



Instructions for Use

INSTANT Virus RNA/DNA Kit



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Order No.:

847-0259200602

50 reactions

847-0259200603

250 reactions



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1 Introduction

1.1 Intended use

The INSTANT Virus RNA/DNA Kit is intended for isolation of viral RNA and DNA from 400 µl of cell-free human body fluids.

The isolation procedure is based on the spin-column based solid phase extraction technology and is optimized for preparation of highly pure viral RNA and DNA from human plasma and serum, cerebrospinal fluid and swab- and stool-supernatants.

INSTANT Virus RNA/DNA Kit is designed to be used in combination with qualitative and quantitative Real-Time PCR methods for diagnostic purpose.

The kit is intended for use by laboratory professionals.

1.2 Test description and principle

The procedure combines lysis of starting material with subsequent binding of viral RNA and DNA to the surface of a Spin Filter membrane. After high- and low-salt washing steps the viral nucleic acid is eluted from the membrane with RNase-free water.

Extraction chemistry and extraction protocol are optimized for a maximum of yield.

The kit contains protocols for implementation of an Internal positive Control (IC) to monitor effectiveness of viral nucleic acid purification, amplification, and detection (→ 8.4 related products).

It is recommended to use Carrier RNA to increase yield of viral nucleic acid extraction from low concentration samples.

Please note that in case of using Carrier RNA the quantification of viral nucleic acids by photometric or fluorometric methods is not possible.











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.

1.3 Notes on the use of the IFU

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

Symbol	Information
	REF Catalogue number.
	Content Contains number of IVD-determinations as indicated.
	Storage temperature Store at temperatures between upper and lower limits as indicated.
	Consult instructions for use This information must be observed to avoid improper use of the kit.
	Used by Expiry date. The product is to be used by the indicated date.
	Lot number The lot number of the kit.
	CE-IVD symbol <i>In vitro</i> diagnostic medical device.
	Manufactured by Contact information of the manufacturer.
	For single use only Single use only. Do not use the product twice.
	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 IFU:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → 1.3 Notes on the use of the IFU).
- 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 IFU, 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, immediately flush eyes or skin with a large amount of water.



FOR SINGLE USE ONLY!

This kit is made for single use only!



ATTENTION!

Don't swallow components of the kit!

The kit shall only be handled by educated staff 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.

Clinical samples must always be considered as potentially infectious and must always be handled under safety conditions. Please observe the federal, state and local safety and environmental regulations.

Liquid and solid waste produced during processing of samples must be considered as potentially infectious and must be handled and discarded according to local safety regulations.

Observe the usual precautions for applications using extracted nucleic acids. All materials and reagents used for RNA isolation should be free of 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 please request the Safety Data Sheet (SDS) at the manufacturer site as shown inside cover page of the IFU.

3 GHS Classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases	EUH
Proteinase K	50-100 % Proteinase	 	315, 319, 334, 335	280, 260, 308+313, 342+311 305+351+338, 302+352, 501	-
Lysis Solution CLS	3-10 % sodium N-lauroyl- sarcosinate 1-3 % guanidinium chloride		315, 318	280, 305+351+338, 310, 302+352, 501	-
Washing Solution HS (conc.)	40-50 % Guanidinium thiocyanate	 	302, 332, 314 412	260, 280 308+310, 305+351+338, 303+361+353 273, 405, 501	032

3.1 Hazard phrases

302	Harmful if swallowed.
314	Causes severe skin burns and eye damage.
315	Causes skin irritation.
318	Causes serious eye damage.
319	Causes serious eye irritation.
332	Harmful if inhaled.
334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

- 335 May cause respiratory irritation.
- 412 Harmful to aquatic life with long lasting effects.

3.2 Precaution phrases

- 260 Do not breathe dust/fume/gas/mist/vapors/spray.
- 273 Avoid release to the environment.
- 280 Wear eye protection / face protection.
- 302+352 IF ON SKIN: Wash with plenty of water.
- 303+361+
353 IF ON SKIN (or hair): Take off immediately all contaminated clothing.
Rinse skin with water/shower.
- 305+351+
338 IF IN EYES: Rinse cautiously with water for several minutes. Remove
contact lenses, if present and easy to do. Continue rinsing.
- 308+310 IF exposed or concerned: Immediately call a POISON CENTER/doctor.
- 308+313 IF exposed or concerned: Call a POISON CENTER/doctor.
- 310 Immediately call a POISON CENTER/doctor.
- 342+311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
- 405 Store locked up.
- 501 Dispose of contents/container in accordance with
local/regional/national/international regulations.

3.3 EU hazard statements

- 032 Contact with acids liberates very toxic gas.

4 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 at low temperature or frozen.
- 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 has to be autoclaved or heated to 100 °C for 15 min subsequently 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 ddH₂O.
- 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, glass ware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

5 Storage and stability

All kit components are shipped at ambient temperature. Upon arrival, store lyophilized **Proteinase K** at 2 °C to 10 °C. Aliquot dissolved **Proteinase K** and store at -40 °C to -15 °C. Repeated freezing and thawing will reduce activity dramatically!

All other components of the INSTANT Virus RNA/ DNA Kit should be stored dry at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

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

After addition of Ethanol store Washing Solution LS and Washing Solution HS tightly capped at room temperature (15 °C to 30 °C).

6 Functional testing and technical assistance

The Roboscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This kit was 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 INSTANT Virus RNA/DNA Kit or other Roboscreen GmbH products, please do not hesitate to contact our technical support team at the manufacturer site as indicated on the inside cover page of the IFU.

7 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 IFU (→ 10 Product specifications). Roboscreen 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 Roboscreen 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 an *in vitro* diagnostic medical product!



8 Kit components

8.1 Included kit components

	Σ 50	Σ 250
REF	847-0259200602	847-0259200603
IC Spiking Tube	Containing Carrier RNA and Internal Extraction Control. Delivered with the detection assays (→ 8.4 Related Products).	
Lysis Solution CLS	30 ml	120 ml
Proteinase K	for 3 x 1.5 ml working solution	for 14 x 1.5 ml working solution
Washing Solution HS (conc.)	15 ml	70 ml
Washing Solution LS (conc.)	16 ml	2 x 36 ml
RNase-free Water	1 x 10 ml	4 x 10 ml
Spin Filter	1 x 50 pcs	5 x 50 pcs
Receiver Tubes	1 x 300 pcs	5 x 300 pcs
Elution Tubes	1 x 50 pcs	5 x 50 pcs
IFU	1	1

8.2 Necessary components not included in the kit

- 96–99.8 % ethanol (molecular biology grade, undenaturated)
- Isopropanol (molecular biology grade)
- 2.0 ml safe-lock tubes with round bottom
- 15 ml tube (for preparation of Lysis Solution CLS / IC mastermix)
- Phosphate buffered saline (PBS) for preparation of stool samples
- Physiological saline (0.9 % NaCl) for preparation of dry swabs

8.3 Required instrumentation and accessories

- Thermal mixer for 1.5 ml or 2.0 ml tubes or water bath
- Calibrated pipettes and suitable filter tips
- Micro centrifuge ($\geq 10,000 \times g$)
- Vortex mixer

8.4 Related products

- RoboGene HCV RNA Quantification Kit 3.0
 - 847-0207610032 32 rxn
 - 847-0207610096 96 rxn
 - 847-0207610192 192 rxn
- RoboGene HBV DNA Quantification Kit 3.0
 - 847-0207710032 32 rxn
 - 847-0207710096 96 rxn
 - 847-0207710192 192 rxn
- RoboGene HDV RNA Quantification Kit 2.0 (for use with RotorGene™3000/6000/Q)
 - 847-0207400544 32 rxn
 - 847-0207400542 96 rxn
- RoboGene HDV RNA Quantification Kit 2.0 for use with 7500 Fast/ Light Cycler® 480
 - 847-0207400584 32 rxn
 - 847-0207400582 96 rxn
- RoboGene HDV RNA Quantification Kit 3.0
 - 847-0207650032 32 rxn
 - 847-0207650096 96 rxn



IMPORTANT NOTE

For the correct use of the internal control, please read the instruction for use of the used detection assay.

9 Initial steps before starting

- Add to each vial of **Proteinase K** 1.5 ml of **RNase-free Water**, mix thoroughly and store as described in section 5.



NOTE

Do not use *preheated* **RNase-free Water** for reconstitution of **Proteinase K**!

- Add to **Washing Solution LS (conc.)** the indicated volume of absolute ethanol, mix thoroughly and store as described in section 5.

847-0259200602	Add 64 ml ethanol to 16 ml Washing Solution LS (conc.).
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847-0259200603	Add 144 ml ethanol to 36 ml Washing Solution LS (conc.).
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- Add to **Washing Solution HS (conc.)** the indicated volume of absolute ethanol, mix thoroughly and store as described in section 5.

847-0259200602	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).
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847-0259200603	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.).
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- Heat thermal mixer or water bath to 70 °C.
- Aliquot up to 1.0 ml of **RNase-free Water** each into a number of 2.0 ml safe-lock round bottom tubes according to the required elution volume and preheat at 70 °C.



NOTE

Preheating to 70° C is essential for efficient elution of extracted nucleic acids. Make sure that preheating temperature is correct and do not use higher aliquot volumes, since this could lead to a reduced preheating of **RNase-free Water**

- Centrifugation steps should be carried out at room temperature.

10 Product Specifications

1. Starting material:

- Fresh or frozen cell-free body fluids (serum, plasma, cerebrospinal fluid and stool).
- Swab collected specimen taken from epithelial surfaces
- Stabilizers: EDTA or citrate
- 400 µl sample volume

NOTE

Avoid freezing and thawing of starting material.

2. Time for isolation:

- Approximately 30 minutes

11 Isolation of viral nucleic acid from 400 µl sample

11.1 Protocol 1: Isolation from dry swabs and stool

In case of using dry swabs or stool, please carry out the following preparation instructions before continuing with protocol 2.

Preparation from dry swabs

1. Place the swab into a 2.0 ml safe-lock round bottom reaction tube containing at least 500 µl physiological saline (0.9 % NaCl) and incubate for 15 minutes at room temperature.
2. Stir with swab to dissolve the sample in the physiological saline.
3. Wring out residual liquid of the swab at the inner wall of the tube and remove the swab.
4. Proceed with **400 µl of the particle-free sample** as described in protocol 2.

Sampling of stool

1. Collect 1 gram or 1 ml of stool with standard specimen collection tubes.
2. Samples may be transported at room temperature, do not exceed the time 1 day after sample collection. Otherwise ensure transport under cooled conditions.
3. Samples may be stored deeply frozen for several months at -20° C to -70° C. Sample stability depends on the chosen storage temperature.

Preparation of stool sample

1. Transfer about 0.1 g of the stool sample into a 2.0 ml safe-lock round bottom reaction tube and add 1000 µl of PBS.

2. Resuspend by vortexing the sample for 5 seconds and centrifuge it at max. speed for 3 minutes.
3. Proceed with **400 µl of the particle-free sample** as described in protocol 2.



NOTE

If the stool sample is very solid extend resuspension time and separate the sample into smaller pieces by pipetting up and down. It may be necessary to cut off the pipette tip to increase the opening. If obtained eluates are cloudy, we recommend clarifying the eluates by centrifuging for 3 minutes at maximum speed (20,000 x g).

11.2 Protocol 2: Isolation of viral nucleic acids using IC Spiking Tube



NOTE

Fill the needed volume of **RNase-free Water** into a 2.0 ml safe-lock round bottom reaction tube as described in section 9 and incubate at 70 °C until the elution step.

1. Vortex and centrifuge the sample prior to use.



NOTE

Centrifugation helps to clarify the sample and prevents clogging of the Spin Filter.

2. Pipet in the specified order **80 µl** of reconstituted **Proteinase K** into an empty 2.0 ml safe-lock round bottom reaction tube and add **400 µl of the sample**. Mix well by pipetting up and down.



NOTE

Make sure that Proteinase K was equilibrated at room temperature prior to use.

3. Then add **400 µl Lysis Solution CLS** and finally **10 µl IC solution** from the IC Spiking Tube to the sample and mix vigorously by pulsed vortexing for 10 seconds.



NOTE

We recommend to prepare a Lysis Solution CLS / IC mastermix in order to apply an identical amount of IC to all samples.

4. Incubate the mixture at **70 °C for 10 minutes**. Agitate mixture from time to time during lysis.



NOTE

We recommend to use a thermal mixer for heating and continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during incubation. Agitating increases efficiency of lysis.

5. After lysis centrifuge the 2.0 ml tube shortly to remove condensate from the lid of the tube.

6. Add **800 µl Isopropanol** (not included in the kit) to the lysed sample, mix well by pipetting up and down several times or by vortexing. Centrifuge shortly.



NOTE

It is important that sample and Isopropanol are mixed vigorously to get a homogeneous solution.

7. Apply **800 µl of the sample** to the Spin Filter placed into a Receiver Tube. Close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.



NOTE

If the solution has not completely passed through the Spin Filter, prolong the centrifugation time or centrifuge at higher speed (up to 14,000 x g).

8. Apply the **residual sample** to the Spin Filter placed into a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
9. Open the Spin Filter and add **500 µl Washing Solution HS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
10. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
11. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.



NOTE

Avoid cross-contamination during addition of Washing Solutions HS and LS and RNase-free Water. Change pipette tips and do not touch membrane or inner surfaces of the Spin Filter when pipetting tips are used repeatedly.

12. Centrifuge at max. speed for 5 minutes to remove all traces of ethanol. Discard the Receiver Tube.
 13. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and pipet **60 µl of RNase-free Water** preheated to 70 °C on the binding membrane.
-



NOTE

For proper elution make sure that pre-heated RNase-free Water contacts the Spin Filter membrane completely. Do not touch the membrane with the pipet tip.

14. Close the cap and incubate Spin Filter and Elution Tube at **70°C for 2 minutes**.
-



NOTE

Elution efficiency depends on the thermal effect of the preheated RNase-free Water. Make sure that the Spin Filter is heated to 70° C prior to final centrifugation.

15. Centrifuge at 8,000 x g (~10,000 rpm) for 1 minute. Discard the Spin Filter, close the Elution Tube and keep it at low temperature or frozen until further use.

11.3 Protocol 3: Isolation of nucleic acids using IC Spiking Tube (modified)



NOTE

Fill the needed volume of **RNase-free Water** into a 2.0 ml safe-lock round bottom reaction tube as described in section 9 and incubate at 70 °C until the elution step.

1. Vortex and centrifuge the sample prior to use.
-



NOTE

Centrifugation helps to clarify the sample and prevents clogging of the Spin Filter.

2. Pipet in the specified order **40 µl of Proteinase K** into an empty 2.0 ml safe-lock round bottom reaction tube and add **400 µl of the sample**. Mix vigorously by pulsed vortexing for 10 seconds.
-



NOTE

Make sure that Proteinase K was equilibrated at room temperature prior to use.

3. Then add **400 µl Lysis Solution CLS** and finally **10 µl of IC solution** from the IC RNA Spiking Tube to the sample and mix vigorously by pulsed vortexing for 10 seconds.
-



NOTE

We recommend to prepare a Lysis Solution CLS / IC RNA mastermix in order to apply an identical amount of IC to all samples.

4. Incubate the mixture at **70 °C for 15 minutes**. Agitate mixture from time to time during lysis.
-



NOTE

We recommend to use a thermal mixer for heating and continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during incubation. Agitating increases efficiency of lysis.

5. After lysis centrifuge the 2.0 ml tube shortly to remove condensate from the lid of the tube.

6. Add **800 µl Isopropanol** (not included in the kit) to the lysed sample, mix well by pipetting up and down several times or by vortexing.



NOTE

It is important that sample and Isopropanol are mixed vigorously to get a homogeneous solution.

7. Apply **800 µl of the sample** to the Spin Filter placed into a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.



NOTE

If the solution has not completely passed through the Spin Filter, prolong the centrifugation time or centrifuge at higher speed (up to 14,000 x g).

8. Apply the **residual sample** to the Spin Filter placed into a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
9. Open the Spin Filter and add **500 µl Washing Solution HS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
10. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
11. Open the Spin Filter and add **650 µl Washing Solution LS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.



NOTE

Avoid cross-contamination during addition of Washing Solutions HS and LS and RNase-free Water. Change pipette tips and do not touch membrane or inner surfaces of the Spin Filter when pipetting tips are used repeatedly.

12. Centrifuge at max. speed for 5 minutes to remove all traces of ethanol. Discard the Receiver Tube.
13. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **60 µl RNase-free Water** (preheated to 70 °C).



NOTE

For proper elution make sure that pre-heated RNase-free Water contacts the Spin Filter membrane completely. Do not touch the membrane with the pipet tip.

14. Close the cap and incubate Spin Filter and Elution Tube at **70°C for 2 minutes**.



NOTE

Elution efficiency depends on the thermal effect of the preheated RNase-free Water. Make sure that the filter membrane is heated to 70° C prior to final centrifugation.

15. Centrifuge at 8,000 x g (~10,000 rpm) for 1 minute. Discard the Spin Filter, close the Elution Tube and keep it at low temperature or frozen until further use.

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient lysis and/or too much starting material	Increase lysis time. Reduce amount of starting material. Increase centrifugation speed.
Sample contains clotted material	Increase centrifugation speed.
Low amount of extracted viral RNA/DNA	
Insufficient lysis	Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
Incomplete elution	Check if incubation temperature is 70° C. Repeat elution step once again. Take a higher volume of RNase-free Water.
Insufficient mixing with Isopropanol	Mix sample with Isopropanol by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted viral RNA	
Too much RNase-free Water	Elute the viral RNA with a slightly lower volume of RNase-free Water.

For your notes: