MAG / LS-IC	empty

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

12.12 Extraction - Scheme 12 Deck layouts for 200 µl protocol

Extraction - 200 Decklayout - START			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm	P1 - Samples / LS	filled with PK, LS-IC and samples
Pos. 2	CM96/1000 + S97	tips with protective plate	new tips
Pos. 3	Gripper + S37		
Pos. 4			
Pos. 5	CM96/1000 + S97	tips with protective plate	new tips
		top: P6 - EB Transfer	empty
Pos. 6		bottom: P7 - EB	filled with EB
Pos. 7		P3 - Waste	empty
Pos. 8		P4 – WS A	filled with WS A
Pos. 9		P5 – WS B	filled with WS B
Pos. 10	Magnet Adapter		
Pos. 11		P2 – Process	filled with BS and MAG
Pos. 12		P8 - Eluates	empty

Decklayout - FINISH			
Position	**FX Part**	**Plate or Reservoir**	**Status**
Pos. 1	Bioshake-T elm		
Pos. 2	CM96/1000 + S97	tips with protective plate	used tips
Pos. 3	Gripper + S37		
Pos. 4		P1 - Samples / LS	filled with waste
Pos. 5	CM96/1000 + S97	tips with protective plate	used tips
		top: P2 - Process	filled with waste
Pos. 6		bottom: P7 - EB	filled with waste
Pos. 7		P3 - Waste	filled with waste
Pos. 8		P4 – WS A	filled with waste
Pos. 9		P5 – WS B	filled with waste
Pos. 10	Magnet Adapter	P6 - EB Transfer	filled with waste
Pos. 11			
Pos. 12		P8 - Eluates	filled with eluates

* Red marked items can be removed and/or discarded if disposables.
Essential plates who got filled during process are marked in green.

13 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.
- Inhibitory substances in starting material. Please use the kit only for samples that match the requirements declared in section Intended use . Depending on downstream process additional restrictions could be valid. Please pay attention to the corresponding IFU of the manufacturer!
- Use Internal Controls for verification of 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 eluted RNA directly in downstream procedures.

Problems with CyBio FeliX → please refer to „Operating Manual CyBio FeliX”	
Head doesn`t 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 head regularly so that the pipette tips can be picked up correctly
Bubbles in the plates	Be sure that plates are filled evenly

	→ Always use the indicated amount of buffers/ reagents for a 96-well plate → Wells of Sample - Plate that are not filled with specimen or controls have to be filled with respective volume water instead. Alternatively remove the specific tips for the unused wells (only possible with 96-Channel Magazine OL3810-13-023). → 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.