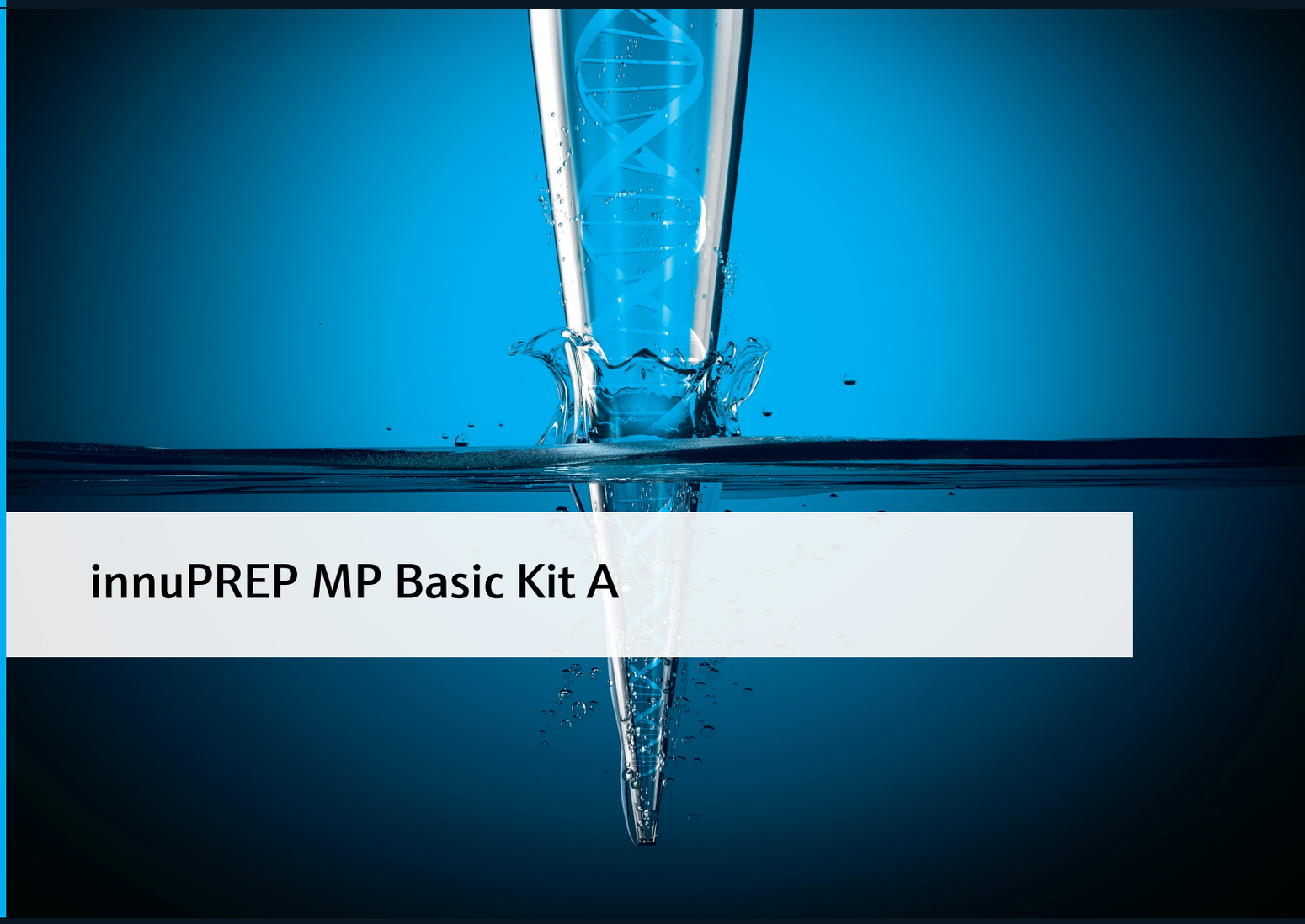
Instructions for Use
Life Science Kits & Assays



innuPREP MP Basic Kit A

Innuscreen GmbH

Order No.:
845-KS-4900100 100 reactions

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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 x 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 before 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 and the kit

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

Symbol	Information
REF	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> reactions.
15°C 30°C	**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.
	Expiry date
LOT	**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 kit 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 and the kit" p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before 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 personnel 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. This kit could be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow 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 on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com

3 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 hours or more before use. Autoclaving will not inactivate RNase activity completely. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC (diethyl pyrocarbonate). The glassware has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C followed by autoclaving or heating to 100 °C for 15 minutes to remove residual DEPC.
- 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 water.
- 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.

4 Storage conditions

All kit components are shipped at ambient temperature.

Upon arrival store lyophilized and dissolved **Proteinase K** and **MAG Suspension M** at 4 °C to 8 °C and lyophilized **Carrier Mix** at -22 °C to -18 °C. Aliquot dissolved **Carrier Mix** and do not freeze and thaw it more than 3 times!

The mixture of **Lysis Solution RL** and **Carrier Mix** is stable for a maximum of 7 days if stored at 4 °C to 8 °C.

All other components of the innuPREP RNA Virus PLUS Kit - KFFLX should be stored dry, at room temperature (15 °C to 30 °C). If stored at room

temperature, the kit is stable until the expiration date printed on the label on the kit box.

If there are any precipitates within the provided solutions solve these precipitates by careful warming. Before every use make sure that all components have room temperature.

5 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This kit has been produced and tested in an ISO 13485 certified facility.

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 please do not hesitate to contact us. For technical support or further information in Germany please contact info.innu@ist-ag.com. For other countries please contact your local distributor.

6 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Intended use” p. 2). Since the performance characteristics of our kits have not been validated for any specific application. IST Innuscreen GmbH 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 IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
The kit is for research use only!

7 Kit components

7.1 Included kit components

	Σ 100
REF	845-KS-4900100
Lysis Solution RL	60 ml
Binding Solution RBS	50 ml
Carrier Mix	1 x lyophilized powder
RNase-free Water	2 ml
Proteinase K	for 2 x 1.5 ml working solution
MAG Suspension M	2 x 2 ml
Washing Solution HS (conc.)	30 ml
Washing Solution LS (conc.)	36 ml
RNase-free Water	15 ml
Manual	1

7.2 Components not included in the kit

- 1.5 ml tubes
- 96–99.8 % ethanol (non denaturated or methylated)
- ddH_2O for dissolving **Proteinase K**
- optional PBS
- optional physiological saline for Influenca A testing

8 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

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

9 Initial steps before starting

- Add 1.25 ml RNase-free Water to each vial **Carrier Mix**, mix thoroughly and store as described above.
- Add the indicated amount of ddH_2O to each vial **Proteinase K**, mix thoroughly and store as described above.

845-KS-4900100	Add 1.5 ml ethanol to lyophilized Proteinase K.

- Add the indicated amount of absolute ethanol to each bottle **Washing Solution HS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KS-4900100	Add 30 ml ethanol to 30 ml Washing Solution HS (conc.).

- Add the indicated amount of absolute ethanol to **Washing Solution LS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KS-4900100	Add 144 ml ethanol to 36 ml Washing Solution LS (conc.).

- Centrifugation steps should be carried out at room temperature.
- Prepare Lysis Solution RL / Carrier Mix as described (→ p. 11).

10 Usage of Carrier Mix

10.1 Detection

Besides carrier RNA, the **Carrier Mix** contains an Internal Control DNA and RNA (IC DNA and IC RNA). Both can be detected by real-time PCR using the corresponding assays.

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

10.2 Preparation of Lysis Solution RL / Carrier Mix

Prepare mixture of **Lysis Solution RL** and **Carrier Mix** according to the table below.

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

If customized extraction controls are used, please add these components to the mixture of **Lysis Solution RL / Carrier Mix**.

NOTE
Store mixture of **Lysis Solution RL / Carrier Mix** at 4–8 °C for a maximum of 7 days.

For Protocol **11.1/ C. Swabs from nasopharyngeal sample (Variant 2)** and for Protocol **11.1/ D. Tissue biopsies** with a higher sample Lysis Solution mixture use the following table:

Component	5 samples	10 samples	n samples
Lysis Solution RL	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

11 Protocol for isolation of DNA or RNA from different fluid samples

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

11.1 Samples lysis (depending on type of starting material)

IMPORTANT NOTE
Prepare Lysis Solution RL / Carrier Mix as described on page 11 before starting!

NOTE
We recommend using 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.

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.

C. Swabs from nasopharyngeal sample (for Influenca 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.

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.

D. Tissue biopsies

1. Transfer 500 µl of Lysis Solution RL / Carrier Mix into a 1.5 ml tube.
2. 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.
3. Place the tube into a thermal mixer or another shaker and incubate under continuous shaking for 30 minutes at room temperature. Lysis time can be increased up to 1 hour.
4. 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.

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.

11.2 Binding of DNA/RNA to magnetic particles

NOTE
Vortex the MAG Suspension M for 1 minute before use!

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

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

1. Mix the sample completely by vortexing for 15 seconds.
2. Incubate sample at room temperature for 5 minutes for binding of nucleic acid to the magnetic particles.
3. Magnetic separation of beads and removal of supernatant using a magnetic rack or another magnetic particle separation equipment.

11.3 Washing of magnetic particles with bound DNA/RNA

1. Add **500 µl of Washing Solution HS** and wash MAG particles by vortexing or by pipetting up and down.
2. Perform magnetic separation of beads and remove the Washing Solution HS.
3. Add **750 µl of Washing Solution LS** and wash MAG particles by vortexing or by pipetting up and down.
4. Perform magnetic separation of beads and remove the Washing Solution LS.
5. Add **750 µl of Washing Solution LS** and wash MAG particles by vortexing or by pipetting up and down.
6. Perform magnetic separation of beads and remove the Washing Solution LS.

NOTE
After the last washing step remove Washing Solution as complete as possible.

11.4 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.

11.5 Elution of bound DNA/RNA

1. Add **40 µl – 100 µl RNase-free water** (elution volume depends on expected amount of target nucleic acid).
2. Re-suspend the magnetic particles completely and incubate at room temperature (if possible under continuous shaking) for 1 – 5 minutes.
3. Perform magnetic separation of beads.
4. Transfer the eluted DNA/RNA into a new 1.5 ml tube.

IMPORTANT NOTE

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 storing the extracted RNA at −80°C.

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted viral DNA/RNA	
Insufficient lysis	Increase lysis time. Reduce amount of starting material.
Incomplete elution	Prolong the incubation time with RNase-free water to 5 minutes or repeat elution step once again. Take a higher volume of RNase-free water.
Insufficient mixing with Binding Solution RBS	Mix sample with Binding Solution RBS 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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