ROBOSCREEN DIAGNOSTICS

Instructions for Use

INSTANT Virus RNA/DNA Kit – IPC16

Rev. 0 _ 05 / 2021

CE

IVD

Order No.:

847-0259200802 96 reactions

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Content

1 Introduction

1.1 Intended use

The INSTANT Virus RNA/DNA Kit - IPC16 is designed for automated isolation and purification of viral nucleic acids from cell free fluid biological samples. The kit uses magnetic-particles based technology in combination with InnuPure® C16 touch device. The kit is intended to be handled by professional users in a laboratory environment.

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 INSTANT Virus RNA/DNA Kit - IPC16 nucleic acid extraction kit in conjunction with diagnostic tests have to be interpreted in accordance with the guidelines for interpretation of the used diagnostic test.

1.2 Test description and principle

All steps of the extraction process are fully automated and run completely on the InnuPure® C16 touch. The MAG Suspension, the PK and the sample are transferred into the Reagent Plate of the kit, which is already prefilled with all extraction reagents needed for the extraction process. The extraction process is based on binding the nucleic acid to surface-modified magnetic particles. After lysis, binding and washing steps the nucleic acid is eluted from the magnetic particles with RNase-free water and is now ready to use for downstream applications. The extraction chemistry in combination with the InnuPure® C16 *touch* protocol is optimized to get maximum yield and quality.

Furthermore, 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 recommended products).

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 Restrictions

This kit is validated for automated purification of viral nucleic acids from the following starting material:

- Fresh or frozen cell-free biological fluids (e.g. serum, plasma, sputum, swab supernatant)
- Stabilizers: EDTA or citrate

The kit is to be used in conjunction with the InnuPure® C16 *touch* with a starting volume of 400 µl per specimen.

If other than the recommended sample types or volumes are used incorrect results may be obtained. The product is to be used only by personnel specially instructed and trained in *in vitro* diagnostics procedures. Do not use expired components or mix with components from different batches.

Depending on downstream process additional restrictions could be valid. Please pay attention to the corresponding instruction for use!

NOTE

Avoid repeated freezing and thawing of starting material.

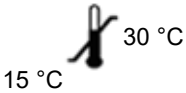
1.4 Guarantees and technical assistance

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.

1.5 Symbols and Abbreviations

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 or component.
	**CE-symbol**
	**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
- 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.

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.

2.1 Warning and precautions

For more information on GHS classification please request the Safety Data Sheet (SDS) at www.roboscreen.com site as shown inside cover page of the instruction for use.

☞ **ATTENTION!**

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

- Pay Attention while handling the materials and reagents contained in the kit. Always wear gloves while handling the reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.
- Do not eat or drink components of the kit!
- Human samples have to be considered as potentially infectious. Thus, always wear lab coat and gloves.

- Discard sample and assay waste according to your in-house safety regulations. Please observe the federal, state and local safety and environmental regulations.
- If the buffer bottles or reagent plates are damaged or leaking, wear gloves and protective goggles when discarding the bottles/plates to avoid any injuries. Roboscreen GmbH 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.
- Follow the usual precautions for applications using extracted nucleic acids.
- Do not add bleach or acidic components to the waste after sample preparation!
- Always use clean and nuclease-free equipment. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

2.2 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 surfaces 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.

- 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. 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.
- 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.

3 GHS Classification

Comp-onent	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases	EUH
PK	Proteinase K 50-100 %		315, 319, 334, 335	280, 260, 308+313, 342+311 305+351+338, 302+352,501	-
LS	Dihydrate (Ethylenedinitrilo) Tetraacetic Acid, Disodium Salt 0,1-1,0 % Guanidinium thiocyanate 40-50 %		302, 332, 314, 412	260, 280, 308+310, 305+351+338, 303+361+353, 273, 405, 501	032
Reagent Plate P (BS)	Guanidinium thiocyanate 30-40 % Propan-2-ol 10-25 %		225, 302, 332, 336, 314, 412	210, 260, 280, 308+310, 305+351+338, 303+361+353, 273, 405, 501	032
Reagent Plate P (WS A)	Guanidinium chloride 40-50% Propan–2-ol 25-35%		225, 302, 315 ,319, 336	210, 260, 280, 308+310, 305+351+338, 303+361+353, 501	

3.1 Hazard phrases

225 Highly flammable liquid and vapour.

302 Harmful if swallowed.

314 Causes severe skin burns and eye damage.

315 Causes skin irritation.

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.

336 May cause drowsiness or dizziness.

412 Harmful to aquatic life with long lasting effects.

3.2 Precaution phrases

210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

260 Do not breathe dust/fume/gas/mist/vapors/spray.

280 Wear protective gloves/protective clothing/eye protection/face protection.

308+310 IF exposed or concerned: Immediately call a POISON CENTER/doctor.

308+313 IF exposed or concerned: Call a POISON CENTER/doctor.

342+311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.

305+351+338

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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 [or shower].

273 Avoid release to the environment.

405 Store locked up.

501 Dispose of contents/container to special waste collection point.

3.3 EU hazard statements

032 Contact with acids liberates very toxic gas.

4 Performance Assessment

Extraction of viral nucleic acids is a very common application in the field of patient sample analysis. Reliable yields of high-quality purified DNA and/or RNA are substantial for further downstream applications to ensure a trustworthy patient diagnosis. In order to offer a CE-IvD-marked solution for automated extraction of both viral DNA and RNA we combined the highly efficient extraction technology of the INSTANT Virus RNA/DNA Kit – IPC16 with the InnuPure C16 *touch* extraction platform. By applying this extraction method, it is possible to perform automated nucleic acid extraction with up to 16 samples in one run with minimal manual effort. The purified nucleic acids can then be quantitatively detected using various real-time PCR kits.

Performance of nucleic acid extraction from RNA and DNA viruses using the INSTANT Virus RNA/DNA Kit – IPC16 and the InnuPure C16 *touch extraction platform* was validated in combination with RoboGene HCV RNA Quantification Kit 3.0 (article number 847-0207610-032/096/132) and RoboGene HBV DNA Quantification Kit 3.0 (article number 847-0207710-032/096/132).
For both parameters 400 µl of plasma were used as sample material. The Elution volume was set to 60 µl and for qPCR amplification 10 µl of the eluates were applied.

4.1 Analytical sensitivity

The analytical sensitivity of the INSTANT Virus RNA/DNA Kit – IPC16 measured in combination with the Hepatitis C Virus (RNA Virus) was determined by analyzing dilution series of the PEI Reference Material HCV RNA (#3443/04, genotype 1). Analytical sensitivity obtained with different qPCR devices is summarized in table 1.

Table 1: Determined device specific limits of detection analyzed by hit rate of the Hepatitis C Virus

PCR device	CFX96		qTOWER³		Light Cycler 480	
CFX96 (CFX)	Numbers analyzed	Hit Rate	Numbers analyzed	Hit Rate	Numbers analyzed	Hit Rate
100 IU/ml	24/24	100 %	24/24	100 %	24/24	100 %
75 IU/ml	24/24	100 %	21/24	88 %	21/24	88 %
50 IU/ml	18/24	75 %	21/24	88 %	23/24	96 %
25 IU/ml	17/24	71 %	17/24	71 %	15/24	63 %
12.5 IU/ml	14/24	58 %	8/24	33 %	11/24	46 %
6.25 IU/ml	8/24	33 %	6/24	25 %	6/24	25 %
3.125 IU/ml	4/24	17 %	4/24	17 %	4/24	17 %
Negativ	24/24	100 %	24/24	100 %	24/24	100 %

Summary of analytical sensitivities for RNA Virus Hepatitis C Virus via hit rate

Table 2: Estimation of Analytical Sensitivity for RNA Virus Hepatitis C Virus by hit rate. Extraction was performed with INSTANT Virus RNA/DNA Kit – IPC 16 in combination with the extraction platform InnuPure C16 touch. Detection was performed with RoboGene HCV RNA Quantification Kit 3.0.

CFX	Between 50 and 75 IU/ml
qTOWER³	Between 75 and 100 IU/ml
Light Cycler 480	Between 75 and 100 IU/ml

The analytical sensitivity of the INSTANT Virus RNA/DNA Kit – IPC 16 measured in combination with the Hepatitis B Virus (DNA Virus) was determined by analyzing dilution series of the PEI Reference Material HBV DNA (#3620/05, genotype D). Analytical sensitivity obtained with different qPCR devices is summarized in table 3.

Table 3: Determined device specific limits of detection analyzed by hit rate of the Hepatitis B Virus

PCR device	CFX96		qTOWER³		Light Cycler 480	
CFX96 (CFX)	Numbers analyzed	Hit Rate	Numbers analyzed	Hit Rate	Numbers analyzed	Hit Rate
100 IU/ml	24/24	100 %	24/24	100 %	24/24	100 %
75 IU/ml	24/24	100 %	24/24	100 %	24/24	100 %
50 IU/ml	24/24	100 %	24/24	100 %	24/24	100 %
25 IU/ml	24/24	100 %	24/24	100 %	24/24	100 %
12.5 IU/ml	19/24	79 %	20/24	83 %	20/24	83 %
6.25 IU/ml	15/24	63 %	19/24	79 %	15/24	63 %
3.125 IU/ml	13/24	54 %	13/24	54 %	12/24	50 %
Negativ	24/24	100 %	24/24	100 %	24/24	100 %

Summary of analytical sensitivities for DNA Virus Hepatitis B Virus via hit rate

Table 4: Estimation of Analytical Sensitivity for DNA Virus Hepatitis B Virus by hit rate. Extraction was performed with INSTANT Virus RNA/DNA Kit – IPC 16 in combination with the extraction platform InnuPure C16 touch. Detection was performed with RoboGene HBV DNA Quantification Kit 3.0.

CFX	Between 25 and 12,5 IU/ml
qTOWER³	Between 25 and 12,5 IU/ml
Light Cycler 480	Between 25 and 12,5 IU/ml

4.2 Linear range

The linear range was exclusively determined by extraction of the RNA Parameter Hepatitis C Virus. Therefore, dilution series from $1x10^6$ to $1 x 10^2$ IU/ml was analyzed. Experimental assessment was performed twice, extracting each dilution in duplicates. All samples were quantified using CFX96, qTOWER³ and LightCycler® 480 for real-time PCR devices.

Obtained quantification results covered a linear range over 4 $\log_{10}$ steps from $1x10^6$ to $1 x 10^2$ IU/ml with excellent linearity.

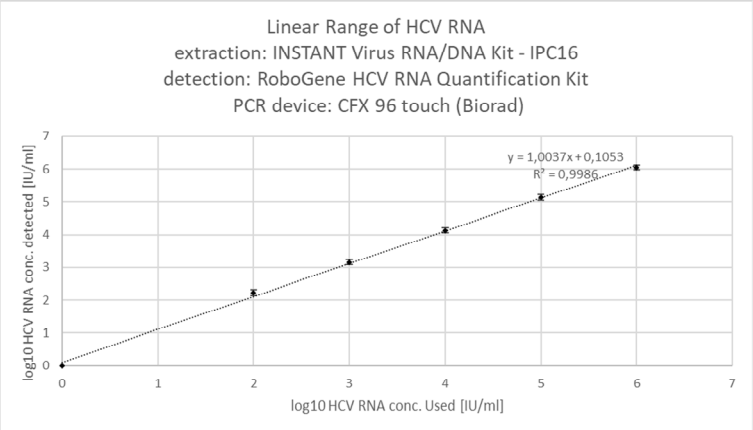


Figure 1 Linear Range obtained with the CFX96 device. Mean quantification values are shown and variabilities are indicated by standard deviations.

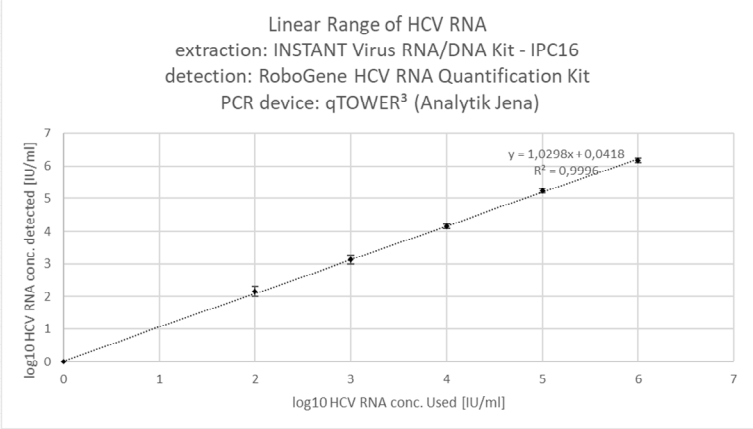


Figure 2 Linear Range obtained with the qTOWER³ device. Mean quantification values are shown and variabilities are indicated by standard deviations.

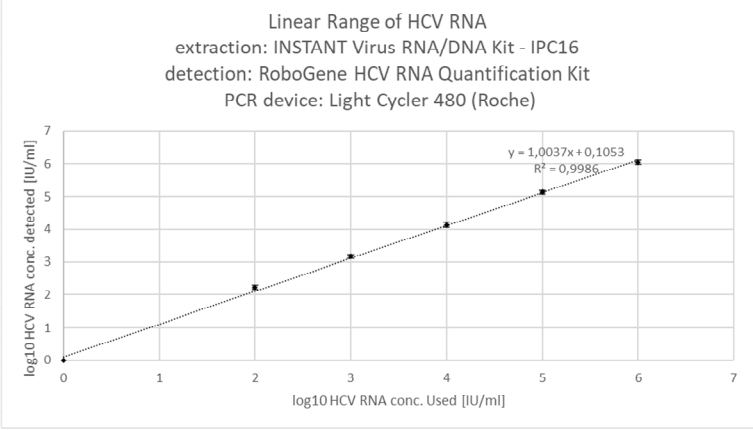


Figure 3 Linear Range obtained with the Light Cycler 480 device. Mean quantification values are shown and variabilities are indicated by standard deviations.

4.3 Precision

The precision data represent the complete test procedure of HBV DNA and HCV RNA comprising nucleic acid extraction with the INSTANT Virus RNA/DNA Kit – IPC 16 and nucleic acid detection using RoboGene HBV DNA Quantification Kit 3.0 or RoboGene HCV RNA Quantification Kit 3.0, respectively.

Dilution series of HBV/HCV-positive patient specimen consisting of 3 different viral load levels and a range of 3-$\log_{10}$ steps were measured on 3 different days with 3 different batches of INSTANT Virus RNA/DNA Kit – IPC16. The analysis was carried out with RoboGene HBV RNA Quantification Kit 3.0 and RoboGene HCV RNA Quantification Kit 3.0 and the CFX96 real-time PCR device.

Results showed overall reliable precision for INSTANT Virus RNA/DNA Kit – IPC16 within the ± 0.6 log10 acceptance interval of different parameters like processing days and batches.

Table 5: Intra-run-precision of the INSTANT Virus RNA/DNA Kit – IPC16 with extraction of HCV RNA and HBV DNA over all factors, different batches and different processing days.

target	conc.	Intra-run-precision - CV [%]						
	[IU/ml]	all factors	lot 1	lot 2	lot 3	day 1	day 2	day 3
HCV	25,000	18	17	13	28	13	32	13
	2,500	16	17	17	12	5	21	21
	250	24	18	29	28	21	17	31
HBV	25,000	7	9	7	2	8	2	9
	2,500	7	13	2	8	11	4	7
	50	28	27	24	33	65	5	19

Table 6: Inter-run-precision of the INSTANT Virus RNA/DNA Kit – IPC16 with extraction of HCV RNA and HBV DNA over all factors, different batches and different processing days.

target	conc.	Inter-run-precision - CV [%]						
	[IU/ml]	all factors	lot 1	lot 2	lot 3	day 1	day 2	day 3
HCV	25,000	27	11	22	19	33	40	15
	2,500	38	26	31	23	55	53	20
	250	40	21	42	16	50	44	39
HBV	25,000	26	17	7	10	16	45	20
	2,500	22	22	16	14	8	39	15
	50	38	35	48	36	51	28	25

5 Kit components, storage and stability

5.1 Kit components

Kit component	Description	Σ 96
REF	847-0259200802	
PK	Proteinase K, lyophilized (for 1.5 ml working solution each)	for 4 x 1.5 ml working solution
MAG	Magnetic Particle Suspension	4 x 1 ml
LS	Lysis Solution	1 x 60 ml
PCR grade H_2O	RNase free water	1 x 10 ml
Reagent Plate P	Pre-filled, sealed	12 pcs (prefilled, sealed)
Elution Strips	collecting vessel for eluates	12 pcs
IFU	Instruction for use	1

5.2 Storage and Stability

All kit components are shipped at ambient temperature. Upon arrival, store lyophilized **PK** and **MAG** at 2 °C to 10 °C. An appropriate amount of **PK** should be dissolved in **PCR grade H2O** shortly before use. Remaining dissolved **PK** can be aliquoted properly and stored at -20 °C. Stored aliquots can be used up to 20 days. Repeated freezing and thawing up to 5 times are possible. To avoid reduced activity freezing and thawing beyond these cycles should be avoided.

All other components of the INSTANT Virus RNA/ DNA Kit – IPC16 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.

5.3 Necessary laboratory equipment required

Component	Manufacturer	Order number
Innu Pure C16 *touch*	Analytik Jena GmbH	845-00020-2
FX Filter Tips 1000 µl	Roboscreen GmbH	847-FX-TIPS-1000

5.4 Recommended Products

Component	Manufacturer	Order number
Carrier RNA	Roboscreen GmbH	847-0206201001

NOTE
For 96 reactions of the INSTANT Virus RNA/DNA Kit – IPC16 2 tubes of Carrier RNA are required.

5.5 General laboratory equipment required

- 1.5 ml tubes
- Pipettes with CV <3 % and suitable filtered tips
- Centrifuge
- Vortex mixer
- Sample rack
- Gloves, lab coat

6 General Procedure

6.1 Collection and handling of clinical plasma/serum samples

- For Plasma collect 5-10 ml blood with standard specimen collection tubes using anticoagulants like EDTA or citrate.
- Store whole blood at 2-25 °C not longer than 6 hours, centrifuge for 20 min at 800-1600 x g to separate plasma or serum from blood cells and transfer to sterile tubes.
- Plasma or serum samples may be transported at ambient temperature; do not exceed 6 hours of time after blood collection.
- For long time storage of samples please use your in-house regulations and validated protocols. Commonly used protocols recommend storage of plasma and serum samples at -70°C or colder, use of screw-cap tubes and avoidance of repeated freezing and thawing.

6.2 Sample preparation for viral NA extraction from swabs

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

Preparation for dry swabs

1. Place the swab into a 2.0 ml reaction tube containing 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**.

6.3 Sample collection and preparation for viral NA extraction from stool

- Collect 1 gram or 1 ml stool, respectively with standard specimen collection tubes
- Samples may be transported at room temperature, do not exceed the time 1 day after sample collection. Otherwise ensure transport on ice
- Samples or RNA may be stored deeply frozen for several months at -20°C to -70°C, stability depending on the storage temperature.

In case of using stool as starting material please carry out the following preparation instructions with the protocol.

Preparation for stool sample

1. Transfer about 0.1 g of the stool sample into a 1.5 ml reaction tube and add 1000 µl 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**.

NOTE

If the stool sample is very solid, the resuspension can be extended; or try to separate the sample into smaller pieces by pipetting up and down. It may be necessary to cut off the pipette tip in order to be able to pipette more easily.

If the eluates obtained are cloudy, we recommend centrifuging them for 3 minutes at maximum speed (20,000 g) to clarify the eluates. This centrifugation does not have a negative effect on the eluates; rather, it improves the results that are obtained with cloudy eluates in subsequent applications.

6.4 Initial steps before starting

6.4.1 Preparation of PK

1. Centrifuge the **PK** tubes briefly at full speed to collect the lyophilized Proteinase K on the bottom of the tube.
2. Add **1500 µl of PCR grade H_2O** to each vial of the **PK**.
3. Close the tubes, mix by vortexing briefly followed by brief centrifugation.

6.4.2 Preparation of Internal Control and Carrier RNA

The application of Carrier RNA and an internal control for RNA and DNA is strongly recommended when using the INSTANT Virus RNA/DNA Kit -IPC16 in combination with diagnostic amplification systems. Amongst other things Carrier RNA prevents RNA degradation. If not added it can lead to reduced yield of viral nucleic acids. The entrainment of an internal control of RNA and DNA serves as a control of an accurate execution of the whole procedure.

Carrier RNA as well as internal control are often part of the detection kit. Therefore, they are not part of the INSTANT Virus RNA/DNA Kit – IPC16. For correct concentration of internal control and carrier RNA please refer to manufacturer's instructions of your further downstream process.

IMPORTANT NOTE

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

In case Carrier RNA is not included within the detection assay and their internal control, Carrier RNA can be obtained separately from Roboscreen GmbH.

6.4.3 Preparation of Carrier RNA (REF 847-0206201001)

The Carrier RNA (not part of the INSTANT Virus RNA/DNA Kit – IPC16) can be used as follow:

1. Centrifuge **Carrier RNA** Tube briefly at full speed to collect the lyophilized Carrier RNA on the bottom of the tube.
2. Add **520 µl PCR grade H_2O** per vial; close the tube, mix by vortexing briefly followed by brief centrifugation.
3. Incubate at 37 °C for 5 min using a shaking platform (800-1,000 rpm), mix by vortexing briefly followed by brief centrifugation.

6.4.4 Preparation of LS – Carrier RNA Mix and/or Internal Control of your downstream process

For the correct ratio of IC solution/Carrier RNA and lysis buffer, mix the solutions as indicated in the following table.

Sample volume	400 µl
Volume LS	(N+1) x 400 µl
Volume IC / Carrier RNA	(N+1) x Vol IC/Carrier RNA per reaction

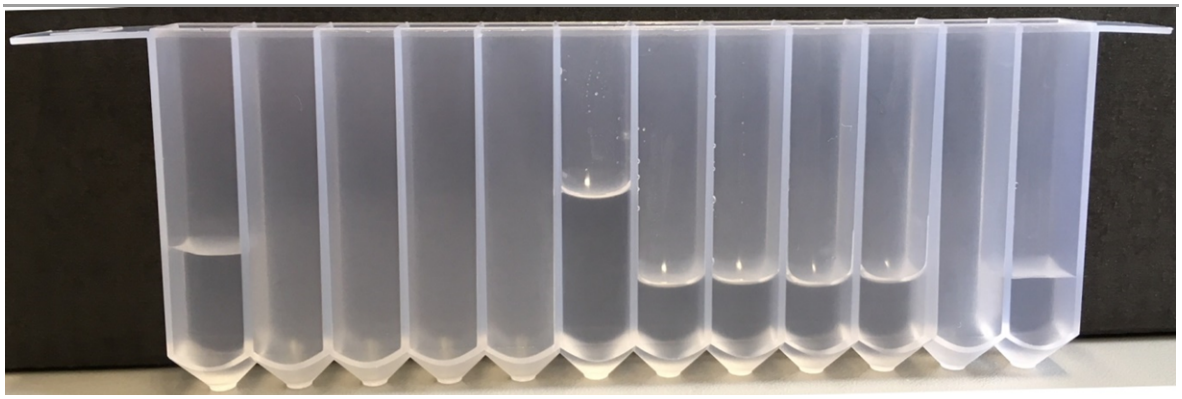
Gently mix solution and avoid foaming, do not vortex.

☞ **NOTE**

Prepare internal controls/ carrier mixes of other suppliers according to the manufacturer's instructions and suitable for required number of reactions plus one (N+1) as shown in the table.

7 Automated extraction using InnuPure® C16 *touch*

7.1 General filling scheme of reagent reservoir



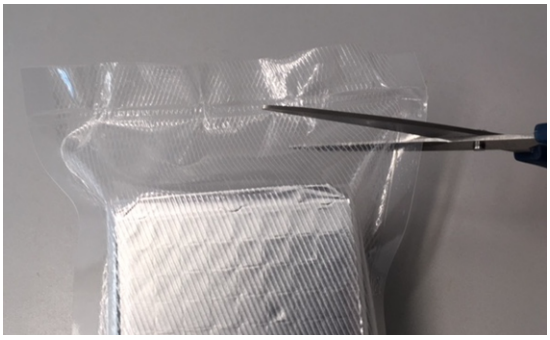
Cavity 1:	RNase-free water	Cavity 7:	Washing Solution
Cavity 2:	Empty	Cavity 8:	Washing Solution
Cavity 3:	Empty	Cavity 9:	Washing Solution
Cavity 4:	Empty	Cavity 10:	Washing Solution
Cavity 5:	Empty	Cavity 11:	Empty
Cavity 6:	Binding Solution	Cavity 12:	Elution Buffer

For Orientation

Cavity 1 is left to the label of the Reagent Plate P.

7.2 Unpacking of Reagent Plate P and piercing of sealing foil

A Unpacking of Reagent Plate P



Reagent Plates are delivered wrapped into plastic bags for transport protection.

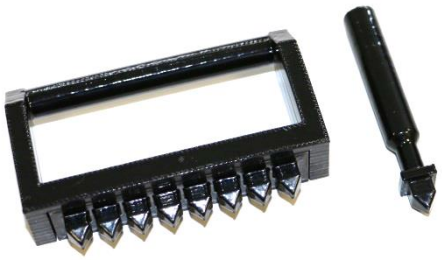
Carefully open the overpack of Reagent Plates by using scissors.

B Piercing of sealing foil

NOTE

Invert the Reagent Plates 3–4 times and thump it onto a table to collect the pre-filled solutions at the bottom of the wells.

Before using Reagent Plates the sealing foil has to be pierced manually. Always wear gloves while piercing of the foil!

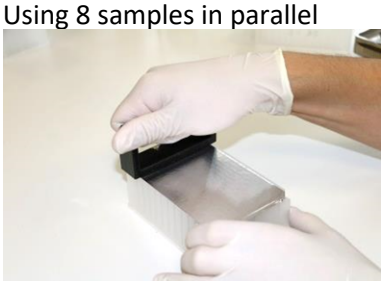
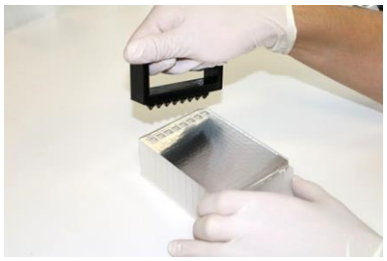
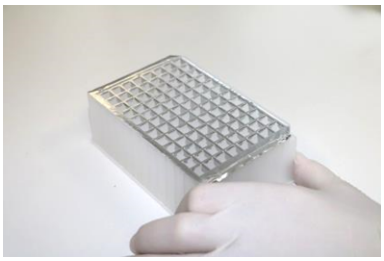


Reagent Plates are prefilled with extraction reagents and are sealed with a foil. Prior to use this foil has to be pierced manually, by using the piercing tools (single piercer or 8fold piercer).

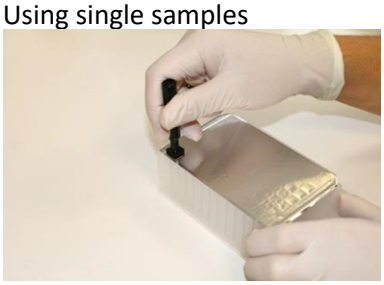
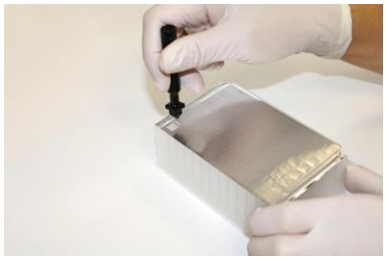
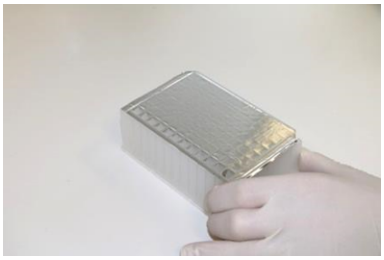
Keep the Reagent Plates in a horizontal position to avoid spilling of the reagents while piercing of the foil.

Open all cavities (one row per sample).

Using 8 samples in parallel

Using single samples

IMPORTANT

Open all cavities of one row per sample!

7.3 Protocol: Isolation of viral nucleic acids from 400 µl starting material

Time for isolation:

Extraction protocol	Protocol on InnuPure® C16 *touch*	Time InnuPure®C16 *touch*	Elution volume
Virus RNA/DNA 400 µl - 06	400 µl	85 min	20–200 µl

Note

The lysis of the starting material is done automatically and is included in the InnuPure® C16 *touch* extraction protocol.

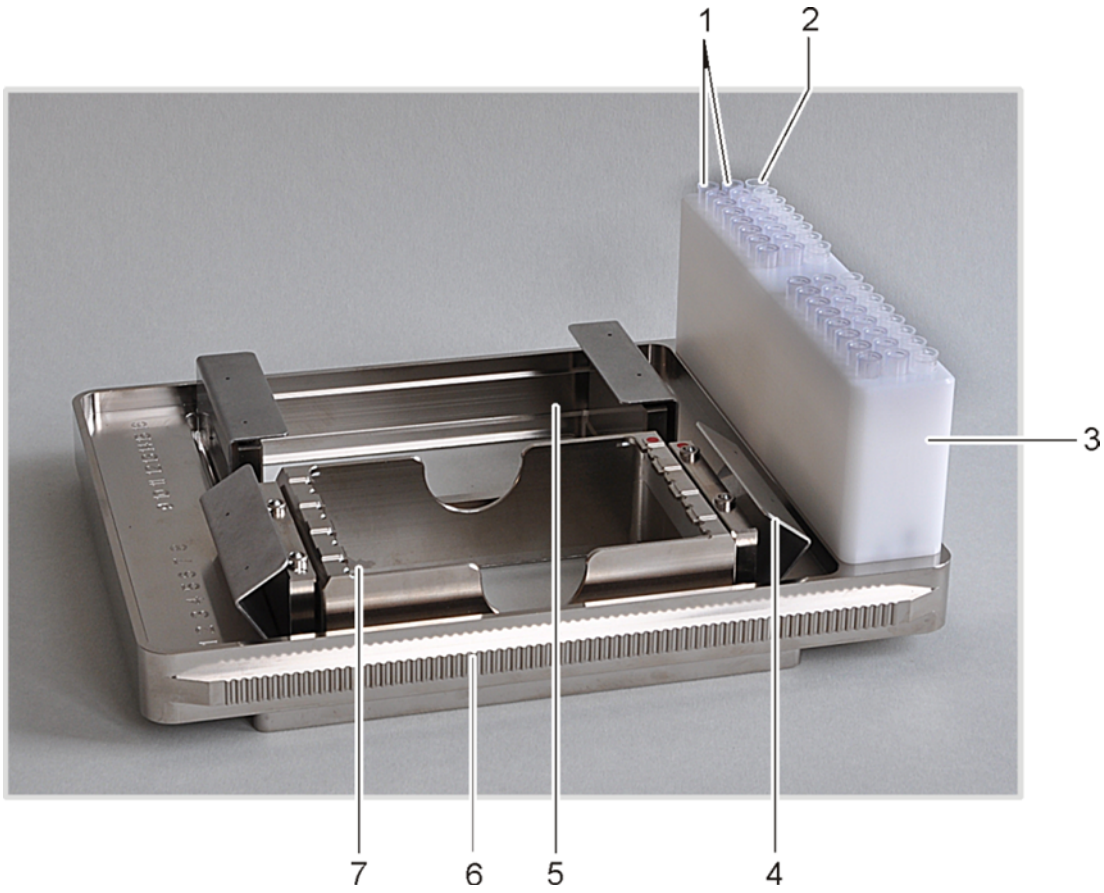
IMPORTANT

It is important to mix the MAG Suspension by vigorous shaking or vortexing before use (approx. 60 seconds)!

1. If there are any precipitates in the sample centrifuge it before use (e.g 6800 x g for 3 minutes). Use the clear supernatant, don't disturb the pellet.
2. Prepare a mixture of **LS** and **IC /Carrier RNA solution** dependent on the number of samples to be processed, e.g. mix 400 µl Lysis Solution V and **10 µl Carrier RNA solution** from Carrier RNA Tube for one sample and use **400 µl** of mixture for preparation.
3. Vortex **MAG** for at least 60 seconds vigorously.
4. Transfer **35 µl** of the **MAG** Suspension directly into the **first cavity** of the Reagent Plate containing RNase-free water. Ensure that the **MAG Suspension** is mixed well before starting. For optimal results it is recommend to mix the **MAG** Suspension after every second pipetting step gain.
5. Transfer **50 µl PK** into the **third cavity**.

6. Vortex and centrifuge the sample shortly prior use.
7. Add 400 µl of the sample directly into the **third cavity** of Reagent Plate.
8. Add 400 µl of the LS / IC/Carrier RNA solution to the **third cavity** of Reagent Plate.

7.4 Sample tray of InnuPure® C16 touch



No. 1:	Filter tips
No. 2:	Elution Strips
No. 3:	Tip block
No. 4:	Holding-down clamp
No. 5:	Sample block for Reagent Plates P
No. 6:	Serrated guide rail (C16 *touch*: non-serrated)
No. 7:	Adapter for Reagent Strips (not applicable for INSTANT Kits)

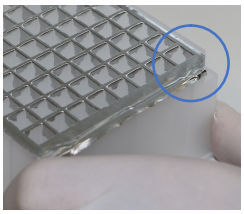
7.5 Preparing sample tray of InnuPure® C16 *touch*

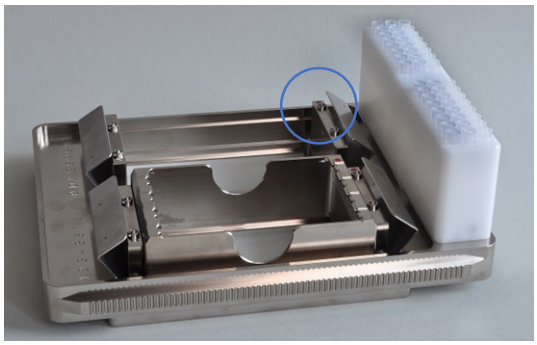
NOTE
The needed number of Reagent Plates depends on the number of samples, which have to be processed.

1. Place the InnuPure® C16 touch sample tray into the priming station and fold the holding-down clamp at the sample tray upwards!
2. Place the Reagent Plate into the holder of the sample tray. Using Reagent Plates, the notched corner of the Reagent Plate has to align with the colored dot at the holder.

Reagent Plate

The notched corners of the Reagent Plate must point to the colored dot on the holder.





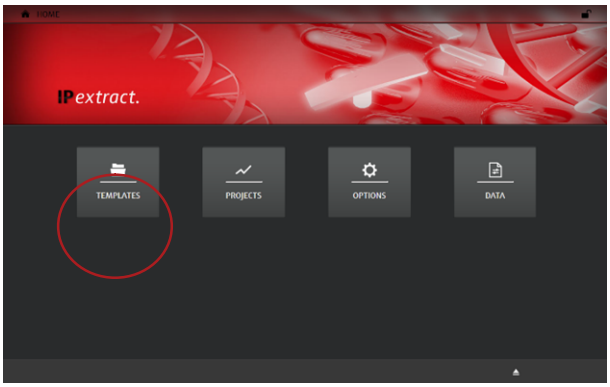
CAUTION
Both holders have to be equipped with a Reagent Plate. If applicable use an empty dummy plate for the respective holder.

3. Fold down the holding-down clamp to prevent the Reagent Plates to be pulled out of the holder during the extraction process.
4. For each extracted sample place two filter tips (847-FX-TIPS-1000) in the smaller drill holes of the tip block.
5. Place the Elution Strips into the wider drill hole at the edge of the tip block.

7.6 Starting the InnuPure® C16 *touch*

NOTE
The following instructions describe the necessary steps for the start of the InnuPure® C16 *touch*. For further features and data entry (e.g. opening templates, entering sample setups, saving projects) refer to the manual of the InnuPure® C16 *touch*.

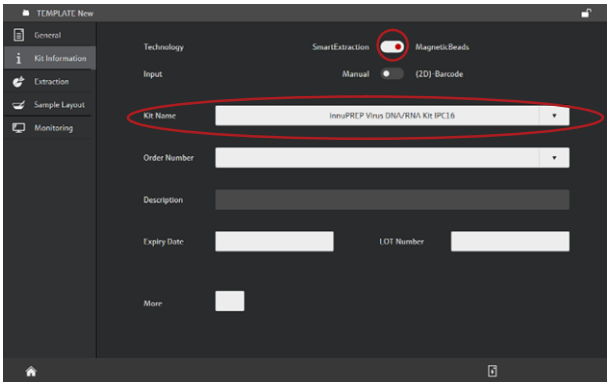
1. Switch on the InnuPure® C16 touch and the tablet computer. Wait until the home screen of IPextract is displayed on the tablet screen.



NOTE
Home screen of IP*extract*

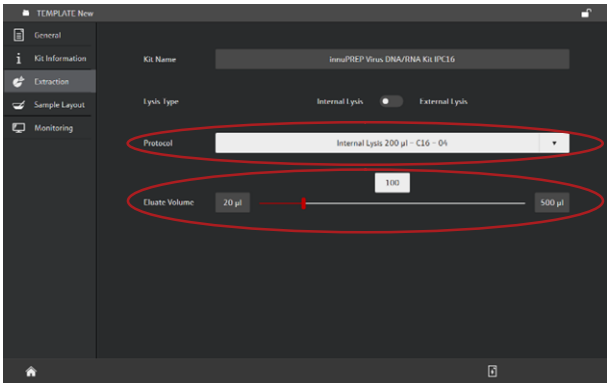
2. Choose [TEMPLATES]→[New Template]→[Kit-based].
3. Enter optional information in the tab “General”.
4. Choose the tab “Kit Information” and switch the “Technology” to “MagneticBeads”!

5. Go to “Kit Name” and choose “**INSTANT Virus RNA/DNA Kit – IPC16**”!



NOTE
“Kit Information” tab

6. Enter optional information in the tab “Kit Information”
7. Choose the tab “Extraction” and choose the following protocol:
   “Virus RNA/DNA 400 µl – 06”.
8. Adjust your desired “Eluate Volume“ using the slider or the text field.

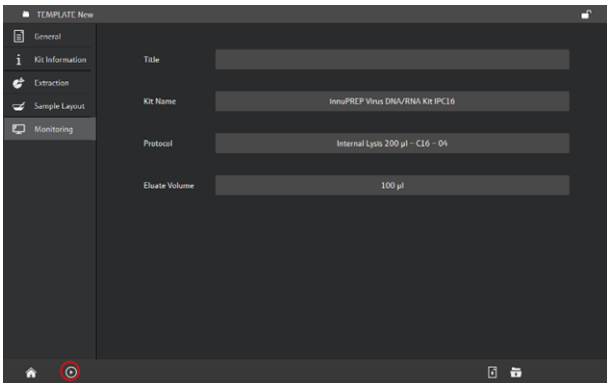


NOTE
“Extraction” tab

The performance data were collected with an elution volume of 60 µl. Therefore, the recommended elution volume is **60 µl**.

It is recommended to carry out your own validations based on this volume, as the result depends heavily on the elution volume.

9. Choose the tab “Monitoring” and start the protocol by tapping the start button.



NOTE
“Monitoring” tab

10. Follow the instructions displayed on the tablet screen.
11. Completion of the protocol is indicated by a message on the tablet screen. Follow the instructions on the screen to remove the sample tray from the device.
12. The Elution Strips contain the extracted DNA or RNA. Close the lids and store eluate under proper conditions.

NOTE
Store the DNA and RNA under adequate conditions, for example store RNA/DNA on ice until amplification. For long term storage we recommend storing either at -15 °C to -40 °C or even > -70°C!

8 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted viral RNA/DNA	
Content of viral nucleic acid in sample insufficient.	Use the right volume of starting material 400 µl. Mix **MAG** Suspension well before usage!
Insufficient lysis of starting material.	Ensure to use the required volume of **PK** for current protocol.
Inadequate extraction.	Inhibiting substances in starting material. Please use the kit only for samples that match the requirements declared in “Product specifications”. Use Internal Controls for verification of extraction procedure.
No use of Carrier RNA	Depending on your overall workflow, it can be beneficial to use carrier RNA for a higher yield. If Carrier RNA is not part of your downstream application, you can provide Carrier RNA separately by Roboscreen GmbH.
Weak amplification of internal Control	
No use of Carrier RNA	Since internal control has a different structure than viral nucleic acid, it can be more vulnerable to RNases / Dnases, so that the amplification can be weaker. If Carrier RNA is not part of your downstream application, you can provide Carrier RNA separately by Roboscreen GmbH.