VIR*Seek* Food Testing Products Available:

- **VIR*Seek* Food Hepatitis A Virus, cat. No. 5728200101**, Real-time RT-PCR kit with 48 reactions for rapid detection of hepatitis A virus (HAV) in food samples
- **VIR*Seek* Food Norovirus Genogroup I, cat. No. 5728200201**, Real-time RT-PCR kit with 48 reactions for rapid detection of norovirus genogroup I (NoVGI) in food samples
- **VIR*Seek* Food Norovirus Genogroup II, cat. No. 5728200301**, Real-time RT-PCR kit with 48 reactions for rapid detection of norovirus genogroup II (NoVGII) in food samples
- **VIR*Seek* Food Murine Norovirus (MNV) Process Control, cat. No. 5728200401**, Murine norovirus spiking material and Real-time RT-PCR kit with 48 reactions for rapid detection of murine norovirus (MNV) process control virus in food samples
- **VIR*Seek* RNA*Extractor* Food, cat. No. 5524400101**, Kit for extraction of viral RNA via silica-coated magnetic beads from different food products (incl. soft fruits, leaf, stem, and bulb vegetables, bottled water and bivalve molluscan shellfish). 48 x 500 µL or 96 X 250 µL sample input volume. All reagents provided.

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VIR*Seek* RNA*Extractor* Kit

Test Kit for Isolation of Viral RNA from Food Products

Cat. No. 5524400101

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1 INTRODUCTION

The VIR*Seek* RNA*Extractor* kit is designed for the isolation of high-quality RNA from viral particles which have been concentrated from food products, including soft fruits, leaf, stem and bulb vegetables, bottled water, bivalve molluscan shellfish, food surfaces and environmental surfaces. Its components are optimized for RNA extraction and removal of potential inhibitors from these sample materials, but the kit may also be used for other sample matrices.

Viral particles present in the samples are ruptured in the first step of the RNA isolation protocol. The RNA isolation workflow is based on reversible adsorption of RNA to the surface of silica-coated magnetic beads under adjusted buffer conditions without the need of laborious and time-consuming centrifugation steps.

The virus concentration and extraction protocol is based on the protocol recommended by the ISO 15216-1:2017.

The kit is intended to be used by analytical laboratories for quality control/ quality assurance testing, food and agricultural testing and research purposes only (e.g. virological monitoring of production processes).

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

1.1 Test Principle

Food samples are collected and processed to extract and concentrate virus particles by applying the recommended protocol according to ISO 15216-1:2017 for the specific sample types. A process control virus, e.g. VIR*Seek* Murine Norovirus (MNV) Process Control, is added during this procedure as a process control for the extraction process.

The VIR*Seek* RNA*Extractor* kit uses the following steps to extract the RNA from the viral particles. Following the chaotropic lysis rupturing the biological compartments contained in the sample, all nucleic acids are free in the solution and bind to silica coated magnetic beads, subsequently added to the lysate.

To yield high quality, ultrapure RNA for subsequent analyses, the nucleic acids adsorbed to the magnetic beads are washed in multiple steps with different washing buffers in order to remove inhibitors. The now purified nucleic acids from the sample (including the viral RNA) are eluted in low-salt elution buffer.

The protocol is optimized for manual purification using magnetic racks. For evaluation of automatization protocols and instruments please contact the Eurofins GeneScan Technologies GmbH customer service (kits@eurofins.com).

The purified RNA can be analyzed immediately by real-time RT-PCR, or may be stored at -20°C (≤6 months) or at -80 °C (≤12 months).

The RNA isolation procedure is compliant with ISO 15216-1:2017 and it can be used downstream of ISO 15216 methods for extraction and concentration of virus particles.

1.2 Components of the Kit

Depending on the sample input the VIR*Seek* RNA*Extractor* kit is sufficient for RNA isolation from 96 (250 µL or surface) or 48 (500 µL) samples.

- 1x Magnetic beads, 1 mL, store at 2°C - 8°C (**Note**: Do not freeze!).
- 1x Carrier Molecule, >600 µL, store at 2°C - 8°C.
- 3x Lysis buffer (LB), 28 mL, store at room temperature.
- 2x WB1, Wash buffer 1, concentrate, 25 mL, store at room temperature.
- 2x WB2, Wash buffer 2, concentrate, 16.5 mL, store at room temperature.
- 2x WB3, Wash buffer 3, concentrate, 11 mL, store at room temperature.
- 6x Elution buffer, 2 mL, store at room temperature.

Note: Shelf life is indicated on the labels of the kit / components.

1.3 Additional Equipment, Consumables and Reagents Required

Equipment:

- 1x Magnetic Bead Holder (e.g. DynaMag™-2 Magnet, Thermo Fisher Scientific, cat. no.12321D).
- 1x Thermo shaker, for 1.5 mL and 2 mL tubes, cooling / heating capable (e.g. Thermal Shake lite, VWR; cat. no. 460-0249P2).
- 1x Vortex mixer (e.g. VWR Collection, cat. no. 444-2790).
- 1x Single channel pipette (1 mL, 100 µL), (e.g. Transferpette® S 100 - 1000 µL (Brand®), Eurofins GeneScan Technologies GmbH, cat. no. 5617703301).
- 1x Single channel pipette (100 µL, 10 µL), (e.g. Transferpette® S 10 - 100 µL (Brand®), Eurofins GeneScan Technologies GmbH, cat. no. 5617703201).
- 1x Single channel pipette (up to 10 µL), (e.g. Transferpette® S, 0.5 - 10 µL (Brand®), Eurofins GeneScan Technologies GmbH, cat. no. 5617703101).
- Optional: Vacuum pump based aspirator (e.g. BioSan FTA-2i, aspirator with trap flask, 181-3282 VWR).

Consumables:

- Absolute ethanol (≥96 %, p.a.), RNase-free.
- Water, molecular biology grade, RNase-free.
- Reaction tubes, 1.5 mL, RNase-free (e.g. DNA LoBind Tubes, Eppendorf, cat. no. 0030108051).
- Reaction tubes, 2 mL, RNase-free (e.g. DNA LoBind Tubes, Eppendorf, cat. no. 0030108078).
- Pipette tips, RNase-free, check compatibility with pipettes utilized.
- Tubes, RNase-free (for preparation of carrier RN / lysis buffer- mixture), check for compatibility with desired sample volume (section 3.2).
- Decontaminating agent (e.g. RNase Away®, Carl Roth GmbH, cat. no. A998).
- Roti® Nucleic Acid-free (Carl Roth GmbH, cat. no. HP69).
- Gloves, powder free.
- Required if according to ISO 15216:. Process Control Virus

2 HOW TO USE THIS PRODUCT

Important Notes

- Store all reagents as indicated in section 1.2.
- Please make sure to store the magnetic beads 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.
- Extraction of viruses from different food matrices should be performed according to the ISO 15216-1:2017 protocol.

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 analyzed 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* 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 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. RNase's 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 laid out 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 color 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:

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

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

Possible Cause	Solution
Inhibition of real-time RT-PCR due to remaining ethanol	Ensure that beads are dried after last washing step prior to elution of RNA from the beads. Increase bead drying time, if needed.
Inhibition of real-time RT-PCR due to remaining salts from the wash buffer	Ensure that no precipitates are present in the wash buffer, store wash buffer at room temperature and dissolve precipitates before usage if needed.
Inhibitors from the sample material are still present in RNA eluate	In order to avoid carry-over of inhibitors, make sure to thoroughly aspirate supernatants after each washing step to minimize carry-over of inhibitors or salts during the RNA isolation procedure. For some matrices inhibition cannot be overcome during RNA isolation. In these cases dilute sample RNA eluate 1:10 in RNase free water or elution buffer and repeat real-time RT- PCR.
Insufficient washing of magnetic beads	Ensure that magnetic beads are completely resuspended during the washing steps.

Observation: Carry-over of magnetic beads into the RNA eluate

Possible Cause	Solution
Carry-over of magnetic beads may be caused by too high aspiration speed or incomplete capture during the elution step.	Prolong separation time and reduce aspiration speed, respectively. If RNA eluate contains high amounts of magnetic beads, place tube containing RNA eluate on magnetic rack and transfer eluted RNA to a new tube.

Observation: Clumping of magnetic beads

Possible Cause	Solution
Biological components from certain sample materials may cause serious problems like clumping beads during RNA isolation.	For inhomogeneous samples (lumpy consistence, e.g. from dark lettuce / leafy greens or berries) we recommend an additional chloroform / butanol purification step after the sample preparation protocol as described in the ISO 15216 for soft fruits.

Observation: No binding of the magnetic beads on the magnetic rack

Possible Cause	Solution
When ethanol is added to a high concentrated polysaccharide solution in the RNA-binding step, the formation of a gel can be observed, especially in samples prepared from soft fruits.	To ensure an adequate enzymatic cleavage of the polysaccharide structure, we recommend using the pectinase from Aspergillus aculeatus (≥3000 U) when performing the protocol of ISO 15216.

4 TROUBLESHOOTING

Observation: Low yield of isolated RNA

Possible Cause	Solution
Ethanol was not added to the lysate	Make sure that ethanol was added to the lysate during the RNA binding step.
Ethanol was not added to WB1, WB2 and / or WB3	Add absolute ethanol to WB1, 2, and 3 according the instructions in section 3.2.
Carrier RNA was not added to lysis buffer	Carrier RNA facilitates binding of viral RNA to the magnetic beads. Ensure that carrier RNA was added to lysis buffer according the instructions in section 3.2. Lack of Carrier RNA can result in reduced RNA yield.
Carrier RNA went through too many freeze-thaw cycles	Minimize freeze-thaw cycles of carrier RNA to avoid its degradation. To minimize the number of cycles aliquot dissolved carrier RNA and store at -20 °C (as described in section 3.2).
Insufficient resuspension of magnetic beads	Resuspend magnetic beads thoroughly by vortexing 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 3.2).
Loss of magnetic beads during procedure	To avoid aspirating the magnetic beads when removing supernatant from captured beads wait until magnetic bead capture is completed. This may take several minutes and should be checked visually. When working with an aspirator, ensure that aspiration speed is not too high.
Magnetic beads were dried too long	Do not let beads over-dry as this makes elution of RNA from beads more difficult.
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.

3 VIRAL RNA ISOLATION PROTOCOL

The VIR*Seek* RNA*Extractor* standard protocol is suitable for a maximum sample volume of 250 µL per individual lysis tube (see section 3.4). For 500 µL sample volume (compliant to ISO 15216-1:2017) use two tubes each containing 250 µL for lysis and merge the magnetic beads / RNA complexes in the first wash step, as described in the protocol (see section 3.5).

3.1 Sample Collection and Preparation of Food Samples

For virus isolation / concentration from food matrices, we recommend the preparation of food samples according to ISO 15216-1:2017 protocols.

Please note that frozen fruits should be defrosted prior to performing ISO 152115-1:2017 protocols. Furthermore, the TGBE buffer should be at room temperature.

Note: To avoid the formation of a gel during the preparation of food samples containing high amounts of polysaccharides (e.g. soft fruits like strawberries), we recommend using pectinase from *Aspergillus aculeatus* (≥3000 U).

Some sample matrices such as dark green salads can cause magnetic bead clumping. In this case we recommend an additional chloroform / butanol purification step after the sample preparation protocol as described in ISO 15216-1:2017 for soft fruits.

3.2 Preparation of Reagents

Before using the kit the first time, prepare wash buffers WB1 - 3 (each provided as concentrate)

Wash buffer 1 (WB1)

a. Add 25 mL absolute ethanol (≥96 %, p.a., RNase-free) to the bottle containing wash buffer WB1 concentrate.
b. Mix well by shaking / swirling / agitating.
c. Mark WB1 bottle after addition of ethanol.
d. Store tightly closed reagent bottle (to prevent evaporation of ethanol) at room temperature.
Note: The wash buffer concentrate may contain precipitates of salts. After adding ethanol the salt will dissolve**.**

Wash buffer 2 (WB2)

a. Add 38.5 mL absolute ethanol (≥96 %, p.a., RNase-free) to the bottle containing wash buffer WB2 concentrate.
b. Mix well by shaking / swirling / agitating.
c. Mark WB2 bottle after addition of ethanol.
d. Store tightly closed reagent bottle (to prevent evaporation of ethanol) at room temperature.

Wash buffer WB3

a. Add 44 mL absolute ethanol (≥96 %, p.a., RNase-free) to the bottle containing wash buffer WB3 concentrate.
b. Mix well by shaking / swirling / agitating.
c. Mark WB3 bottle after addition of ethanol.
d. Store tightly closed reagent bottle (to prevent evaporation of ethanol) at room temperature

Addition of Carrier Molecule to Lysis buffer LB

Final lysis buffer (containing Carrier Molecule) should be prepared fresh. Calculate the volume of lysis buffer (LB) and Carrier Molecule according to the following formulas. Mix calculated volumes of Carrier Molecule and LB in an RNase-free tube.

Calculation for "Protocol 250" (see 3.4):

$V_L = n \times 750\ \mu L$
$V_C = n \times 5\ \mu L$

n: number of samples to be processed
V_L: volume of lysis buffer to be added; in µL
V_C: volume of Carrier Molecule-solution to be added; in µL

Calculation for "Protocol 500" (see 3.5):

$V_L = n \times 1500\ \mu L$
$V_C = n \times 10\ \mu L$

n: number of samples to be processed
V_L: volume of lysis buffer to be added; in µL
V_C: volume of Carrier Molecule-solution to be added; in µL

3.3 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 required according to ISO 15216. Please refer to the ISO 15216 for general instructions on how to use a process control. We recommend the use of the murine norovirus as process control virus and the corresponding Eurofins GeneScan Technologies GmbH real-time RT-PCR kit (VIR*Seek* Murine Norovirus (MNV) Process Control, cat. no. 5728200401).

3.4 Protocol 250: Isolation of Viral RNA from 250 µL Samples with the VIR*Seek* RNA*Extractor* Kit

This protocol is for the isolation of viral RNA from samples with a maximum volume of 250 µL per lysis tube. The VIR*Seek* RNA*Extractor* protocol can be completed at room temperature. All aspiration steps can be performed with a pipette or an aspirator with an appropriate aspiration speed.

Before starting

- If working with the VIR*Seek* RNA*Extractor* kit for the first time, read "Working Guidelines" (see section 2.2).
- 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).
- Add Carrier Molecule to lysis buffer according to section 3.2.
- Prepare wash buffers WB1 - 3 according to section 3.2.
- Heat up thermo shaker to 50°C (use block module for 2 mL reaction tubes) in time.
- Viral particles have to be extracted and concentrated from the sample for RNA extraction.

1. Sample lysis

a. Pipette 750 µL freshly prepared lysis buffer containing Carrier Molecule (see section 3.2) into a 2 mL RNase-free reaction tube.
b. Transfer up to 250 µL well-mixed sample into the reaction tube containing final LB and mix by pipetting up and down.
c. Incubate tube for 10 min on thermo shaker (block module for 2.0 mL reaction tubes) (300 rpm / 50 °C).

2. RNA binding

a. Cool down thermo shaker to room temperature (block module for 2.0 mL reaction tubes) in time.
b. Add 1 volume ethanol (≥96 %, p.a., RNase-free) to the sample tube.
c. Vortex magnetic bead suspension to completely re-suspend beads before pipetting.
d. Add 10 µL magnetic bead suspension to tube and re-suspend beads by pipetting up and down (8 - 10 x).
e. Incubate tube for 10 min on thermo shaker (800 rpm / RT).

3. 1st wash step

a. Place tube on magnetic rack to bind magnetic beads. Leave tube on rack until the mixture becomes clear (1 - 2 min).
b. After complete capture of the beads, aspirate and discard supernatant.
c. Remove tube from the magnetic rack.
d. Add 900 µL prepared WB1 (see section 3.2).
e. Wash beads by repeatedly pipetting up and down (8 - 10 x).

4. 2nd wash step

a. Place tube on magnetic rack to bind magnetic beads. Leave tube on rack until the mixture becomes clear (1 - 2 min).
b. After complete capture of the beads, aspirate and discard supernatant.
c. Remove tube from the magnetic rack.
d. Add 1000 µL of prepared WB2 (see section 3.2).
e. Wash beads by repeatedly pipetting up and down (8 - 10 x).
f. Transfer suspension into a new 1.5 mL RNase-free tube.

5. 3rd wash step

a. Place tube on magnetic rack to bind magnetic beads. Leave tube on magnetic rack until the mixture becomes clear (1 - 2 min).
b. After complete capture of the beads, aspirate and discard supernatant.
c. Remove tube from the magnetic rack.
d. Add 1000 µL prepared WB3 (see section 3.2).
e. Wash beads by repeatedly pipetting up and down (8 - 10 x) and immediately place tube on magnetic rack to bind magnetic beads.

Note: To facilitate magnetic bead drying, place the tubes on the magnetic rack immediately after washing. This will prevent aggregation of the magnetic beads on the bottom o the tube.

6. Dry beads

a. Heat up thermo shaker to 40°C (block module for 1.5 mL reaction tubes) in time.
b. Leave tube on magnetic rack until the mixture becomes clear (1 - 2 min).
c. After complete capture of the beads, aspirate and discard supernatant.
d. Check tube for any remaining liquid. If there is a notable volume, use an appropriate pipette to remove it.
e. Let beads dry on magnetic rack (with open cap) at room temperature for 5 min. Then dry for another 5 min at 40°C on thermo block to remove remaining ethanol.

Caution: Do not let beads over-dry!

f. Ensure that no liquid remains in the tube and beads are dried. If there is a notable volume or beads are still wet, use a fine-tipped pipette to remove any liquid and / or prolong bead drying time on thermo block.

Note: Complete removal of ethanol is very important for successful downstream applications (e.g. real-time RT-PCR, where ethanol acts as an inhibitor).

7. RNA elution

a. Heat up thermo shaker (block module for 1.5 mL reaction tubes) in time.
b. Add 100 µL pre-warmed (60°C) water or elution buffer to tube and re-suspend beads by repeatedly pipetting until beads are in solution.
c. Incubate tube for 5 min on thermo shaker (1300 rpm, 60 °C).
d. Place tube on magnetic rack to bind magnetic beads. Leave tube on magnetic rack until the mixture becomes clear.
e. Transfer the supernatant, which contains the viral RNA, to a new RNase-free reaction tube.
f. Use directly or store isolated RNA at -20 °C (<6 months) or -80 °C (<12 months), respectively.

3.5 Protocol 500: Isolation of Viral RNA from 500 µL Samples with the VIRSeek RNAExtractor Kit

This protocol is for the isolation of viral RNA from samples with a maximum sample volume of 500 µL.

Note: Use two reaction tubes, each containing 250 µL sample, for lysis and merge the beads in the first wash step, as described in the following protocol.

VIR*Seek* RNA extraction protocol can be completed at room temperature. All aspiration steps can be performed with a pipette or an aspirator with an appropriate aspiration speed.

Before starting

- If working with the VIR*Seek* RNA*Extractor* kit for the first time, read "Working Guidelines" (see section 2.2).
- 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).
- Add Carrier Molecule to lysis buffer according to section 3.2.
- Prepare wash buffers WB1 - 3 according to section 3.2.
- Heat up thermo shaker to 50°C (use block module for 2.0 mL reaction tubes) in time.

1. Sample lysis

a. Pipette 750 µL prepared final lysis buffer containing Carrier Molecule (see section 3.2) into two 2 mL RNase-free tubes (A and B).
b. Transfer 250 µL well-mixed sample into each of the two tubes containing final LB and mix by pipetting up and down.
c. Incubate tubes for 10 min on thermo shaker (300 rpm / 50 °C).

2. RNA binding

a. Cool down thermo shaker to room temperature (block module for 2.0 mL reaction tubes) in time.
b. Add 1 volume ethanol (≥96 %, p.a., RNase-free) to the sample tubes.
c. Vortex magnetic bead suspension to completely re-suspend beads before pipetting.
d. Add 10 µL magnetic bead suspension to tubes and re-suspend beads by pipetting up and down (8 - 10 x). e. Incubate tubes for 10 minutes on thermo shaker (800 rpm / RT)

3. 1st wash step

a. Place tubes on magnetic rack to bind magnetic beads. Leave tubes on magnetic rack until the mixture becomes clear (1 - 2 min).
b. After complete capture of the beads, aspirate and discard supernatant.
c. Remove tubes from the magnetic rack.
d. Add 900 µL prepared wash buffer 1 (see section 3.2).
e. Wash beads by repeatedly pipetting up and down (8 - 10 x).
f. Transfer suspension from tube B into tube A and mix by pipetting.

The remaining washing and drying steps as well as the RNA elution should be performed as described in section 3.4 starting with "4. 2nd wash step".

3.6 Protocol Food Surface: Isolation of Viral RNA from Surface Samples with the VIR*Seek* RNA*Extractor* Kit

This protocol is for the isolation of viral RNA from food surface samples (e.g. sweet pepper, tomato, windfalls, grapes) and environmental surface samples. VIRSeek RNA extraction protocol can be completed at room temperature. All aspiration steps can be performed with a pipette or an aspirator with an appropriate aspiration speed.

Before starting

- If working with the VIRSeek RNAExtractor kit for the first time, read "Working Guidelines" (see section 2.2).
- 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).
- Add Carrier Molecule to lysis buffer according to section 3.2.
- Prepare wash buffers WB1 - 3 according to section 3.2.
- Heat up thermo shaker to 50 °C (use block module for 2.0 mL reaction tubes) in time

1. Sample lysis

a. Pipet 750 µL prepared LB containing Carrier Molecule (see section 3.2) into a 2 mL RNase-free tube.
b. Dip swab in the lysis buffer, press against tube wall to release liquid (according to ISO 15216-1:2017).
c. Repeat step b. 3 - 5 times.
d. Incubate tube for 10 min on thermo shaker (300 rpm / 50 °C).

The remaining washing and drying steps as well as the RNA elution should be performed as described in section 3.4. Starting with "2. RNA binding".