ROBOSCREEN DIAGNOSTICS

Instructions for Use

INSTANT Virus RNA/DNA Kit

Rev. 0 _ 09 / 2020

CE

IVD

Order No.:

847-0259200602	50 reactions
847-0259200603	250 reactions

CE

Manufacturer:

Roboscreen GmbH	Phone: +49 341 989734 0	www.roboscreen.com
Hohmannstraße 7	Fax: +49 341 989734 199	info@roboscreen.com
04129 Leipzig		
Germany		

IFU INSTANT Virus RNA/DNA Kit Rev. 0 09 / 2020

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1 Introduction

1.1 Intended use

The INSTANT Virus RNA/DNA Kit has been designed for isolation both of viral RNA and DNA from different kinds of starting material. The extraction procedure is based on a new kind of chemistry (patent pending). The INSTANT Virus RNA/DNA Kit is optimized for the rapid preparation of highly pure viral RNA and DNA from cell free fluid biological samples, for example: plasma and serum and works with 400 µl liquid samples.

The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream applications, like amplification reactions and further analytical procedures. Diagnostic results generated using the extraction procedure in conjunction with diagnostic tests should be interpreted with regard to other clinical or laboratory results. To reduce irregularities in diagnostic results, internal controls for downstream applications should be used.

1.2 Test description and principle

The procedure combines lysis of starting material with subsequent binding of viral RNA and DNA onto the surface of a Spin Filter membrane. After several washing steps the viral RNA and DNA is eluted from the membrane by using RNase-free water. Extraction chemistry and extraction protocol are optimized to get maximum of yield. Further, the kit should be used with corresponding detection assays, containing Carrier RNA and an Internal Extraction Control to monitor the purification, amplification, and detection processes (see related products).

Please note that the eluates contain both viral RNA/DNA 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 RNA/DNA with other methods like specific quantitative Real-time PCR.

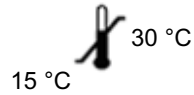
The detection limit for certain viruses depends on the individual procedures, for example in-house PCR or commercial used detection assays.

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 this manual

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 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.
	**CE-IVD symbol** *In vitro* diagnostic medical device.
	**Manufactured by** Contact information of manufacturer.
	**For single use only** Do not use components 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 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").
- 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, 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 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. Roboscreen GmbH has not tested the liquid waste generated during kit usage 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 regulations.

Clinical samples 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 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 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 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 ddH_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.

5 Storage conditions

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 reduced 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.

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 manual (→ "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 (→ "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 Components not included in the kit

- 1.5 ml tubes
- 96–99.8 % ethanol (molecular biology grade, undenaturated)
- Isopropanol (molecular biology grade)
- 2 ml tubes; optional

8.3 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 Quantifiation Kit 2.0 (for use with RotorGene™3000/6000/Q
  - 847-0207400544 32 rxn
  - 847-0207400542 96 rxn
- RoboGene HDV RNA Quantifiation Kit 2.0 for use with 7500 Fast/ Light Cycler® 480
  - 847-0207400584 32 rxn
  - 847-0207400582 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

- Heat thermal mixer or water bath to 70 °C.
- Add to each vial of **Proteinase K** 1.5 ml ddH_2O, mix thoroughly and store as described above.
- Add to **Washing Solution LS (conc.)** the indicated amount of absolute ethanol, mix thoroughly and store as described above.

847-0259200602	Add 64 ml ethanol to 16 ml Washing Solution LS (conc.).
847-0259200603	Add 144 ml ethanol to 36 ml Washing Solution LS (conc.).

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

847-0259200602	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).
847-0259200603	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.).

- Preheat aliquots of the **RNase-free Water** at 70 °C according to the desired elution volumes.

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

- Centrifugation steps should be carried out at room temperature.

10 Product Specifications

1. Starting material:

- Fresh or frozen cell-free body fluids (e.g. serum, plasma, sputum, cerebrospinal fluid).
- Stabilizers: EDTA or citrate
- 400 µl sample volume

NOTE
Avoid freezing and thawing of starting material.

2. Time for isolation:

- Approximately 30 minutes

11 Protocols for Isolation of viral RNA/DNA from 400 µl sample

11.1 Protocol 1: Isolation of viral RNA/DNA from swabs

The procedure for using swabs as starting material is identical to the procedure of protocol 2.

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

Preparation for dry swabs

1. Place the swab into a 2.0 ml 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.

11.2 Protocol 2: Isolation using IC Spiking Tube

NOTE
Pre-fill the needed amount of **RNase-free Water** into a 2.0 ml reaction tube and incubate the **RNase-free Water** at 70 °C until the elution step.

1. Vortex and centrifuge the sample prior to use.
2. Open a 2.0 ml tube (not provided) and add 80 µl **Proteinase K.** Add **400 µl of the sample** and mix well by pipetting up and down.
3. Mix **400 µl Lysis Solution CLS** with **10 µl IC solution** from the IC Spiking Tube **IC**. Add the mixture to the sample, mix vigorously by pulsed vortexing for 10 seconds. Incubate the mixture at **70 °C for 10 minutes**. After lysis centrifuge the 2.0 ml tube shortly to remove condensate from the lid of the tube.

NOTE
We recommend to use a shaking platform for a continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during incubation. No shaking will reduce the lysis efficiency.

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

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

5. Apply **850 µl of the sample** to the Spin Filter located in 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, centrifuge again at higher speed or prolong the centrifugation time.

6. Apply the **residual sample** to the Spin Filter located in 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 2.0 ml Receiver Tube.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

7. 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 2.0 ml Receiver Tube.

8. 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.

9. 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.

10. Centrifuge at max. speed for 5 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

11. 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. Close the cap and incubate at 70°C for 2 minutes. Centrifuge at 8,000 x g (~10,000 rpm) for 1 minute. Discard the Spin Filter, close the Elution Tube and place it on ice until further use.

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

NOTE
Pre-fill the needed amount of **RNase-free Water** into a 2.0 ml reaction tube and incubate the **RNase-free Water** at 70 °C until the elution step.

1. Vortex and centrifuge the sample prior to use.
2. Add **40 µl of Proteinase K** to an empty 2.0 ml tube (not provided). Add **400 µl of the sample** and mix vigorously by pulsed vortexing for 10 seconds.
3. Mix **400 µl Lysis Solution CLS** with **10 µl IC solution** from the IC Spiking Tube **IC RNA**. Add the mixture to the sample, mix vigorously by pulsed vortexing for 10 seconds. Incubate the mixture at **70 °C for 15 minutes**. After lysis centrifuge the 2.0 ml tube shortly to remove condensate from the lid of the tube.

NOTE
We recommend to use a shaking platform for a continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during incubation. No shaking will reduce the lysis efficiency.

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

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

5. Apply **800 µl of the sample** to the Spin Filter located in 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 2.0 ml Receiver Tube.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

6. Apply the **residual sample** to the Spin Filter located in 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 2.0 ml Receiver Tube.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

7. 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 2.0 ml Receiver Tube.
8. 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.
9. 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.
10. Centrifuge at max. speed for 5 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
11. 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** (preheat to 70 °C). Close the cap and incubate at room temperature for 2 minutes. Centrifuge at 8,000 x g (~10,000 rpm) for 1 minute. Discard the Spin Filter, close the Elution Tube and place it on ice 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. Increase centrifugation speed. Reduce amount of starting material.
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	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 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 lower volume of RNase-free Water.

For your notes:

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