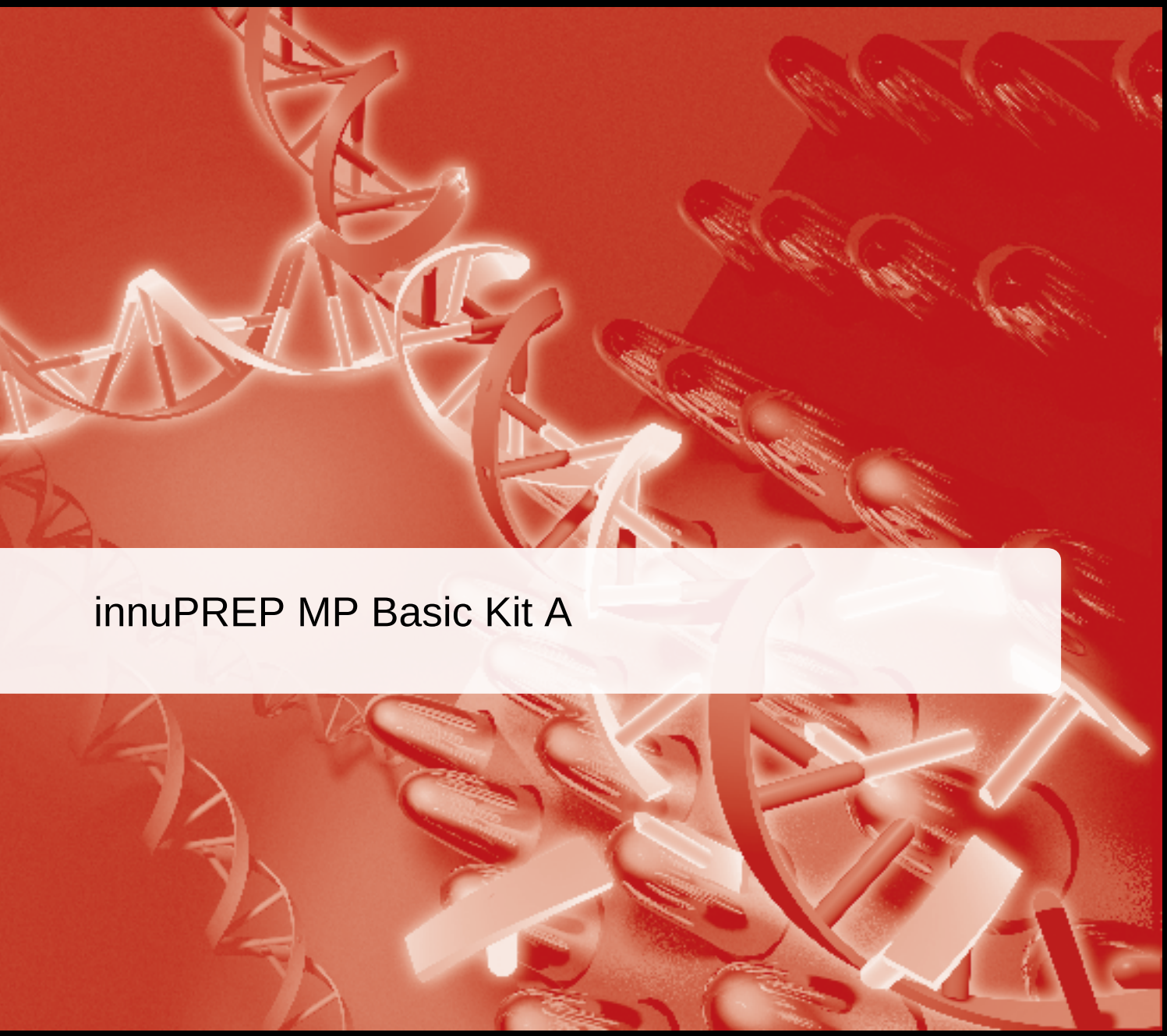
analytikjena

# Manual



## innuPREP MP Basic Kit A

Life Science unlimited

**Order No.:**

| | |
|---|---|
| 845-KS-4900010 | 10 reactions |
| 845-KS-4900100 | 100 reactions |
| 845-KS-4900500 | 500 reactions |

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!



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# Contents

# 1 Introduction

## 1.1 Intended use

The innuPREP MP Basic Kit A has been designed as a tool for isolation of viral DNA or RNA (or bacterial DNA or RNA) from different kinds of body fluids. The reagents can be processed very easy using different types of home-made or commercially available magnetic separation racks. The described protocol is a general protocol and can be modified by the customer. This allows a highly flexible adaptation on the demand to isolate DNA or RNA from other kinds of starting material etc.

The separation technology is based on a new kind of chemistry which enables binding of DNA and RNA in one step on the surface of magnetic particles.

The extraction process combines the lysis of starting material following binding of DNA and RNA on the surface of magnetic particles. After a washing step and particle drying the surface fixed nucleic acid is eluted in water. The procedure is very simple, universally applicable and highly efficient. Further, the kit contains a Carrier Mix with Carrier RNA as well as an internal control DNA and RNA. The internal control DNA and RNA can be used in combination with a corresponding real time PCR detection kit (innuDETECT Internal Control DNA/RNA Assay).

The kit works with 200 µl liquid sample (cell-free body fluids), 1 – 5 mg Biopsies, Cell cultures (max. $5 \times 10^6$), nasopharyngeal swabs and stool samples (0.05 – 0.1 g). The extracted nucleic acids are suitable for downstream applications like PCR, real-time PCR or any kind of enzymatic reaction.

We highly recommend the usage of the internal control DNA or RNA (IC DNA/RNA) or own internal standards (low-copy) respectively, as well as positive and negative controls to monitor the purification, amplification, and detection processes (see related products).

Please note that the eluates contain both nucleic acids and Carrier RNA. In case of using Carrier RNA the quantification of nucleic acids (isolated with this kit) by photometric or fluorometric methods is not possible. It is recommended to quantify extracted DNA and RNA with other methods like specific quantitative PCR or real-time PCR. Furthermore, Carrier RNA may inhibit PCR reactions. Thus the amount of Carrier RNA has to be carefully optimized depending on the individual PCR system used.

**Consult instruction for use**

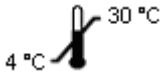
This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

## 1.2 Notes on the use of this manual

For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:


**REF**
Catalogue number


**Content**
Contains sufficient reagents for <N> reactions




**Storage conditions**
Store at room temperature or shown conditions respectively

**Consult instructions for use**
This information must be observed to avoid improper use of the kit and the kit components.

**Used by**
Expiry date.


**Lot number**
The number of the kit charge

**Manufactured by**
Contact information of manufacturer

**For single use only**
Do not use components for a second time.

**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 systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → "Notes on the use of this manual" p. 4).
- Working steps are numbered.

# 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 manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

**For single use only!**

This kit is made for single use only!

**Attention!**

Don't eat or drink components of the kit!

The kit shall only be handled by educated personal in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. Analytik Jena AG 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.

Clinical sample must always be considered as potentially infectious. Samples from risk patients must always be labeled and handled under consequent safety conditions. Please observe the federal, state and local safety and environmental regulations. Observe the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

**Attention!**

Do not add bleach or acidic components to the waste after sample preparation!

**Note**

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.

For more information, please ask for the material safety data sheet (MSDS).

# 3 Storage conditions

The innuPREP MP Basic Kit A should be stored dry, at room temperature (4 - 35 °C) and is stable for at least 12 months under these conditions. Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming. For further information see table kit components (→ "Kit components" p. 7).

# 4 Function testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. The components of each innuPREP MP Basic Kit A were tested by isolation of DNA and RNA in combination with internal control DNA and RNA (IC DNA/RNA) and subsequent detection of IC DNA and IC RNA by real-time PCR.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the innuPREP MP Basic Kit A or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. For other countries please contact your local distributor.

# 5 Product use and warranty

The user is responsible to validate the performance of the Analytik Jena AG kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. Analytik Jena AG kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by the Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

**Note**

For research use only!

# 6 Kit components

**Important**

Store lyophilized Proteinase K at 4 °C!

Divide dissolved Proteinase K into aliquots and storage at – 20 °C is recommended. Repeated freezing and thawing will reduce the activity dramatically!

Store MAG Suspension M at 4 °C – 8 °C.

Store lyophilized Carrier Mix at – 20 °C.

It is recommended to divide dissolved Carrier Mix stock solution into aliquots for storage at –20 °C. Do not freeze and thaw Carrier Mix stock solution more than 3 times.


**Storage conditions**

All other components are stored at room temperature.

| | Σ 10 | Σ 100 | Σ 500 |
|---|---|---|---|
| REF | 845-KS-4900010 | 845-KS-4900100 | 845-KS-4900500 |
| **Lysis Solution RL** | 10 ml | 60 ml | 2x 150 ml |
| **Binding Solution B** | 10 ml | 50 ml | 250 ml |
| **Carrier Mix** | 1x lyophilized powder | 1x lyophilized powder | 5x lyophilized powder |
| **RNase-free water** | 2.0 ml | 2.0 ml | 5x 2.0 ml |
| **Proteinase K** | for 1 x 0.3 ml working solution | for 2 x 1.5 ml working solution | for 7 x 1.5 ml working solution |
| **MAG Suspension M** | 2 ml | 2x 2 ml | 6x 2 ml |
| **Washing Solution HS** | 5 ml (final volume 10 ml) | 30 ml (final volume 60 ml) | 2x 70 ml (final vol. 2x 140 ml) |
| **Washing Solution LS** | 6 ml (final volume 30 ml) | 36 ml (final volume 180 ml) | 180 ml (final volume 900 ml) |
| **RNase-free water** | 2 ml | 15 ml | 2x 25 ml |
| **Manual** | 1 | 1 | 1 |

| | Σ 10 | Σ 100 | Σ 500 |
|---|---|---|---|
| REF | 845-KS-4900010 | 845-KS-4900100 | 845-KS-4900500 |
| **Initial steps** | • Add 5 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed!<br>• Add 24 ml of 96-99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!<br>• Dissolve Proteinase K by addition of 0.3 ml of $ddH_2O$, mix thoroughly and store as described below!<br>• Add 1.25 ml RNase-free Water to each tube Carrier Mix, mix thoroughly by pipetting up and down! | • Add 30 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed!<br>• Add 144 ml of 96-99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!<br>• Dissolve Proteinase K by addition of 1.5 ml of $ddH_2O$, mix thoroughly and store as described below!<br>• Add 1.25 ml RNase-free Water to each tube Carrier Mix, mix thoroughly by pipetting up and down! | • Add 70 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed!<br>• Add 720 ml of 96-99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!<br>• Dissolve Proteinase K by addition of 1.5 ml of $ddH_2O$, mix thoroughly and store as described below!<br>• Add 1.25 ml RNase-free Water to each tube Carrier Mix, mix thoroughly by pipetting up and down! |

# 7 Recommended steps before starting

- Ensure that the Washing Solution HS, Washing Solution LS, Proteinase K and Carrier Mix have been prepared according to the instruction (→ "Kit components" p. 7)
- Centrifugation steps should be carried out at room temperature
- Avoid freezing and thawing of starting material.

# 8 Components not included in the kit

- 1.5 ml tubes
- 2 ml tubes; optional
- 96 – 99.8 % ethanol

**Note:** Use only absolute/pure ethanol, NO methylated or denatured alcohol!

- dd$H_2O$ for dissolving Proteinase K
- PBS; optional (for isolation of viral RNA from stool sample)
- Physiological saline; optional (0.9 % NaCl for Influenca A testing)

# 9 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 surface 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.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free.)
- 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 or more hours before use. Autoclaving alone will not inactivate many RNases 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 (diethyl pyrocarbonate). The glassware has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C and then it has to be autoclaved or heated to 100 °C for 15 min to remove residual DEPC.
- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5 % SDS), thoroughly rinsed with RNase-free water, rinsed with ethanol and finally allowed to dry.
- All buffers have to be prepared with DEPC-treated RNase-free dd$H_2O$.
- 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.

# 10 Carrier Mix

## 10.1 Storage conditions and handling

The Carrier Mix contains a carrier RNA and an internal control DNA and RNA (IC DNA/RNA).

- Add dissolved Carrier Mix to Lysis Solution RL immediately
- Unused Carrier Mix should kept frozen at – 20 °C
- Do not freeze and thaw the Carrier Mix more than 3 times
- Mixture of Lysis Solution RL and Carrier Mix is stable for 1 day at 4 °C
- Internal control DNA and RNA can be detected by real-time PCR using the corresponding assay, as shown in the following table:

| Name | Amount | Order No. |
|---|---|---|
| innuDETECT Internal Control DNA Assay | 100 rxn | 845-ID-0006100 |
| innuDETECT Internal Control RNA Assay | 100 rxn | 845-ID-0007100 |
| innuDETECT Internal Control DNA/RNA Assay | 100 rxn | 845-ID-0008100 |

## 10.2 Preparation of Lysis Solution RL / Carrier Mix

1. Add 1.25 ml RNase-free Water to each tube Carrier Mix.
2. Mix thoroughly by pipetting up and down!
3. After the preparation of Carrier Mix stock solution prepare the mixture of Lysis Solution RL / Carrier Mix as described in the following table:

| Component | 5 samples | 10 samples | n samples |
|---|---|---|---|
| **Lysis Solution RL** | 1.8 ml | 3.6 ml | 360 µl x sample |
| **Carrier Mix** | 60 µl | 120 µl | 12 µl x sample |
| **Final volume** | 1.86 ml | 3.72 ml | 372 µl x sample |

**Note:** If customized Extraction Controls should be used, please add these components to the Lysis Solution RL / Carrier Mix! Don't add the Carrier Mix without the Lysis Solution RL to the sample. The addition of Carrier Mix directly to the sample may lead to degradation of the control DNA/RNA. If you want to use own extraction tubes coated with carrier nucleic acids and IC RNA and/or DNA (e.g. Extraction Tubes from AJ Roboscreen GmbH) do not use the Carrier Mix.

**Note**

For Protocol **12.1 C Swabs from nasopharyngeal sample Variant 2** and for Protocol **12.1 D Tissue biopsies** with a higher sample Lysis Solution mixture use the following table:

| Component | 5 samples | 10 samples | n samples |
|---|---|---|---|
| **Lysis Solution RL** | 3.0 ml | 6.0 ml | 600 µl x sample |
| **Carrier Mix** | 60 µl | 120 µl | 12 µl x sample |
| **Final volume** | 3.2 ml | 6.12 ml | 612 µl x sample |

# 11 Product specifications

## 1. Starting material:

- Serum, plasma, synovial fluids, saliva, cell culture supernatants and other cell-free bodily fluids (200 µl)
- Biopsies (1 – 5 mg)
- Cell cultures (max. 5 x $10^6$)
- Nasopharyngeal swabs
- Stool samples (0.05 – 0.1 g)

## 2. Time for isolation:

Approximately 20 minutes after lysis

## 3. Positive test results obtained for the following targets:

- Rift valley fever virus (RNA virus model)
- Vaccinia virus (DNA virus model)
- ***Yersinia pestis*** (Gram- bacteria)
- ***Bacillus anthracis*** spores (Gram+ bacteria)
- Ebola virus
- Bovine viral diarrhea virus (BVDV)
- Marburg virus
- Yellow fever virus
- Norovirus
- Sigma virus
- Influenza A & influenza B virus
- ***Francisella tularensis***
- ***Bacillus cereus***
- ***Bacillus thuringiensis***

# 12 General protocol for isolation of DNA or RNA from different types of fluid samples

**Note**
This protocol can't be used to process whole blood samples!

## 12.1 Samples lysis (depending on type of starting material)

**Important**
Prepare Lysis Solution RL / Carrier Mix as described on page 10/11 before starting!

**Note**
We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during incubation. No shaking will reduce the lysis efficiency.

**A. Cell-free bodily fluids** (serum, plasma, synovial fluid, liquor, saliva)

Transfer **300 µl Lysis Solution RL** / **Carrier Mix** into a 1.5 or 2.0 ml tube. Add **200 µl** of the **sample and 20 µl of Proteinase K** to the tube. Place the tube into a thermal mixer or into another shaker and incubate under continuous shaking for 15 minutes at room temperature (RT).

**B. Cell culture supernatant**

Transfer **300 µl Lysis Solution RL** / **Carrier Mix** into a 1.5 or 2.0 ml tube. Add **200 µl** of the **cell culture supernatant** (cell culture medium) **and 20 µl of Proteinase K** to the tube. Place the tube into a thermal mixer or into another shaker and incubate under continuous shaking for 15 minutes at room temperature (RT).

**C. Swabs from nasopharyngeal sample** (for ***INFLUENZA A*** testing)

**Variant 1:**

Place the swab into a 1.5 ml reaction tube containing physiological saline (0,9 % NaCl) and incubate for 15 minutes at room temperature. Afterwards shake the swab vigorously, squeeze it and remove the swab.

Transfer **300 µl Lysis Solution RL** / **Carrier Mix** into a new 1.5 or 2.0 ml tube. Transfer **200 µl** of the **physiological saline and**

**20 µl of Proteinase K** into the tube filled with Lysis Solution RL / Carrier Mix. Place the tube into a thermal mixer or into another shaker and incubate under continuous shaking for 15 minutes at room temperature (RT).

**Variant 2:**

Transfer **500 µl Lysis Solution RL** / **Carrier Mix** into a 1.5 ml or 2.0 ml tube. Place the swab into the tube containing Lysis Solution RL / Carrier Mix, incubate short and afterwards shake the swab vigorously, squeeze it and remove the swab. Add **20 µl of Proteinase K** to the tube and place the tube into a thermal mixer or into another shaker and incubate under continuous shaking for 15 minutes at room temperature (RT).

## D. Tissue biopsies

Transfer **500 µl of Lysis Solution RL** / **Carrier Mix** into a 1.5 ml tube. Add **1 – 5 mg** of the **tissue biopsy and 20 µl of Proteinase K** to the tube containing Lysis Solution RL / Carrier Mix. Close the cap and mix by vortexing for 10 sec. Place the tube into a thermal mixer or another shaker and incubate under continuous shaking for 30 minutes at room temperature (RT). Lysis time can be increased up to 1 hour. After lysis centrifuge the sample at max. speed for 1 minute to spin down unlysed material and transfer **500 µl** of **clear supernatant** into a new 1.5 ml or 2.0 ml tube.

## E. Stool sample (tested for Norovirus extraction)

**Variant 1:**

Transfer about **0.05 – 0.1 g** of **stool sample** into a 1.5 ml reaction tube. Add **250 µl PBS** (not included in the kit). Vortex the tube for 10 seconds. Centrifuge the tube at max. speed for 3 minutes. During centrifugation time add **300 µl Lysis Solution RL** / **Carrier Mix** into a new 1.5 ml or 2.0 ml tube. Transfer the **clear supernatant** of **sample (max. 250 µl)** after centrifugation to the tube containing Lysis Solution RL / Carrier Mix **and add 20 µl of Proteinase K**. Place the tube into a thermal mixer or into another shaker and incubate under continuous shaking for 15 minutes at room temperature (RT).

**Variant 2:**

In some cases the initial feacal sample is mixed with special ELISA Buffer for subsequent ELISA detection of Norovirus. Use **250 µl of the sample**, transfer it into a 1.5 ml reaction tube and centrifuge the tube at maximum speed for 3 minutes. During centrifugation time add **300 µl Lysis Solution RL / Carrier Mix** into a new 1.5 ml or 2.0 ml tube. Transfer the **clear supernatant** of **sample (max. 250 µl)** after centrifugation to the tube containing Lysis Solution RL / Carrier Mix **and add 20 µl of Proteinase K**. Place the tube into a thermal mixer or into another shaker and incubate under continuous shaking for 15 minutes at room temperature (RT).

## 12.2 Binding of DNA/RNA to magnetic particles

**Important**
Vortex the MAG Suspension M for 1 minute before use!

Add **450 µl of Binding Solution B and 20 µl of MAG Suspension M** to the lysed sample.

**Note:** The Binding Solution B is very viscously, please pipette carefully. It is important that the sample and the Binding Solution B are mixed vigorously to get a homogeneous solution.

Mix the sample completely by vortexing for 15 seconds.

Incubate sample at room temperature for 5 minutes for binding of nucleic acid to the magnetic particles.

Magnetic separation of beads and removal of supernatant using a magnetic rack or another magnetic particle separation equipment.

## 12.3 Washing of magnetic particles with bound DNA/RNA

Add **500 µl of Washing Solution HS** and wash MAG particles by vortexing or by pipetting up and down.

Perform magnetic separation of beads and remove the Washing Solution HS.

Add **750 µl of Washing Solution LS** and wash MAG particles by vortexing or by pipetting up and down.

Perform magnetic separation of beads and remove the Washing Solution LS.

Add **750 µl of Washing Solution LS** and wash MAG particles by vortexing or by pipetting up and down.

Perform magnetic separation of beads and remove the Washing Solution LS.

**Important**
After the last washing step remove Washing Solution as complete as possible!

## 12.4 Drying of magnetic particles with bound DNA/RNA

To remove the ethanol from Washing Solution LS completely, place the opened tube with the magnetic beads at room temperature or in a thermal mixer at 50 °C.

The drying step is important for all following downstream application.

**The ethanol must be removed completely!**

Time for drying depends on temperature and should be observed individually. Using thermal mixer and 50°C, the drying process is normally finished within 5 minutes. Drying at room temperature needs a longer incubation time.

## 12.5 Elution of bound DNA/RNA

Add **40 µl – 100 µl RNase-free water** (elution volume depends on expected amount of target nucleic acid).

Re-suspend the magnetic particles completely and incubate at room temperature (if possible under continuous shaking) for 1 – 5 minutes.

Perform magnetic separation of beads.

Transfer the eluted DNA/RNA into a new 1.5 ml tube.

**Important notes!**

1. If the DNA/RNA eluate contains carryover of magnetic particles, place the tube on a magnet or centrifuge the tube at maximum speed for 1 minute. Pipette the supernatant with DNA/RNA into a new tube.
2. Store the DNA/RNA under adequate conditions. We recommend to store the extracted RNA at –80°C.

# 13 Troubleshooting

| Problem / probable cause | Comments and suggestions |
|---|---|
| **Low amount of extracted viral DNA/RNA** | |
| • Insufficient lysis | Increase lysis time.<br>Reduce amount of starting material. |
| • Incomplete elution | Prolong the incubation time with RNase-free water to 5 minutes or repeat elution step once again.<br>Take a higher volume of RNase-free water. |
| • Insufficient mixing with Binding Solution B | Mix sample with Binding Solution B by pipetting or by vortexing prior to add MAG Suspension M. |
| **Low concentration of extracted viral DNA/RNA** | |
| • Too much RNase-free water | Elute the viral RNA with lower volume of RNase-free water. |
| • No Carrier RNA added | Add Carrier RNA, as described in the manual |

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