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VIR*Seek* RNA*Extractor* AE1

TEST KIT FOR AUTOMATED ISOLATION OF VIRAL RNA
FROM ENVIRONMENTAL SAMPLES INCLUDING WASTEWATER
USING AUTOMATED PLATFORMS

Cat. no. 5524400801, 5524400805

For 96 or 5 x 96 extractions

VIR*Seek*

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1 INTRODUCTION

The VIR*Seek* RNA*Extractor* AE1 kit is designed for the automated isolation of high-quality RNA from viral particles which have been concentrated from environmental surface swabs or from wastewater samples. Its components are optimised for RNA extraction and removal of potential inhibitors from these sample materials.

In light of the COVID-19 pandemic tools to support SARS-CoV-2 surveillance systems are deemed necessary such as to monitor the viral load on inanimate surfaces or in wastewater samples. Although, main route of transmission of SARS-CoV-2 is from person-to-person an indirect route of transmission by touching inanimate surfaces is discussed and surface swabs are used for monitoring. Furthermore, wastewater-based epidemiology has become an important approach in order to comprehensively monitor dynamics of SARS-CoV-2 infections in highly populated areas. Data have shown that concentrations of viral RNA do correlate with trends in reported COVID-19 cases, showing the usefulness of this public health tool. Respective workflows are available in order to concentrate the virus from those sample matrices before a subsequent RNA isolation using the VIR*Seek* RNA*Extractor* AE1.

The VIR*Seek* RNA*Extractor* AE1 protocol is validated for use with the ThermoFisher Scientific KingFisher™ Flex System and additionally with the GSD Auto-Pure96 System from Gold Standard Diagnostics for wastewater samples. Other automated purification platforms can well be used, after an on-site local validation at the respective laboratory site.

The kit is intended to be used by analytical laboratories for environmental testing and research purposes only (e.g. monitoring of viral contaminations on environmental surfaces or in wastewater).

The kit is not intended for clinical diagnostics and should therefore be regarded as “For Research Use Only”.

1.1 Test Principle

The RNA isolation process of the VIR*Seek* RNA*Extractor* AE1 kit is based on the wellestablished Boom method and uses magnetic beads for the isolation of nucleic acids from environmental sample types such as wastewater pellets. The viral particles are lysed and inactivated by incubation with lysis buffer containing chaotropic salts and detergents, thereby making the RNA accessible for further isolation/ purification. Furthermore, the lysis buffer has been optimised with regards to stabilisation of small RNA amounts, leading to high RNA recovery rates and resulting in an adequate sensitivity. The released viral RNA then binds to magnetic beads that are mixed with the lysate. In order to obtain high quality, ultrapure RNA for subsequent analysis, this binding step is followed by multiple washing steps using different wash buffers. Finally, the purified viral RNA is eluted from the magnetic beads in a low-salt elution buffer and can be used directly for subsequent analyses or stored at -20 °C (for up to six months) or at -80 °C (for up to twelve months).

From a technical point of view, the extracted RNA can be analysed using different real-time RT-PCR systems. However, all validation experiments have been performed in combination with the VIR*Seek* and VIR*Type* SARS-CoV-2 real-time RT-PCR kits.

1.2 Components of the Kit

The VIR*Seek* RNA*Extractor* AE1 kit contains reagents sufficient for RNA isolation from 96 or 5 x 96 samples.

Cat. no. 5524400801 (96 reactions)

- 1x **NQ2V**, magnetic beads, 1.1 mL, store at 2 °C - 8 °C (**Note**: Do not freeze!).
- 1x **NQ1V**, carrier molecule, dissolved, 600 µL, store at 2 °C - 8 °C.
- 2x **LK2V**, lysis buffer, 28 mL, store at 18 °C - 25 °C.
- 2x **WK5V**, wash buffer concentrate, 25 mL, store at 18 °C - 25 °C.
- 4x **WK6V**, wash buffer concentrate, 11 mL, store at 18 °C - 25 °C.
- 1x **HZ2V**, elution buffer, 12 mL, store at 18 °C - 25 °C.

Cat. no. 5524400805 (5 x 96 reactions)

- 1x **NQ2V**, magnetic beads, 5.5 mL, store at 2 °C - 8 °C (**Note**: Do not freeze!).
- 1x **NQ1V**, carrier molecule, dissolved, 3 mL, store at 2 °C - 8 °C.
- 1x **LK2V**, lysis buffer, 264 mL, store at 18 °C - 25 °C.
- 1x **WK5V**, wash buffer concentrate, 240 mL, store at 18 °C - 25 °C.
- 2x **WK6V**, wash buffer concentrate, 106 mL, store at 18 °C - 25 °C.
- 1x **HZ2V**, elution buffer, 55 mL, store at 18 °C - 25 °C.

Note: Shelf life is indicated on the labels of the kit/components. Shelf-life after opening is defined as being 6 months but no longer that the overall shelf-life as indicated on the labels of the kit.

Note: Volumes as indicated above are calculated with a defined overfilling and may vary.

1.3 Additional Equipment, Consumables and Reagents Required

Equipment, consumables and reagents listed are exclusively for the RNA extraction workflow.

Equipment:

- 1x **KingFisher™ Flex System** for automated RNA isolation (ThermoFisher Scientific, cat. no. 5400600)
  OR
  GSD Auto-Pure96 for automated RNA isolation (Gold Standard Diagnostics)
- 1x **Single channel pipette (1 mL, 100 µL**), (e.g. Transferpette® S 100 - 1000 µL, Brand®)
- 1x **Single channel pipette (100 µL, 10 µL),** (e.g. Transferpette® S 10 - 100 µL, Brand®)

Optional:

- 1x **Stepper pipette** (e.g. HandyStep® S Brand®)
- 1x **Thermo shaker**, for 96 well plates, cooling/heating capable
  (e.g. ThermoMixer® C, Eppendorf and suitable block inserts)
  Only required if virus lysis is not performed on the automated extraction platform; see section 2.6.2)

Instrument-specific consumables:

- **KingFisher™ Flex**
  - **Deepwell Plates**, RNase-free
  - **TipComb**, RNase-free
- **GSD Auto-Pure96**
  - Deepwell Plates (e.g. from Gold Standard Diagnostics, cat. No. AS-17061-02)
  - Tip Comb (e.g. Gold Standard Diagnostics, cat. No. AS-17061-01)

Consumables:

- **Absolute ethanol** (≥ 99 %, p.a.), RNase-free.
- **Reaction tubes,** 50 mL, RNase-free.
- **Pipette tips**, RNase-free, check compatibility with pipettes utilised.
- **Tips for stepper pipet,** RNase-free, check compatibility with pipettes utilised.
- **RNase Decontaminating Agent** (e.g. RNase Away®, Carl Roth GmbH).
- **Nucleic Acid Decontamination Agent** (e.g. Roti® Nucleic Acid-free, Carl Roth GmbH,).
- **Gloves**, powder free.

Optional:

Process Control Virus (e.g. MS2 phage as included in the VIR*Seek* SARS-CoV-2 Mplex I Wastewater or murine norovirus as included in the VIR*Seek* SARS-CoV-2 Mplex II Wastewater see section 1.4). Required for determination of extraction efficiency.

1.4 Complete Portfolio of the VIR*Seek* SARS-CoV-2 Solution

- **VIR*Seek* SARS-CoV-2 Mplex**, cat. no. 5728201101
  - Qualitative real-time RT-PCR kit with 96 reactions for rapid screening for SARS-CoV-2 (N1/N2-gene targets) in environmental and food surface samples including an extraction control.
- **VIR*Seek* SARS-CoV-2 Ident 2**, cat. no. 5728200705
  - Qualitative real-time RT-PCR kit with 48 reactions for rapid detection of SARS-CoV-2 specific RdRp sequence in environmental and food surface samples.
- **VIR*Seek* SARS-CoV-2 Mplex I Wastewater**, cat. no. 5728401801
  - Qualitative real-time RT-PCR kit with 48 reactions for rapid detection of SARS-CoV-2 specific N1/N2-gene sequence in wastewater samples including MS2 as an extraction control.
- **VIR*Seek* SARS-CoV-2 Mplex II Wastewater**, cat. no. 5728401901
  - Qualitative real-time RT-PCR kit with 48 reactions for rapid detection of SARS-CoV-2 specific RdRP sequence in wastewater samples including MNV as an extraction control.

2 HOW TO USE THIS PRODUCT

Important Notes:

- Store all reagents as indicated in section 1.2.
- Please make sure to store NQ2V (Magnetic Beads) and the NQ1V (Carrier Molecule) at 2 °C - 8 °C as soon as you receive them.
- Do not use the reagents beyond the expiration dates printed on the labels.
- Never store kit components in the vicinity of samples or post-PCR products.

2.1 General and Safety Precautions

- All samples should be handled with caution as they are potentially infectious.
- In order to safeguard the health of laboratory personnel, it is essential that the whole of this method be carried out only by skilled personnel using good laboratory practices.
- A BSL-2 laminar flow biosafety cabinet is recommended for activities with potential for producing aerosols of pathogens.
- Relevant national Health and Safety Regulations relating to the viruses analysed must be adhered to.
- Do not eat, drink or apply cosmetics in the work area in which the test is performed.
- Do not pipette by mouth.
- Avoid contact of kit components with injured skin.
- Always wear laboratory gloves, goggles, and a suitable lab coat when working with chemicals.
- The VIR*Seek* RNA*Extractor* AE1 kit contains chaotropic agents (guanidine thiocyanate) which may cause severe damage upon contamination or ingestion.

2.2 Working Guidelines

- Comply with Good Laboratory Practice (refer to EN ISO 7218 standard).
- Refer to EN ISO 22174:2005 for the general requirements for the *in-vitro* amplification of nucleic acid sequences.
- Perform cleaning protocol (outlined in section 2.4).
- Use RNA & DNA-, nuclease-free and sterile lab ware.
- Wear gloves and change frequently.

2.3 RNA Handling - Specific Working Guidelines

It is important to create and maintain an RNase-free environment when working with RNA. RNases are very thermostable enzymes degrading RNA – even in small quantities. Laboratory personnel are the main source of RNase contamination as RNases are expressed in human keratinocytes and are present on skin and hairs.

- Separate the different procedures spatially.
  Ideally use separate rooms for sample preparation and PCR setup to maintain a strict "one-way-system", thus avoiding cross-contamination in the work stream.
  At least dedicate different areas, equipment and consumables to each procedure.
- Establish a working area, designated as "RNase-free", in which only RNA work is performed. If the RNase-free working area is inside a lab with non-RNase-free working areas, clearly indicate RNase-free parts, e.g. using colour tape.
- Use dedicated RNase-free lab equipment (e.g. pipettes) for RNA related work. Glassware has to be cleaned and decontaminated before use. For decontamination we recommend baking glassware at > 200 °C for ≥ 4 hours.
- Only use RNase-free tips and consumables which are guaranteed to be RNase-free.
- Clean the RNA extraction area as described in the cleaning protocol (see section 2.4)
- Wear disposable gloves (latex or vinyl gloves) to prevent contamination with RNases which are present on human skin. Change gloves frequently during the procedure or after touching skin, hair, common surfaces etc.
- Wear a lab coat to prevent contamination from clothes.
- Only open one tube at a time and always change pipette tips between liquid transfers to avoid cross-contamination.
- Tightly close all reagent bottles after use in order to prevent evaporation of ethanol.

2.4 Cleaning Protocol

Before commencing work and after completing the work, ensure that the RNA extraction area is cleaned as follows:

Cleaning steps	Cleaning protocol
1.	Decontaminate surfaces with Roti® Nucleic Acid-free* or 1 % HCl to remove DNA / RNA contamination.
2.	Clean the work surfaces and non-disposable laboratory equipment (pipettes, shaker, thermo shaker etc.) with an RNase decontamination solution* (e.g. RNase AWAY®, Carl Roth, cat. no. A998) to remove RNase contaminations.

** Follow the manufacturer's instructions.*

2.5 Waste Disposal

Dispose of any waste which is potentially contaminated with pathogenic virus particles according to your internal and local regulations.

For disposal of reagents and chemicals please refer to the material safety data sheet (MSDS).

2.6 Before you Begin

- If working with the VIR*Seek* RNA*Extractor* AE1 kit for the first time, read “Working Guidelines” (see section 2.2 & 2.3).
- For additional equipment and consumables refer to section 1.3.
- Clean working area and non-disposable laboratory equipment with appropriate RNase decontaminating reagent (see section 2.4).
- When lysis is performed outside the KingFisher™ Flex or the GSD Auto-Pure96 system, heat up thermo shaker to 60 °C in time.

2.6.1 Preparation of Reagents

Wash Buffer

Before using the kit the first time, prepare wash buffers WK5V and WK6V (each provided as concentrate).

After preparation, the washing buffers should be stored at 18 °C - 25 °C and can be used until indicated by the expiration date.

Wash buffer WK5V

a. Add 25 mL or 240 mL absolute ethanol (≥ 99 %, p.a., RNase-free) to each bottle containing wash buffer concentrate WK5V (for 1 x 96 or 5 x 96, respectively).

b. Mix well by shaking/swirling/agitating.

c. Mark WK5V bottle after addition of ethanol.

d. Store tightly closed reagent bottle (to prevent evaporation of ethanol) at 18 °C - 25 °C.

Note: The wash buffer concentrate may contain precipitates of salts. After adding ethanol, the salt will dissolve. Alternatively, heat up to 50 °C until all crystals have completely dissolved prior to use.

Wash buffer WK6V

a. Add 44 mL or 424 mL absolute ethanol (≥ 99 %, p.a., RNase-free) to the bottle containing wash buffer concentrate WK6V (for 1 x 96 or 5 x 96, respectively).

b. Mix well by shaking/swirling/agitating.

c. Mark WK6V bottle after addition of ethanol.

d. Store tightly closed reagent bottle (to prevent evaporation of ethanol) at 18 °C - 25 °C.

Final Lysis Buffer

Final lysis buffer should be prepared fresh before each extraction by addition of carrier molecule NQ1V to lysis buffer LK2V.

Note: If LK2V has crystallised please heat up to 50 °C until all crystals have completely dissolved prior to use.

Calculate the required volumes of lysis buffer LK2V and carrier molecule NQ1V according to following formula (consider +10 % excess) and mix in an RNase-free 50 mL tube.

Calculation: n reactions = n * 495 µL LK2V + n * 5 µL NQ1V

n: number of samples and controls to be processed

Binding Mixture

Calculate the required volumes of absolute ethanol and magnetic beads NQ2V according to following formula (consider +10 % excess) and mix well in an RNase-free tube by pipetting up and down until beads are completely dissolved.

Calculation: n reactions = n * 490 µL absolute ethanol + n * 10 µL NQ2V

n: number of samples and controls to be processed

Note: Please vortex the magnetic beads NG2V rigorously, before adding to the binding mixture.

2.6.2 VIR*Seek* RNA*Extractor* AE1 Protocol for the KingFisher™ Flex System

Prior to using the VIR*Seek* RNA*Extractor* AE1 kit for the first time please program the following workflow into the KingFisher™ Flex System according to the system's manual. The script 'VIRSeek_V03' and 'VIRSeek_wLysis_V03' will be provided by Eurofins GeneScan Technologies upon request.

Note: In case lysis is performed outside of the KingFisher™ Flex System, steps 2 and 3 are omitted in the automated workflow.

For the scripts please contact your local sales support

Table 1. VIR*Seek* RNA*Extractor* AE1 workflow protocol for KingFisher™ Flex System

Step		**Content of plate**	**Details**	
1	Pick-up tip comb	Empty	-	-
Steps 2 and 3 are only relevant, if the samples lysis is performed within the KingFisher™ Flex System				
2	Lysis	500 µL final lysis buffer (LK2V incl. NQ1V) containing sample from swab or wastewater pellet	Release beads	yes
			Preheat	yes
			Heating during mixing	yes
			Temperature	60 °C
			1. Mixing time	00:10:00
			1. Mixing speed	Medium
			Collect beads	no
3	Pause		Take out plate & manually add 500 µL Binding Mix (containing 490 µL Ethanol and 10 µL NQ2V) to the lysate	
4	Bind	500 µL lysate 10 µL NQ2V 490 µL Ethanol	Release beads	no
			1. Mixing time	00:05:00
			1. Mixing speed	Half mix
			Collect beads, count	5
			Collect time [s]	1
5	Collect Beads		Collect beads, count	1
			Collect time [s]	5
6	Wash step 1	900 µL WK5V	Release beads	yes
			hh:mm:ss	00:00:05
			Speed	Fast
			1. Mixing time	00:00:10
			1. Mixing speed	Bottom mix
			2. Mixing time	00:00:10
			2. Mixing speed	Fast
			Loop count	2
			Collect beads, count	5
			Collect time [s]	1
7	Collect Beads		Collect beads, count	3
			Collect time [s]	5
8	Wash step 2	1000 µL WK6V	Release beads	yes
			hh:mm:ss	00:00:05
			Speed	Fast

			1. Mixing time	00:00:15
			1. Mixing speed	Bottom mix
			2. Mixing time	00:00:15
			2. Mixing speed	Fast
			Loop count	1
			Collect beads, count	5
			Collect time [s]	10
9	Wash step 3	1000 µL WK6V	Release beads	yes
			hh:mm:ss	00:00:05
			Speed	Fast
			1. Mixing time	00:00:10
			1. Mixing speed	Bottom mix
			2. Mixing time	00:00:10
			2. Mixing speed	Fast
			Loop count	1
			Collect beads, count	5
			Collect time [s]	10
10	Dry		Outside well	00:02:00
11	Dry		above well Eluate	00:03:00
12	Elute	100 µL HZ2V	Release beads	yes
			hh:mm:ss	00:00:05
			Speed	Fast
			Preheat	yes
			Heating during mixing	yes
			Temperature	60 °C
			Mixing time	00:05:00
			Mixing speed	Medium
			Collect beads, count	5
			Collect time [s]	30
13	Leave tip comb	WK6V	-	-

2.6.3 VIR*Seek* RNA*Extractor* AE1 Protocol for the GSD Auto-Pure96

Prior to using the VIR*Seek* RNA*Extractor* AE1 kit for the first time please program the following work into the GSD Auto-Pure96 according to the system's manual. The script 'VIRSeek' and 'VIRSeekLysis' will be provided by Eurofins GeneScan Technologies upon request.

Note: In case lysis is performed inside the GSD Auto-Pure96 System, the automated workflow starts with the 'VIRSeekLysis' script, described in Table 2, followed by the extraction protocol 'VIRSeek', described in Table 3.

For the scripts please contact your local sales support.

Table 2. Workflow script 'VIRSeekLysis' for the initial lysis step using the VIR*Seek* RNA*Extractor* AE1 on the Auto-Pure96 System

Step	Name	Plate	Mix Time (Min)	Mix Amp (%)	Wait Time (Min)	Volume (µL)	Mixing speed	Temp (°C)	Segments	Cycle times	Magnet speed	Liq-lvl (sec)	Anti splash (sec)	1st (sec)	2nd (sec)	3rd (sec)	4th (sec)	5th (sec)
1	Load	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Lysis	2	10.0	80	0	500	3	60	0	1	-	5	5	-	-	-	-	-
3	Unload	1	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-

Table 3. Workflow script 'VIRSeek' for the extraction of RNA using the VIR*Seek* RNA*Extractor* AE1 with the Auto-Pure96 System excluding initial lysis step

Step	Name	Plate	Mix Time (Min)	Mix Amp (%)	Wait Time (Min)	Volume (µL)	Mixing speed	Temp (°C)	Segments	Cycle times	Magnet speed	Liq-lvl (sec)	Anti splash (sec)	1st (sec)	2nd (sec)	3rd (sec)	4th (sec)	5th (sec)
1	Load	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Bind	2	5.0	80	0	1000	9	OFF	5	2	2	5	5	2	2	2	2	2
3	Wash 1	3	0.7	80	0	900	9	OFF	5	4	1	5	5	5	5	5	5	5
4	Wash 2	4	0.7	80	0	1000	9	OFF	5	1	1	0	2	2	2	2	2	2
5	Wash 3	5	0.7	80	5	1000	9	OFF	5	1	1	0	2	2	2	2	2	2
6	Elution	8	5.0	80	0	100	2	60	2	1	1	0	2	30	30	0	0	0
7	Unload	4	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-

2.6.4 Sample Collection and Preparation of Samples

This protocol is designed for the isolation of viral RNA from environmental surface swabs and wastewater samples.

The environmental surfaces should be swabbed according to the WHO document “Surface sampling of coronavirus disease (COVID-19): A practical “how to” protocol for health care and public health professionals” (version 1.1). The guideline recommends the use of sterile swabs with synthetic tips and plastic shafts. In short, the swab is dipped into Phosphate Buffered Saline (PBS buffer) or sterile 0.9 % NaCl solution, and then a surface area of 5 x 5 cm is wiped with the moistened swab, while slowly twisting the swab. The swab is then placed back into the storage container until further processing (for more details refer to the respective sample collection proceedings).

For wastewater samples a sample pre-treatment step needs to be considered including a concentration of the virus particles e.g. by using PEG precipitation. Further information can be extracted in the ‘Wastewater Treatment Quick Reference Guide’ from Eurofins GeneScan Technologies upon request. As an outcome of such a pre-treatment step, a wastewater pellet is resuspended in 500 µL final lysis buffer and transferred into a deep-well plate (lysis plate).

2.7 Isolation of Viral RNA

2.7.1 Process Control Virus

For the assessment and control of the virus extraction procedure, the presence of a process control virus throughout the virus collection and the RNA isolation is recommended.

We recommend the use of the MS2 process/extraction control provided in VIR*Seek* SARS-CoV-2 Mplex I Wastewater or MNV process/extraction control virus provided in VIR*Seek* SARS-CoV-2 Mplex II Wastewater (see section 1.4).

Thereby we recommend adding either 20 µL of MS2 phage or 10 µL of a 1:100 diluted MNV into 500 µL of final lysis buffer when monitoring the extraction efficiency.

2.7.2 Preparation of Plates

Before running the extraction, several deep-well plates suitable for the used extraction platform have to be prepared according to the following instructions.

- Lysis plate:
  Processing of surface swabs:
  For each sample and control(s) pipet 500 µL freshly prepared final lysis buffer (see section 2.6.1) into the wells of a deep-well plate suitable for the used extraction platform.

  Processing of wastewater samples:
  Only pipet 500 µL freshly prepared final lysis buffer (see section 2.6.1) for the control(s) into the wells of a deep-well plate suitable for the used extraction platform. For the wastewater samples resuspend (PEG) pellet (see section 2.6.4) in 500 µL final lysis buffer and transfer it into the deep-well plate.

- Buffer plates:
  For each sample and control(s) prepare four separate deep-well plates suitable for the used automated extraction platform containing:

Plate	Buffer volume per deep well
Wash plate 1	900 µL WK5V - with Ethanol added!
Wash plate 2	1000 µL WK6V - with Ethanol added!
Wash plate 3	1000 µL WK6V - with Ethanol added!
Elution plate	100 µL HZ2V

Note: Make sure that the set-up/position of aliquoted buffers of all these plates are aligned with each other!

2.7.3 Sample Lysis and RNA Extraction for Moistened Swabs

1. Prepare the lysis buffer LK2V with carrier molecule NQ1V (see section 2.6.1).
2. Aliquot 500 µL of final lysis buffer into a suitable deep well plate (see section 1.3).
3. Stir the swab tip 3x in the respective well of the lysis plate to resuspend the virus particles, squeeze out the swab on the walls of the well or tube above the liquid level and discard the swab.
4. Optional: if an extraction control is used, it is recommended to add the extraction control at this stage to the deep wells (see section 2.7.1).
5. Place the lysis plate in a thermal mixer preheated to 60 °C and incubate for 10 minutes at medium speed (e.g. 350 rpm).
6. Prepare binding mixture containing absolute ethanol and magnetic beads NQ2V (see section 2.6.1).
7. Add 500 µL binding mixture to each well containing samples or control(s).
8. Place the deep well plates in the KingFisher™ Flex or Auto-Pure96 respectively and start the program (for KingFisher™ Flex see section 2.6.2 (*VIRSeek_V03*), for Auto-Pure96 see section 2.6.3, Table 3(*VIRSeek*)).
9. After the run, remove the plates, according to the protocol request.

Note : If sample lysis in the automated extraction instrument is desired, refer to the respective script (for KingFisher™ Flex see 2.6.2, Table 1, for Auto-Pure96 see 2.6.3, Table 2 ('VIRSeekLysis'). For KingFisher™ Flex load all prepared wash- and elution- plates and the lysis plate following steps 1-4 but without Ethanol and beads on the instrument and start the program 'VIRSeek_wLysis_V03'. The instrument will pause after the lysis (Table 1, Step 3) and prompt you to add 500 µL binding mixture to the lysate. For Auto-Pure96 only load the lysis plate following steps 1-4 but without Ethanol and beads on the instrument and start the program 'VIRSeekLysis' (see Table 2). After the program is finished, take out the lysis plate and add 500 µL binding mixture to the lysate. Then proceed with step 8.

Note: If the eluates are not analysed immediately, seal with adequate foil and store the elution plate at -20 °C for up to 6 months or at -80 °C for long term storage (up to 12 months).

2.7.4 Sample Lysis and RNA Extraction from wastewater pellets

1. Prepare the lysis buffer LK2V with carrier molecule NQ1V (see 2.6.1).
2. Resuspend pellet in 500 µL final lysis buffer and transfer lysate into a deep well plate. For the controls aliquot 500 µL final lysis buffer into the deep well plate see section 2.7.2.
3. Optional: if an extraction control is used, it is recommended to add the extraction control at this stage see section 2.7.1.
4. Place the lysis plate in a thermal mixer preheated to 60 °C and incubate for 10 minutes at medium speed (e.g. 350 rpm).
5. Prepare binding mixture containing absolute ethanol and magnetic beads NQ2V (see section 2.6.1).
6. Add 500 µL binding mixture to each well containing samples or control(s).
7. Place the deep well plates in the KingFisher™ Flex or Auto-Pure96 respectively and start the program (for KingFisher™ Flex see section 2.6.2 (VIRSeek_V03), for Auto-Pure96 see section 2.6.3, Table 3 (VIRSeek)).
8. After the run, remove the plates, according to the protocol request.

Note: If sample lysis in the automated extraction instrument is desired, refer to the respective script (for KingFisher™ Flex see 2.6.2, Table 1, for Auto-Pure96 see 2.6.3, Table 2 ('VIRSeekLysis'). For KingFisher™ Flex load all prepared wash- and elution- plates and the lysis plate following steps 1-3 but without Ethanol and beads on the instrument and start the program 'VIRSeek_wLysis_V03'. The instrument will pause after the lysis (Table 1, Step 3) and prompt you to add 500 µL binding mixture to the lysate. For Auto-Pure96 only load the lysis plate following steps 1-3 but without Ethanol and beads on the instrument and start the program 'VIRSeekLysis' (see Table 2). After the program is finished, take out the lysis plate and add 500 µL binding mixture to the lysate. Then proceed with step 7.

Note: If the eluates are not analysed immediately, seal with adequate foil and store the elution plate at -20 °C for up to 6 months or at -80 °C for long term storage (up to 12 months).

3 TROUBLESHOOTING

Observation:

A low or no signal due to low purity / inhibition were observed during real-time RT-PCR

Possible Cause	Solution
Ethanol was not added to the lysate	Make sure that ethanol was added to the lysate after the lysis step.
Ethanol was not added to WK5V and WK6V	Add absolute ethanol to WK5V and WK6V according the instructions in section 2.6.1.
NQ1V was not added to LK2V	Carrier molecule NQ1V facilitates binding of viral RNA to the magnetic beads NQ2V. Ensure that carrier molecule NQ1V was added to lysis buffer LK2V according the instructions in section 2.6.1. Lack of carrier molecule NQ1V can result in reduced RNA yield.
Insufficient resuspension of NQ2V	Resuspend magnetic beads NQ2V thoroughly before pipetting.
Inappropriate storage of kit components	Kit components should be stored under appropriate conditions (see section 1.2) to ensure successful RNA isolation. Make sure that reagent bottles are stored tightly closed after addition of ethanol (see section 2.6.1).
Isolated RNA is degraded due to RNase contamination	To prevent RNase contamination, wear a lab coat and suitable gloves, which should be changed frequently during the procedure. Only use RNase-free pipette tips. Decontaminate work surfaces and non-disposable laboratory equipment (pipettes, vortex mixer) with appropriate reagents before starting.
RNA degradation during sample storage	Sample processing should be done immediately after collection/preparation. Otherwise ensure that samples are stored suitably.
Isolated RNA is degraded due to freeze-thaw cycles	Minimize freeze-thaw cycles of the samples to avoid RNA degradation.
Inhibitors from the sample material are still present in RNA eluate	For some matrices inhibition cannot be overcome during RNA isolation. In these cases dilute sample RNA eluate 1:2 in RNase free water or elution buffer and repeat real-time RT-PCR.

4 PRODUCT WARRANTIES, SATISFACTION GUARANTEE

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5 IMPORTANT NOTES

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TECHNICAL SUPPORT SERVICE

For technical assistance and more information please reach out to your local sales support as stated on the Eurofins Technologies website or reach out to your local distributor.

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