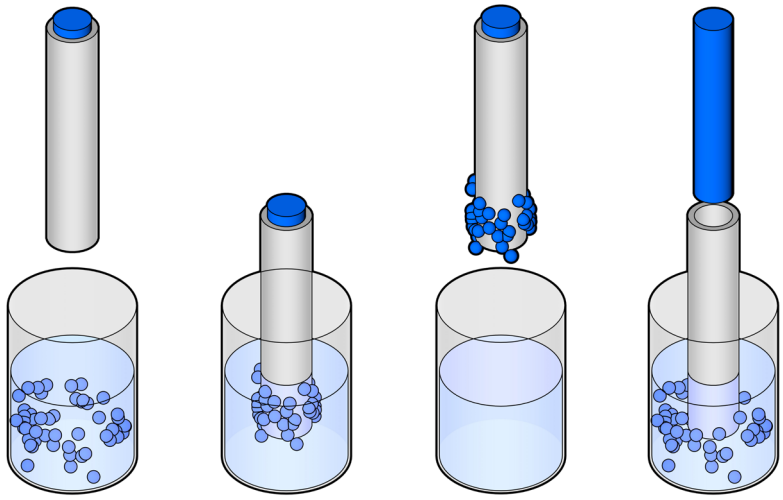


foodproof®

Magnetic Preparation Kit VI

Automated viral RNA/DNA extraction

PRODUCT INSTRUCTIONS

Documentation for the automated isolation of viral RNA and DNA from samples like serum, plasma, feces and swab samples for direct use in PCR.

Product No. KIT230190

foodproof®

Magnetic Preparation Kit VI

Automated viral
RNA/DNA extraction

Store Box A at 15 to 25 °C
Store Box B at -15 to -25 °C

FOR *IN VITRO* USE AND
RESEARCH USE ONLY

Product No. KIT230190
480 reactions

Product Instructions:
Revision A, January 2024

TABLE OF CONTENTS

1. OVERVIEW

The foodproof® Magnetic Preparation Kit VI is designed for the rapid and safe high-throughput extraction of viral RNA/DNA from up to 200 µL sample material, e.g. serum, plasma, feces and swab samples. After preparing and loading the plates into the foodproof RoboPrep® 32, the foodproof RoboPrep® 96 or the KingFisher™ Flex, all lysis and purification steps are performed automatically without the need for external lysis or centrifugation steps. The kit uses Proteinase K, chaotropic salts and carrier-tRNA for the lysis, and magnetic beads for the washing and purification steps, resulting in a high yield of highly purified RNA/DNA.

1.1. General Information

Number of Reactions

The kit is designed for 480 reactions.

Storage Conditions

Store Box A at 15 to 25 °C
Store Box B at -15 to -25 °C

The components of the foodproof Magnetic Preparation Kit VI are guaranteed to be stable through the expiration date printed on the label.

Improper storage will adversely impact RNA/DNA purification if precipitates form in the solutions.

After Proteinase K has been dissolved, the solution should be aliquoted and stored at -15 to -25 °C. The solution is stable at -15 to -25 °C for 12 months.

1.2. Applicability

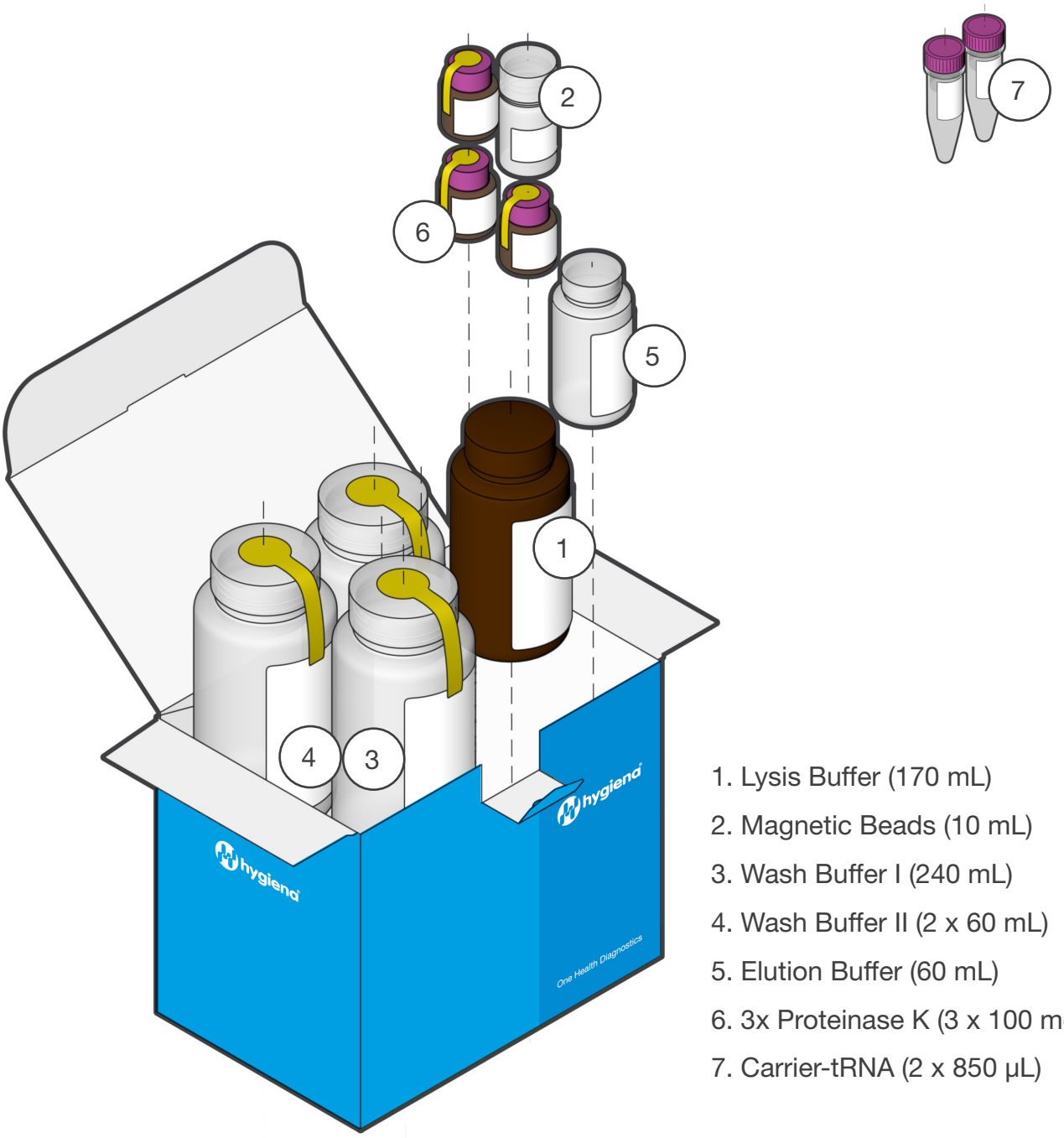
The foodproof Magnetic Preparation Kit VI is optimized for the isolation of RNA and DNA for a wide range of samples. The extracted RNA/DNA is highly purified and suitable for qualitative and quantiative applications using any PCR system.

1.3. Kit Contents

This a schematic representation of the foodproof Magnetic Preparation Kit VI with all its components. All solutions except for the magnetic beads, are clear, and should not be used if precipitates form. If this happens, simply warm up solutions to 15 to 25 °C or in a 37 °C water bath until the precipitates have dissolved.

BOX A

BOX B



1. Lysis Buffer (170 mL)
2. Magnetic Beads (10 mL)
3. Wash Buffer I (240 mL)
4. Wash Buffer II (2 x 60 mL)
5. Elution Buffer (60 mL)
6. 3x Proteinase K (3 x 100 mg)
7. Carrier-tRNA (2 x 850 µL)

2. INSTRUCTIONS

This section provides all information for a seamless extraction from a variety of matrices.

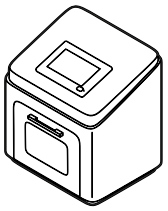
2.1. Required Material

Most of the required equipment and reagents are available through Hygiena® Diagnostics. Please contact us for further information.

It is highly recommended only to use the materials described below to guarantee the robustness of the method.

Equipment

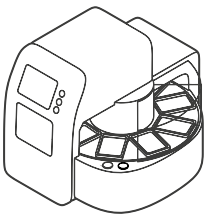
☐ **RoboPrep® 32,** capable of 32 samples per run
Product No. MCH230000



Only for automated extraction procedure 2.3.1.

OR

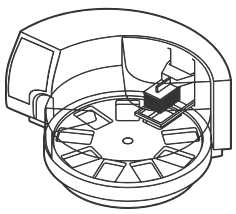
☐ **RoboPrep® 96,** capable of 96 samples per run
Product No. MCH230001



Only for automated extraction procedure 2.3.2.

OR

☐ **KingFisher™ Flex,** capable of 96 samples per run



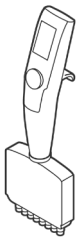
Only for automated extraction procedure 2.3.3.

Recommended:

☐ **Multichannel pipette** and filter tips

e.g. 8-Channel Pipette VIAFLO - INTEGRA Biosciences
with
GripTips: 50 to 1,250 µL

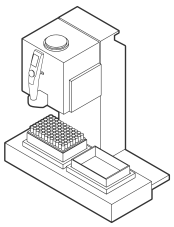
or EP Xplorer Plus Electronic Multichannel Pipette
with
Filter tips: 50 to 1,250 µL



Optional (for high- to very high-throughput demands)**:**

☐ **Benchtop pipetting system** and deep well tips

VIAFLO 96 base unit,
96-channel pipetting head, 50-1,250 µL, spring-loaded plate holder A & B for 96 well plates - all INTEGRA Biosciences; with
GripTips in racks: 50 to 1,250 µL

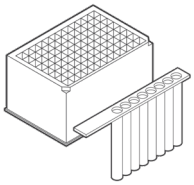


Consumables

☐ **MPK VI Consumable Pack for RoboPrep® 32**, 192 reactions

12x deep well plates (2 mL),
24x tip comb

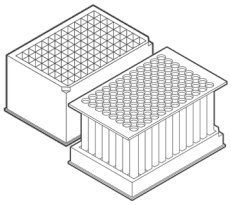
Only for automated extraction procedure 2.3.1.



☐ **MPK VI Consumables for RoboPrep® 96**, 480 reactions

20x deep well plates (2 mL),
10x elution plate (0.2 mL),
5x tip comb,
10x sealing foil (for 96 microplates)

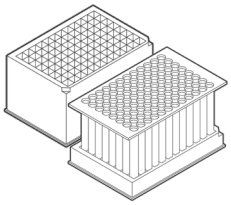
Only for automated extraction procedure 2.3.2.



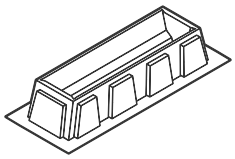
☐ **MPK VI Consumables for KingFisher™ Flex**, 480 reactions

20x deep well plates (2 mL),
10x elution plate (0.2 mL),
5x tip comb,
10x sealing foil (for 96 microplates)

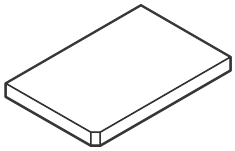
Only for automated extraction procedure 2.3.3.



☐ Sterile **reservoir**, 100 mL
(20 units per bag, 100 units per box)

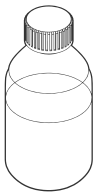


☐ **Plate cover** (50 units per box)

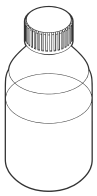


Reagents

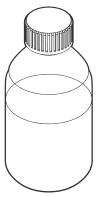
☐ **Ethanol, absolute** (96 - 98 %)



☐ **Isopropanol, absolute** (96 - 98 %)



☐ **Water, double-distilled**

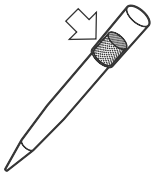


2.2. Precautions and Preparations

Follow all universal safety precautions governing work with biohazardous materials, e.g., wear lab coats and gloves at all times. Properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

For more information, please refer to the appropriate safety data sheet (SDS). The SDS is available online at www.hygiena.com/sds.

☐ Always use filter tips in order to avoid cross-contamination.



☐ Prepare **Proteinase K** before using it the first time (calculate the required amount of bottles). Dissolve Proteinase K in 5 mL double-distilled water, aliquot solution.

Store aliquots at –15 to –25 °C, stable for 12 months.

☐ Prepare **Wash Buffer I** before using it the first time.

Add 240 mL absolute ethanol, mix well, and store at 15 to 25 °C.

Label and date bottle after ethanol is added and tick off the corresponding box on the label.

☐ Prepare **Wash Buffer II** before using it the first time.

Add 420 mL absolute ethanol, mix well, and store at 15 to 25 °C.

Label and date bottle after ethanol is added and tick off the corresponding box on the label.

2.3. Workflows

The following procedures describe the automated RNA/DNA extraction from virus with the foodproof Magnetic Preparation Kit VI in combination with our two extraction devices: The RoboPrep 32 for low to medium throughput (32 samples per run) and the RoboPrep 96 or the KingFisher Flex for medium to high throughput (96 samples per run).

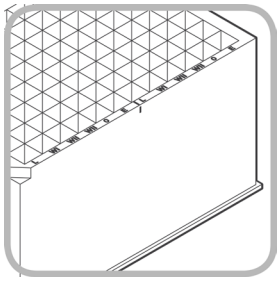
After preparing and loading the plates into the devices, no further manual steps are necessary. A complete run takes approximately 45 minutes.

2.3.1. RoboPrep 32

This protocol describes the RNA/DNA extraction from up to 200 µL sample using the foodproof RoboPrep 32. It consists of two parts: The manual preparation of plates and samples and the automated extraction run.

Please make sure that you have prepared all required reagents (see 2.2. Precautions and Preparations).

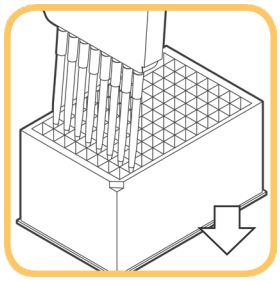
For a 16-sample run you will need the following consumables: 1 deep well plate, 2 tip combs.
The RoboPrep 32 processes two deep well plates simultaneously (a total of 32 samples).



1. MARK PLATE ROWS

Label the rows of the deep well plate for the individual buffers to prevent pipetting errors when preparing the deep well plate.

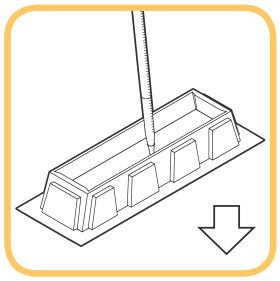
Note: As a naming system you can use the following buffer abbreviations: L: Lysis mix, WI: Wash Buffer I, WII: Wash Buffer II, 0: No buffer, E: Elution Buffer.



2. ADD BUFFER TO RESPECTIVE ROWS

Row 2 (and 8): Add **800 µL Wash Buffer I** to each well.
Row 3 (and 9): Add **800 µL Wash Buffer II** to each well.
Row 4 (and 10): Add **800 µL Wash Buffer II** to each well.
Row 5 (and 11): Remains empty.
Row 6 (and 12): Add **100 µL Elution Buffer** to each well.

Note: We recommend using multichannel pipettes to speed up liquid handling.



3. PREPARE LYSIS MIX

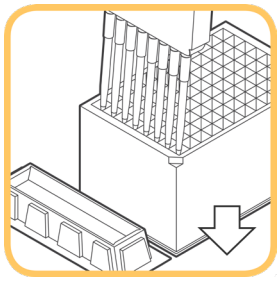
Calculate volume of the lysis mix components (see table below) and add them to a reservoir.

Note: Before pipetting, take care that the magnetic beads are homogenously distributed. To achieve this, vortex bottle with magnetic beads for 5 seconds immediately before adding them to the remaining reagents.

Table: Volumes of the required reagents for the lysis plate are dependent on the sample volume. Please take note of the different measurement units (µL or mL). An excess is already included in the columns for 8 or more samples to facilitate pipetting.

	1 Sample	8 Samples	16 Samples	24 Samples	32 Samples
Lysis Buffer	300 µL	3 mL	5.4 mL	7.8 mL	10.8 mL
Isopropanol	400 µL	4 mL	7.2 mL	10.4 mL	13.6 mL
Proteinase K	25 µL	250 µL	450 µL	650 µL	850 µL
Magnetic Beads*	20 µL	200 µL	360 µL	520 µL	680 µL
Carrier - tRNA	3 µL	30 µL	54 µL	78 µL	102 µL

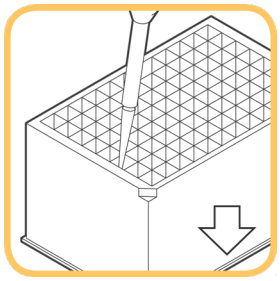
* Vortex for 5 seconds immediately before use.



4. ADD LYSIS MIX TO PLATE

Add **748 µL lysis mix** from the reservoir to row 1 (and 7) of the deep well plate.

Note: Immediately before transferring the lysis mix, carefully rock reservoir and pipet up and down simultaneously to distribute the magnetic beads evenly in the solution.



5. ADD SAMPLES TO LYSIS MIX

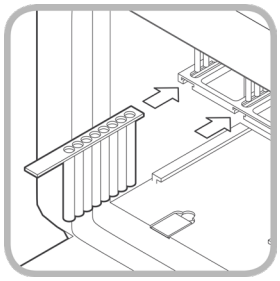
Add **up to 200 µL** sample to lysis mix in row 1 (and 7) of the deep well plate.

Note: Avoid cross-contamination by NOT pipetting up and down during this step.

6. SELECT PROGRAM

Start the RoboPrep 32 instrument.

Select the pre-installed program '**MPK VI**' via touchscreen.

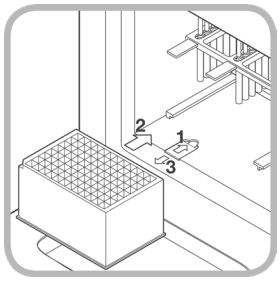


7. INSTALL TIP COMBS

Open the front door.

Insert tip combs.

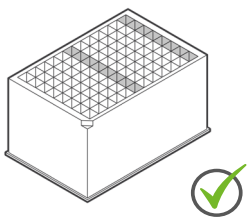
Note: Number of tip combs (tc) depends on number of samples that are processed:
samples ≤ 8: 1 tc, samples ≤ 16: 2 tc, samples ≤ 24: 3 tc, samples ≤ 32: 4 tc.



8. INSERT PLATE(S) AND START RUN

To enter the deep well plate, lower the heating block by pushing the lever backwards (1), slide in the plate (2) and push the lever in the opposite direction to raise the heating block and to fix the plate (3).

Start the run. All the extraction steps will run automatically.



READY FOR DETECTION

Row 6 (and 12) of the plate contain the extracted viral RNA/DNA from the samples.

RECOMMENDED: Use eluted RNA/DNA right after extraction. It is recommended to analyse a 1:10 dilution from feces samples, because the eluate may still contain PCR-inhibiting substances.

For later analysis, transfer extracts to tubes and store at -15 to -25 °C. For long-term storage, keep at -80 °C. After thawing, mix briefly by vortexing and centrifuge at high speed for 1 min.

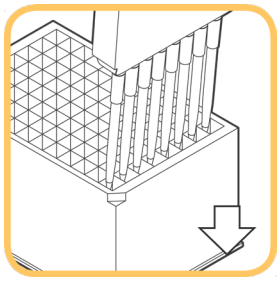
Note: If the elution plate still visibly contains magnetic beads, you may centrifuge it to avoid interference with the detection kit (1 min at high speed). The RNA/DNA is in supernatant.

2.3.2. RoboPrep 96

This protocol describes the RNA/DNA extraction from up to 200 µL sample using the RoboPrep 96. It consists of two parts: The manual preparation of plates and samples and the automated extraction run.

Please make sure that you have prepared all required reagents (see 2.2. Precautions and Preparations).

For a 96-sample run you will need the following consumables:
4 deep well plates, 2 elution plates, 1 tip comb.

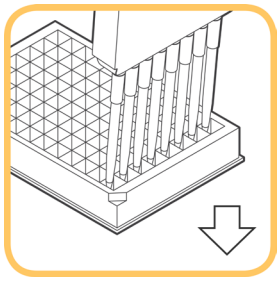


1. PREPARE WASH PLATES

Fill the deep well plates with wash buffer and label them with position on the turntable in the instrument:

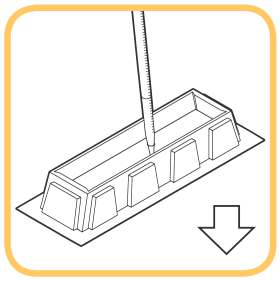
WASH PLATE 1: add **800 µL Wash Buffer I** per well, mark as “3”.
WASH PLATE 2: add **800 µL Wash Buffer II** per well, mark as “4”.
WASH PLATE 3: add **800 µL Wash Buffer II** per well, mark as “5”.

Note: We recommend using multichannel pipettes to speed up liquid handling.



2. PREPARE ELUTION PLATE

Add **100 µL Elution Buffer** per well to the elution plate and label with “8”.



3. PREPARE LYSIS MIX

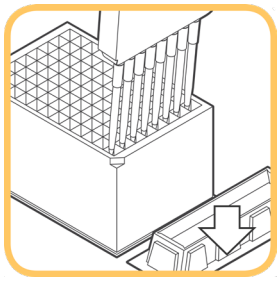
Calculate volume of the lysis mix components needed (see table below) and add them to a reservoir.

Note: Before pipetting, take care that the magnetic beads are homogenously distributed. To achieve this, vortex bottle with magnetic beads for 5 seconds immediately before adding them to the remaining reagents.

Table: Volumes of the required reagents for the lysis plate are dependent on the sample volume. Please take note of the different measurement units (µL or mL). An excess is already included in the columns for 48 or 96 samples to facilitate pipetting.

	1 Sample	48 Samples	96 Samples
Lysis Buffer	300 µL	15 mL	30 mL
Isopropanol	400 µL	20 mL	40 mL
Proteinase K	25 µL	1.25 mL	2.5 mL
Magnetic Beads*	20 µL	1 mL	2 mL
Carrier - tRNA	3 µL	150 µL	300 µL

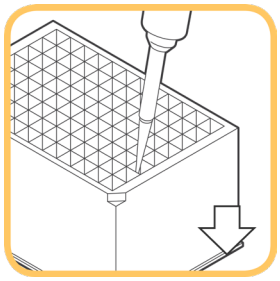
* Vortex for 5 seconds immediately before use.



4. ADD LYSIS MIX TO LYSIS PLATE

Add **748 µL lysis mix** from the reservoir to the lysis plate wells (deep well plate), mark as “2”.

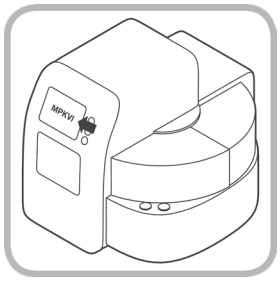
Note: Immediately before transferring the lysis mix, carefully rock reservoir and pipet up and down simultaneously to distribute the magnetic beads evenly in the solution.



5. ADD SAMPLES TO LYSIS PLATE

Add **up to 200 µL** sample to the lysis plate containing the lysis mix.

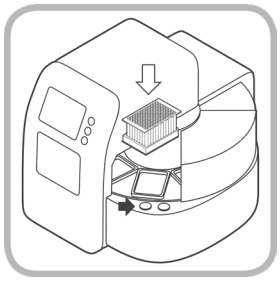
Note: Avoid cross-contamination by NOT pipetting up and down during this step.



6. SELECT PROGRAM

On the touchscreen, press 'Run Prog.' and select the pre-installed program '**MPKVI**' via the shortcut.

Note: Do not press 'Run' before loading, otherwise the robot starts without plates.



7. LOAD INSTRUMENT WITH PLATES

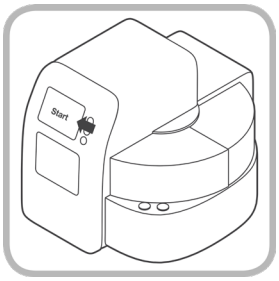
Press 'view' and on the symbol for the rotary table. A table shows, on which positions ('Plate') which plate ('Name') is placed on the rotary table.

Load the plates on position 1, 2, 3, 4, 5 and 8.
The position 6 and 7 remain unoccupied!

Note: Use the two buttons in front of the opening to rotate the turntable to the corresponding position. Place the plates carefully to avoid spilling of liquids.

Table: Plate distribution on the turntable.

Plate (on display: 'Name')	Position on the turntable (on display 'Plate')	Description
- Load -	1	Plate with tip comb
LyBi	2	Lysis plate
Wash1	3	Wash plate 1
Wash2	4	Wash plate 2
Wash3	5	Wash plate 3
Dry / Elution	8	Elution plate

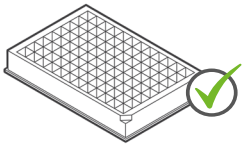


8. START RUN

Once all the plates have been placed in the instrument, confirm again by pressing 'Start'.

All the extraction steps will run automatically.

READY FOR DETECTION



The elution plate contains the extracted viral RNA/DNA from the samples.

RECOMMENDED: Use eluted RNA/DNA right after extraction. It is recommended to analyze a 1:10 dilution from feces samples, because the eluate may still contain PCR-inhibiting substances.

For later analysis, transfer extracts to tubes and store at -15 to -25 °C. For long-term storage, keep at -80 °C. After thawing, mix briefly by vortexing and centrifuge at high speed for 1 min.

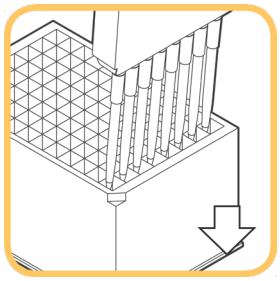
Note: If the elution plate still visibly contains magnetic beads, you may centrifuge it to avoid interference with the detection kit (1 min at high speed). The RNA/DNA is in supernatant.

2.3.3. KingFisher Flex

This protocol describes the RNA/DNA extraction from up to 200 µL sample using the KingFisher Flex. It consists of two parts: The manual preparation of plates and samples and the automated extraction run.

Please make sure that you have prepared all required reagents (see 2.2. Precautions and Preparations).

For a 96-sample run you will need the following consumables:
4 deep well plates, 2 elution plates, 1 tip comb.

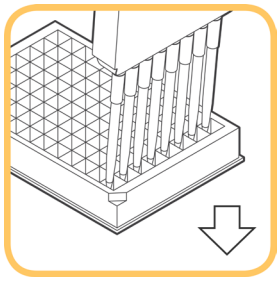


1. PREPARE WASH PLATES

Fill the deep well plates with wash buffer and label them:

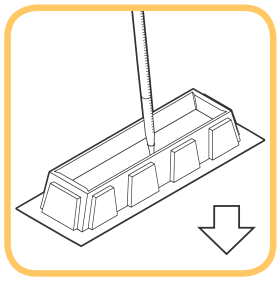
WASH PLATE 1: add **800 µL Wash Buffer I** per well.
WASH PLATE 2: add **800 µL Wash Buffer II** per well.
WASH PLATE 3: add **800 µL Wash Buffer II** per well.

Note: We recommend using multichannel pipettes to speed up liquid handling.



2. PREPARE ELUTION PLATE

Add **100 µL Elution Buffer** per well to the elution plate.



3. PREPARE LYSIS MIX

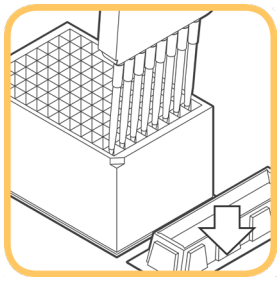
Calculate volume of the lysis mix components (see table below) and add them to a reservoir.

Note: Before pipetting, take care that the magnetic beads are homogenously distributed. To achieve this, vortex bottle with magnetic beads for 5 seconds immediately before adding them to the remaining reagents.

Table: Volumes of the required reagents for the lysis plate are dependent on the sample volume. Please take note of the different measurement units (µL or mL). An excess is already included in the columns for 48 or 96 samples to facilitate pipetting.

	1 Sample	48 Samples	96 Samples
Lysis Buffer	300 µL	15 mL	30 mL
Isopropanol	400 µL	20 mL	40 mL
Proteinase K	25 µL	1.25 mL	2.5 mL
Magnetic Beads*	20 µL	1 mL	2 mL
Carrier - tRNA	3 µL	150 µL	300 µL

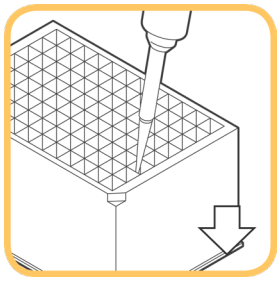
* Vortex for 5 seconds immediately before use.



4. ADD LYSIS MIX TO LYSIS PLATE

Add **748 µL lysis mix** from the reservoir to the lysis plate wells (deep well plate).

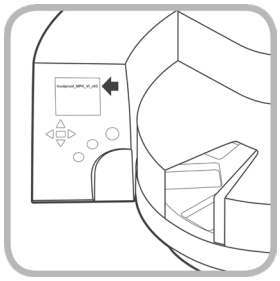
Note: Immediately before transferring the lysis mix, carefully rock reservoir and pipet up and down simultaneously to distribute the magnetic beads evenly in the solution.



5. ADD SAMPLES TO LYSIS PLATE

Add **up to 200 µL** sample to the lysis plate containing the lysis mix.

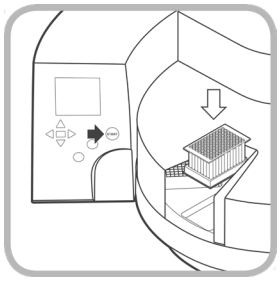
Note: Avoid cross-contamination by NOT pipetting up and down during this step.



6. SELECT PROGRAM

Start the KingFisher Flex instrument.

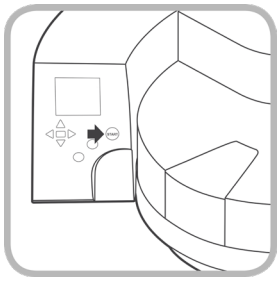
Open folder 'DNA' and select the pre-installed program '**foodproof_MPK_VI_v03**'.



7. LOAD INSTRUMENT WITH PLATES

The program tells you which plate you have to put into the instrument next. Load the instrument with the requested plate and then press 'Start' to load the next plate.

Note: After pressing 'Start', the rotary plate will automatically move into the correct loading position.

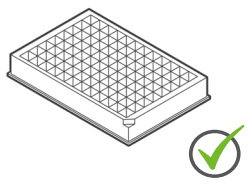


8. START RUN

Once all the plates have been placed in the instrument, confirm again by pressing 'Start'.

All the extraction steps will run automatically.

READY FOR DETECTION



The elution plate contains the extracted viral RNA/DNA from the samples.

RECOMMENDED: Use eluted RNA/DNA right after extraction. It is recommended to analyze a 1:10 dilution from feces samples, because the eluate may still contain PCR-inhibiting substances.

For later analysis, transfer extracts to tubes and store at -15 to -25 °C. For long-term storage, keep at -80 °C. After thawing, mix briefly by vortexing and centrifuge at high speed for 1 min.

Note: If the elution plate still visibly contains magnetic beads, you may centrifuge it to avoid interference with the detection kit (1 min at high speed). The RNA/DNA is in supernatant.

2.4. Troubleshooting

Problem	Possible Cause	Recommendation
Low RNA/DNA yield or purity.	Improper storage of kit components.	Store all buffers (Lysis Buffer, Wash Buffer I, Wash Buffer II, Elution Buffer) at room temperature. Store the magnetic beads at 15 to 25 °C. Store lyophilized Proteinase K at 15 to 25 °C. Store reconstituted Proteinase K as aliquots at -15 to -25 °C. Store Carrier-tRNA at -15 to -25 °C.
	Buffer or other reagents not closed tightly.	Close all reagent bottles tightly after each use to preserve pH and stability, and to prevent contamination.
	Ethanol not added to Wash Buffer I and/or Wash Buffer II.	Add absolute ethanol to the Wash Buffer I and Wash Buffer II before using. After adding ethanol, mix the Wash Buffer I and Wash Buffer II well, and store at room temperature. Always mark the Wash Buffer I and Wash Buffer II bottle to indicate the addition of ethanol.
	Low amount of magnetic beads.	Mix the magnetic beads thoroughly before pipetting to the Lysis Plate.
	Suboptimal reaction conditions.	Don‘t forget to install tip combs. Ensure proper heating conditions. Ensure correct positioning of heating blocks in RoboPrep 32 and KingFisher Flex. Verify correct temperature of the heating block with a thermometer.
RNA/DNA does not perform well in real-time PCR.	Salt carryover during elution.	Check the wash buffers for salt precipitates. If there are any precipitates, dissolve these precipitates by careful warming. Ensure that wash buffers are stored at room temperature.
	DNA extract contains too many PCR inhibitors.	Dilute DNA/RNA extract, e.g., 1:10, or reduce the amount of extracted DNA/RNA.
Eluted RNA/DNA is brown colored.	Small part of the magnetic particles are left in the elution.	Centrifuge at full speed for 1 min and transfer supernatant (contains DNA/RNA) to a new tube.

2.6 Support

If you have questions or experience any problems with our products, please contact us:

www.hygiena.com/support

Our aim is to provide you with a solution as quickly and effectively as possible. We would also like you to contact us if you have any suggestions for improving the product or in case you would like to use our product for a different application. We highly value your feedback.

3. ADDITIONAL INFORMATION

3.1 General Information

Quality Control

All products are regularly monitored by our quality control. You can find the certificate of analysis (COA) on our website. If you would like to carry out your own quality control, you will find the analysis method described in the certificate.

Waste Disposal

All contaminated and potentially infectious material, like enrichment cultures or food samples, should be autoclaved before disposal and eliminated according to local rules and regulations. For proper disposal of unused chemicals, please refer to the SDS.

Warranty and Disclaimer of Liability

“Limited Warranty” and “Disclaimer of Liability”: Hygiena Diagnostics GmbH warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

(1) The product is used according to the guidelines and instructions set forth in the product literature;

(2) Hygiena Diagnostics GmbH does not warrant its product against any and all defects when: the defect is as a result of material or workmanship not provided by Hygiena Diagnostics GmbH; defects caused by misuse or use contrary to the instructions supplied, or improper storage or handling of the product;

(3) All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend only for a period of one year from the date of manufacture. There are no other warranties that extend beyond those described on the face of this warranty;

(4) Hygiena Diagnostics GmbH does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of Hygiena Diagnostics GmbH;

(5) Hygiena Diagnostics GmbH does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, expense for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death;

(6) Hygiena Diagnostics GmbH reserves the right to replace or allow credit for any modules returned under this warranty.

3.2 Trademarks

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Other brand or product names are trademarks of their respective holders.

3.3 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S 400 20.1 L

3.2 Change Index

Version 1, March 2020
First version of the insert.

Version 2, April 2020
RoboPrep 32 protocol added.

Version 3, October 2020
RoboPrep 96 protocol added.

Revision A, January 2024
Rebranding and new layout.
S 400 20.1 L 20 -> INS-KIT230190-RevA

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