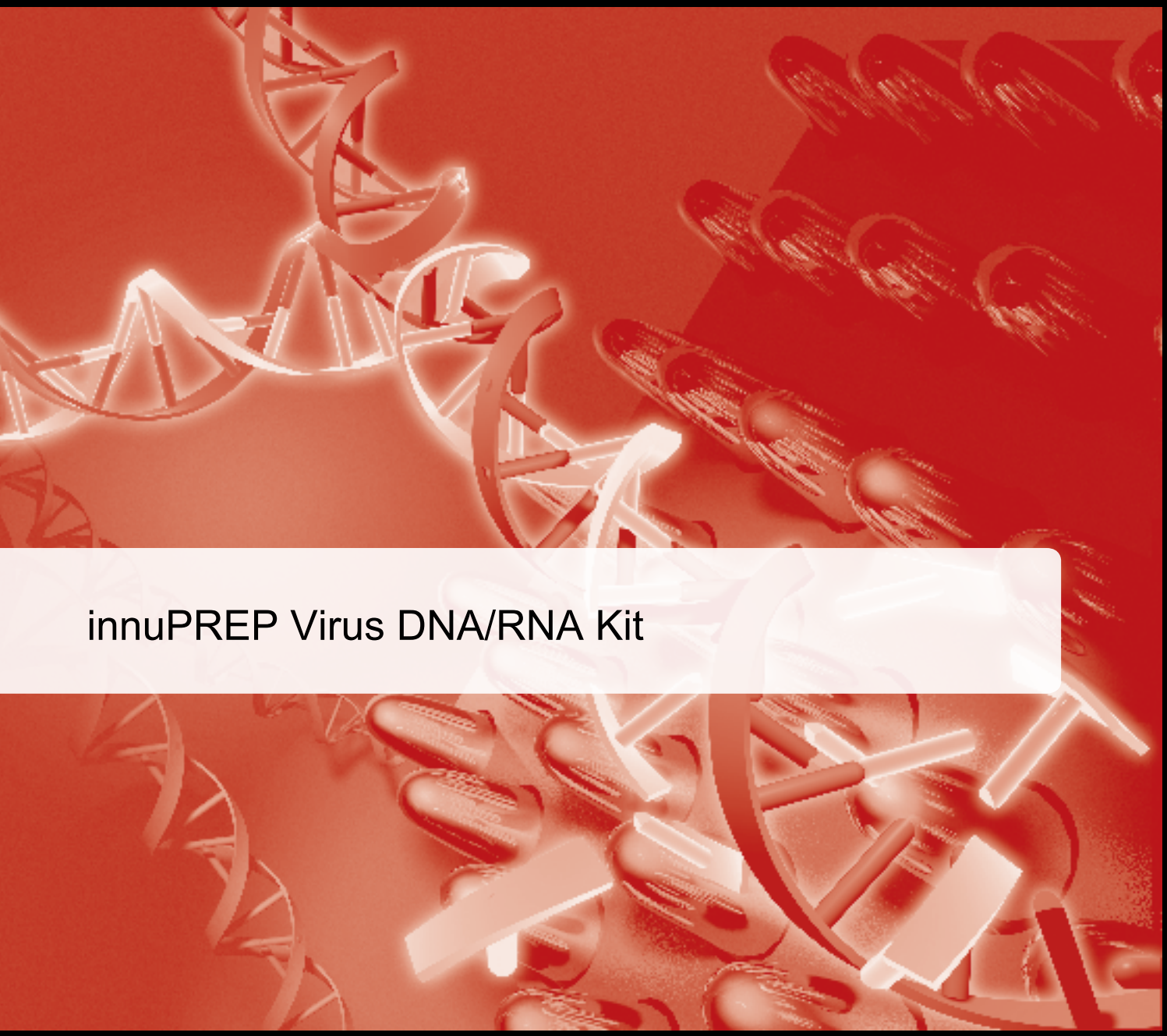
analytikjena

Manual



innuPREP Virus DNA/RNA Kit

Life Science unlimited

Order No.:

845-KS-4800010	10 reactions
845-KS-4800050	50 reactions
845-KS-4800250	250 reactions

Publication No.: HB_KS-4800_e_131218

This documentation describes the state at the time of publishing.
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Contents

1 Introduction

1.1 Intended use

The innuPREP Virus DNA/RNA Kit has been designed for isolation of viral DNA and RNA from different kinds of starting material. The extraction procedure is based on a new kind of chemistry (patent pending). The innuPREP Virus DNA/RNA Kit is optimized for the rapid preparation of highly pure viral nucleic acids from cell free fluid biological samples, for example: plasma, serum, urine, liquor as well as cell culture supernatant, solid materials and swabs.

The procedure combines lysis of starting material with subsequent binding of viral nucleic acids onto the surface of a Spin Filter membrane. After several washing steps the viral nucleic acids are eluted from the membrane by using RNase-free water. Extraction chemistry and extraction protocol are optimized to get maximum of yield. 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 and 400 µl liquid, tissue and swab samples. The extracted viral nucleic acids are suitable for downstream applications like PCR, real-time PCR or any kind of enzymatic reaction.

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

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 viral 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 add Carrier RNA has to be carefully optimized depending on the individual PCR system used.

Note

In some cases the recovery of viral RNA can be better using the innuPREP Virus RNA Kit (see related products).

Consult instruction for use

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

1.2 Notes on the use of this manual

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

REF
Catalogue number

N

Content
Contains sufficient reagents for <N> reactions

Storage conditions
Store at room temperature or shown conditions respectively

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

Used by
Expiry date.

Lot number
The number of the kit charge

Manufactured by
Contact information of manufacturer

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

Note / Attention
Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

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

2 Safety precautions

Note

Read through this chapter carefully prior to guarantee your own safety and a trouble-free operation.

Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

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

For single use only!

This kit is made for single use only!

Attention!

Don’t eat or drink components of the kit!

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

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. Analytik Jena AG has not tested the liquid waste generated during using the kit for potential residual infectious components. This case is highly unlikely, but cannot be excluded completely. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

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

Attention!

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

Note

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany

Phone: +49 (0)761 19 240.

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

3 Storage conditions

The innuPREP Virus DNA/RNA Kit should be stored dry, at room temperature (14– 25 °C) and is stable for at least 12 months under these conditions. Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming. For further information see table kit components (→ "Kit components" p. 7).

4 Function testing and technical assistance

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

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

5 Product use and warranty

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

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

Note

For research use only!

6 Kit components

Important

Store lyophilized Proteinase K at 4 °C!

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

Store lyophilized Carrier Mix at – 20 °C.

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

Storage conditions

All other components are stored at room temperature.

	Σ 10	Σ 50	Σ 250
REF	845-KS-4800010	845-KS-4800050	845-KS-4800250
Lysis Solution CBV	5 ml	25 ml	120 ml
Binding Solution SBS	15 ml	60 ml	250 ml
Carrier Mix	1x lyophilized powder	1x lyophilized powder	3x lyophilized powder
RNase-free water	2.0 ml	2.0 ml	3x 2.0 ml
Proteinase K	for 1 x 0.3 ml working solution	for 1 x 1.5 ml working solution	for 4 x 1.5 ml working solution
Washing Solution HS	5 ml (final volume 10 ml)	15 ml (final volume 30 ml)	70 ml (final volume 140 ml)
Washing Solution LS	6 ml (final volume 30 ml)	16 ml (final volume 80 ml)	2 x 36 ml (final vol. 2 x 180 ml)
RNase-free water	2.0 ml	2 x 2.0 ml	25 ml
Spin Filter (blue)	10	50	5 x 50
Receiver Tubes (2.0 ml)	50	5 x 50	25 x 50
Elution Tubes (1.5 ml)	10	50	5 x 50
Manual	1	1	1

	Σ 10	Σ 50	Σ 250
REF	845-KS-4800010	845-KS-4800050	845-KS-4800250
Initial steps	• Add 5 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 24 ml of 96-99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 0.3 ml of ddH_2O, mix thoroughly and store as described below! • Add 1.25 ml RNase-free Water to the tube Carrier Mix, mix thoroughly by pipetting up and down!	• Add 15 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 64 ml of 96-99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described below! • Add 1.25 ml RNase-free Water to the tube Carrier Mix, mix thoroughly by pipetting up and down!	• Add 70 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed! • Add 144 ml of 96-99.8 % ethanol to each bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described below! • Add 1.25 ml RNase-free Water to each tube Carrier Mix, mix thoroughly by pipetting up and down!

7 Recommended steps before starting

- Heat thermal mixer or water bath at 70 °C
- Pre-heat RNase-free water at 70 °C.

Note: Do not use pre-heat RNase-free water for Carrier Mix.

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

8 Components not included in the kit

- 1.5 ml reaction tubes
- 2.0 ml reaction tubes; optional
- 96 – 99.8 % ethanol
- ddH_2O for dissolving Proteinase K

9 General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free.)
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240 °C for four or more hours before use. Autoclaving alone will not inactivate many RNases completely. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC (diethyl pyrocarbonate). The glassware has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C and then it has to be autoclaved or heated to 100 °C for 15 min to remove residual DEPC.
- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5 % SDS), thoroughly rinsed with RNase-free water, rinsed with ethanol and finally allowed to dry.
- All buffers have to be prepared with DEPC-treated RNase-free 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.

10 Carrier Mix

10.1 Storage conditions and handling

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

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

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

10.2 Preparation of Lysis Solution CBV / Carrier Mix

1. Add 1.25 ml RNase-free Water to each tube Carrier Mix.
2. Mix thoroughly by pipetting up and down!

   After the preparation of Carrier Mix stock solution prepare the mixture of Lysis Solution CBV / Carrier Mix as described in the following tables:

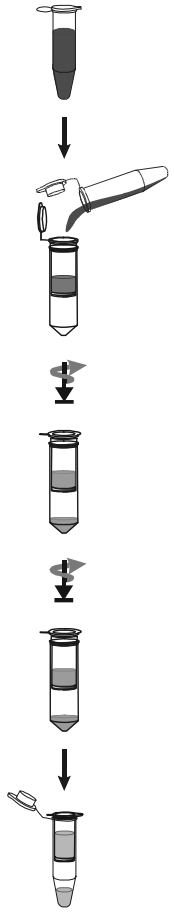
Solution Mix for extraction from 200 µl of serum/plasma or other cell-free body fluids:

Component	5 samples	10 samples	n samples
Lysis Solution CBV	1.2 ml	2.4 ml	240 µl x sample
Carrier Mix	60 µl	120 µl	12 µl x sample
Final volume	1.26 ml	2.52 ml	252 µl x sample

Solution Mix for extraction from 400 µl of serum/plasma or other cell-free body fluids:

Component	5 samples	10 samples	n samples
Lysis Solution CBV	2.4 ml	4.8 ml	480 µl x sample
Carrier Mix	60 µl	120 µl	12 µl x sample
Final volume	2.46 ml	4.92 ml	492 µl x sample

11 General procedure for viral DNA/RNA extraction



Lysis of starting material

Binding of viral nucleic acids onto Spin Filter (blue)

Washing of the bound nucleic acids

Elution of viral DNA/RNA

12 Product specifications

1. **Starting material:**
   - Serum, plasma, cell culture supernatants and other cell-free body fluids (200 µl and 400 µl)
   - Tissue samples and biopsies (max. 20 mg)
   - Swab samples

2. **Time for isolation:**

   Approximately 25 minutes

3. **Positive tested for:**

   E.g. HBV, CMV, EBV, HSV, FMDV

13 Protocol 1: Isolation of viral DNA/RNA from serum, plasma, cell culture supernatants/mediums or cell-free body fluids up to 200 µl

Important

Pre-fill the needed amount of RNase-free Water in a 1.5 ml reaction tube and incubate the RNase-free Water at 70 °C until the elution step.

Prepare Lysis Solution CBV / Carrier Mix as described on page 10 before starting!

1. Pipette **200 µl Lysis Solution CBV / Carrier Mix** into a 2.0 ml reaction tube. Add **200 µl of the sample and 20 µl of Proteinase K**, mix vigorously by pulsed vortexing for 10 sec. Incubate at 70 °C for 10 minutes.

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

   After lysis centrifuge the reaction tube shortly to remove condensate from the lid of the tube.

2. Add **400 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

   Note: It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

3. Apply the sample to the Spin Filter (blue) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

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

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

4. 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.

5. 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.

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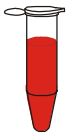
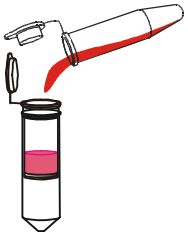
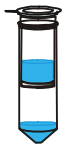
Protocol 1: Isolation of viral DNA/RNA from cell-free body fluids up to 200 µl

Recommended steps before starting

- Heat thermal mixer or water bath (70 °C)
- Prepare Washing Solution HS, Washing Solution LS, Proteinase K and Carrier Mix according to the instruction
- Pre-heat the needed amount of RNase-free water (70 °C)

1.	Starting material	▪ Serum, plasma, cell culture supernatants / medium ▪ Cell-free body fluids	▪ Add 200 µl into an 2.0 ml reaction tube
2.	Lysis	 	▪ Add 200 µl CBV/Carrier Mix and 20 µl PK ▪ Vortex: 10 sec ▪ Incubation: 10 min @ 70 °C ▪ Centrifuge: shortly
3.	Binding of DNA/RNA New Receiver Tubes	 	▪ Add 400 µl SBS ▪ Vortex ▪ Add Spin Filter to Receiver Tube ▪ Add sample to Spin Filter ▪ 10.000 x g (~12.000 rpm): 1 min
4.	Washing New Receiver Tubes	 	▪ Add 500 µl HS ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Add 650 µl LS ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Add 650 µl LS ▪ 10.000 x g (~12.000 rpm): 1 min
5.	Remove Ethanol New Receiver Tube	 	▪ Discard filtrate ▪ Add Spin Filter to Receiver Tube ▪ 10.000 x g (~12.000 rpm): 5 min

✂ Cut at the scattered line and laminate the card for a more convenient handling on the table top ✂

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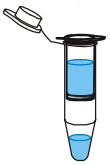
6. Elution



- Add Spin Filter to an Elution Tube
- Add 60 µl pre-heat RNase-free water
- Incubation: 2 min @ RT
- 8.000 x g (~10.000 rpm): 1 min

Order No.:	845-KS-4800010	10 reactions
	845-KS-4800050	50 reactions
	845-KS-4800250	250 reactions

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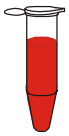
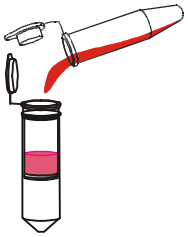
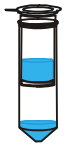
Protocol 2: Isolation of viral DNA/RNA from cell-free body fluids up to 400 µl

Recommended steps before starting

- Heat thermal mixer or water bath (70 °C)
- Prepare Washing Solution HS, Washing Solution LS, Proteinase K and Carrier Mix according to the instruction
- Pre-heat the needed amount of RNase-free water (70 °C)

1.	Starting material	▪ Serum, plasma, cell culture supernatants / medium ▪ Cell-free body fluids	▪ Add 400 µl into an 2.0 ml reaction tube
2.	Lysis	 	▪ Add 400 µl CBV/Carrier Mix and 20 µl PK ▪ Vortex: 10 sec ▪ Incubation: 10 min @ 70 °C ▪ Centrifuge: shortly
3.	Binding of DNA/RNA New Receiver Tubes	 	▪ Add 800 µl SBS ▪ Vortex ▪ Add Spin Filter to Receiver Tube ▪ Add 650 µl sample to Spin Filter ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Load residual sample ▪ 10.000 x g (~12.000 rpm): 1 min
4.	Washing New Receiver Tubes	 	▪ Add 500 µl HS ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Add 650 µl LS ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Add 650 µl LS ▪ 10.000 x g (~12.000 rpm): 1 min
5.	Remove Ethanol New Receiver Tube	 	▪ Discard filtrate ▪ Add Spin Filter to Receiver Tube ▪ 10.000 x g (~12.000 rpm): 5 min

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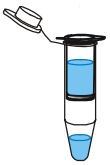
6. Elution



- Add Spin Filter to an Elution Tube
- Add 60 µl pre-heat RNase-free water
- Incubation: 2 min @ RT
- 8.000 x g (~10.000 rpm): 1 min

Order No.:	845-KS-4800010	10 reactions
	845-KS-4800050	50 reactions
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Protocol 3: Isolation of viral DNA/RNA from tissue or biopsies

Recommended steps before starting

- Heat thermal mixer or water bath (70 °C)
- Prepare Washing Solution HS, Washing Solution LS, Proteinase K and Carrier Mix according to the instruction
- Pre-heat the needed amount of RNase-free water (70 °C)

---

1. Starting material

- Tissue samples
- Biopsies

- Add max. 20 mg (small pieces) into a 1.5 ml reaction tube
- Prepare 10 % (w/v) suspension (RNase-free water or PBS)
- Centrifuge: max speed, 2 min
- Transfer 200 µl supernatant to 1.5 ml reaction tube

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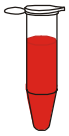
2. Lysis



- Add 200 µl CBV/Carrier Mix and 20 µl PK
- Vortex: 10 sec
- Incubation: 10 min @ 70 °C
- Centrifuge: shortly

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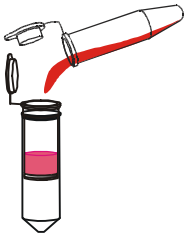
3. Binding of DNA

New Receiver Tubes



- Add 400 µl SBS
- Vortex
- Add Spin Filter to Receiver Tube
- Add sample to Spin Filter
- 10.000 x g (~12.000 rpm): 1 min

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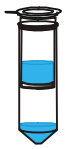
4. Washing

New Receiver Tubes



- Add 500 µl HS
- 10.000 x g (~12.000 rpm): 1 min
- Add 650 µl LS
- 10.000 x g (~12.000 rpm): 1 min
- Add 650 µl LS
- 10.000 x g (~12.000 rpm): 1 min

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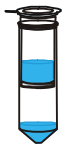
5. Remove Ethanol

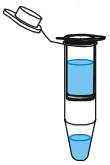
New Receiver Tube



- Discard filtrate
- Add Spin Filter to Receiver Tube
- 10.000 x g (~12.000 rpm): 5 min

✂ Cut at the scattered line and laminate the card for a more convenient handling on the table top ✂

6. Elution



- Add Spin Filter to an Elution Tube
- Add 60 µl pre-heat RNase-free water
- Incubation: 2 min @ RT
- 8.000 x g (~10.000 rpm): 1 min

Order No.:	845-KS-4800010	10 reactions
	845-KS-4800050	50 reactions
	845-KS-4800250	250 reactions

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

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✂ Cut at the scattered line and laminate the card for a more convenient handling on the table top ✂

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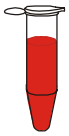
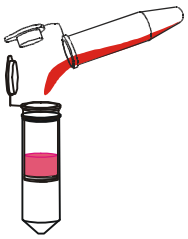
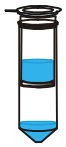
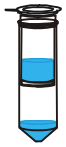
innuPREP Virus DNA/RNA Kit

Protocol 4: Isolation of viral DNA/RNA from swab

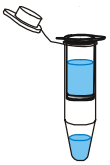
Recommended steps before starting

- Heat thermal mixer or water bath (70 °C)
- Prepare Washing Solution HS, Washing Solution LS, Proteinase K and Carrier Mix according to the instruction
- Pre-heat the needed amount of RNase-free water (50 °C)

1.	Starting material	▪ Swab	▪ Place the swab into an 1.5 ml reaction tube with 0.9 % NaCl ▪ Incubation: 15 min @ RT ▪ Shake swab, squeeze and remove ▪ Add 200 µl sample to 1.5 ml tube
2.	Lysis		▪ Add 200 µl CBV/Carrier Mix and 20 µl PK ▪ Vortex: 10 sec ▪ Incubation: 10 min @ 70 °C ▪ Centrifuge: shortly
3.	Binding of DNA/RNA New Receiver Tubes	 	▪ Add 400 µl SBS ▪ Vortex ▪ Add Spin Filter to Receiver Tube ▪ Add sample to Spin Filter ▪ 10.000 x g (~12.000 rpm): 2 min
4.	Washing New Receiver Tubes	 	▪ Add 500 µl HS ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Add 650 µl LS ▪ 10.000 x g (~12.000 rpm): 1 min ▪ Add 650 µl LS ▪ 10.000 x g (~12.000 rpm): 1 min
5.	Remove Ethanol New Receiver Tube	 	▪ Discard filtrate ▪ Add Spin Filter to Receiver Tube ▪ 10.000 x g (~12.000 rpm): 5 min

✂ Cut at the scattered line and laminate the card for a more convenient handling on the table top ✂

6. Elution



- Add Spin Filter to an Elution Tube
- Add 60 µl pre-heat RNase-free water
- Incubation: 2 min @ RT
- 8.000 x g (~10.000 rpm): 1 min

Cut at the scattered line and laminate the card for a more convenient handling on the table top

Order No.:	845-KS-4800010	10 reactions
	845-KS-4800050	50 reactions
	845-KS-4800250	250 reactions

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6. 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.
7. Centrifuge at 10.000 x g (~12.000 rpm) for 5 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
8. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **60 µl pre-heat RNase-free Water** (70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 8.000 x g (~10.000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free Water (e.g. 30 µl + 30 µl) might increase the yield of extracted viral DNA/RNA.

Note

The viral DNA/RNA can be eluted with a lower or a higher volume of RNase-free Water (depends on the expected yield of viral DNA/RNA). Elution with lower volumes of RNase-free Water increases the final concentration of viral DNA/RNA. Store the extracted viral DNA/RNA at +4 °C. For long time storage placing at –20 °C is recommended.

14 Protocol 2: Isolation of viral DNA/RNA from serum, plasma, cell culture supernatants/mediums or cell-free body fluids up to 400 µl

Important

Pre-fill the needed amount of RNase-free Water in a 1.5 ml reaction tube and incubate the RNase-free Water at 70 °C until the elution step.

Prepare Lysis Solution CBV / Carrier Mix as described on page 10 before starting!

1. Pipette **400 µl Lysis Solution CBV / Carrier Mix** into a 2.0 ml reaction tube. Add **400 µl of the sample and 20 µl of Proteinase K**, mix vigorously by pulsed vortexing for 10 sec. Incubate at 70 °C for 10 minutes.

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

   After lysis centrifuge the reaction tube shortly to remove condensate from the lid of the tube.

2. Add **800 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

   Note: It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.

3. Apply 650 µl of the sample to the Spin Filter (blue) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

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

   Discard the Receiver Tube with the filtrate.

4. Place the Spin Filter into a new 2.0 ml Receiver Tube and load the residual sample onto the Spin Filter. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 min.

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

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

5. 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.

6. 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.
7. 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.
8. Centrifuge at 10.000 x g (~12.000 rpm) for 5 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
9. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **60 µl pre-heat RNase-free Water** (70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 8.000 x g (~10.000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free Water (e.g. 30 µl + 30 µl) might increase the yield of extracted viral DNA/RNA.

Note

The viral DNA/RNA can be eluted with a lower or a higher volume of RNase-free Water (depends on the expected yield of viral DNA/RNA). Elution with lower volumes of RNase-free Water increases the final concentration of viral DNA/RNA. Store the extracted viral DNA/RNA at +4 °C. For long time storage placing at –20 °C is recommended.

15 Protocol 3: Isolation of viral DNA/RNA from tissue samples or biopsies (max. 20 mg)

Important

Pre-fill the needed amount of RNase-free Water in a 1.5 ml reaction tube and incubate the RNase-free Water at 70 °C until the elution step.

Prepare Lysis Solution CBV / Carrier Mix as described on page 10 before starting!

1. Cut max. **20 mg of the sample** into small pieces and prepare a 10 % (w / v) suspension of tissue in buffer (e.g. RNase-free Water or PBS) using commercial homogenization tools (bead based homogenization or other homogenization tools).
2. Centrifuge the suspension at max. speed for 2 minutes in order to remove particles. Use the clear particle-free supernatant for further processing.
3. Add **200 µl Lysis Solution CBV / Carrier Mix** into a 1.5 ml reaction tube, add **200 µl of the sample and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 10 sec. Incubate at 70 °C for 10 minutes.

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

   After lysis centrifuge the reaction tube shortly to remove condensate from the lid of the tube.
4. Add **400 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

   Note: It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.
5. Apply the sample to the Spin Filter (blue) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minutes.

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

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

6. 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.
7. 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.
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. Centrifuge at 10.000 x g (~12.000 rpm) for 5 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
10. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **60 µl pre-heat RNase-free Water** (70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 8.000 x g (~10.000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free Water (e.g. 30 µl + 30 µl) might increase the yield of extracted viral DNA/RNA.

Note

The viral DNA/RNA can be eluted with a lower or a higher volume of RNase-free Water (depends on the expected yield of viral DNA/RNA). Elution with lower volumes of RNase-free Water increases the final concentration of viral DNA/RNA. Store the extracted viral DNA/RNA at +4 °C. For long time storage placing at –20 °C is recommended.

16 Protocol 4: Isolation of viral DNA/RNA from swabs

Important

Pre-fill the needed amount of RNase-free Water in a 1.5 ml reaction tube and incubate the RNase-free Water at 70 °C until the elution step.

Prepare Lysis Solution CBV / Carrier Mix as described on page 10 before starting!

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. Proceed with **200 µl of the particle-free sample** for further steps.
2. Add **200 µl Lysis Solution CBV / Carrier Mix** into a 1.5 ml reaction tube, add **200 µl of the sample and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 10 sec. Incubate at 70 °C for 10 minutes.

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

   After lysis centrifuge the reaction tube shortly to remove condensate from the lid of the tube.
3. Add **400 µl Binding Solution SBS** to the lysed sample, mix by vortexing or by pipetting up and down several times.

   Note: It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution.
4. Apply the sample to the Spin Filter (blue) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

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

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.
5. 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.

6. 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.
7. 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.
8. Centrifuge at 10.000 x g (~12.000 rpm) for 5 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.
9. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **60 µl pre-heat RNase-free Water** (70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 8.000 x g (~10.000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free Water (e.g. 30 µl + 30 µl) might increase the yield of extracted viral DNA/RNA.

Note

The viral DNA/RNA can be eluted with a lower or a higher volume of RNase-free Water (depends on the expected yield of viral DNA/RNA). Elution with lower volumes of RNase-free Water increases the final concentration of viral DNA/RNA. Store the extracted viral DNA/RNA at +4 °C. For long time storage placing at –20 °C is recommended.

17 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
• Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis centrifuge the lysate to pellet unlysed material. Reduce amount of starting material.
Low amount of extracted viral DNA/RNA	
• 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 Binding Solution SBS	Mix sample with Binding Solution SBS by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted viral DNA/RNA	
• Too much RNase-free Water	Elute the viral DNA/RNA with lower volume of RNase-free Water.
• No Carrier RNA added	Add Carrier RNA (Carrier Mix) to the sample, as described in the manual above.

18 Related Products

Name	Amount	Order No.
Detection of IC DNA		
innuDETECT Internal Control DNA Assay	100 rxn	845-ID-0006100
Detection of IC RNA		
innuDETECT Internal Control RNA Assay	100 rxn	845-ID-0007100
Detection of IC DNA and IC RNA		
innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100
Nucleic acid purification		
innuPREP Virus DNA Kit	10 rxn	845-KS-4600010
	50 rxn	845-KS-4600050
	250 rxn	845-KS-4600250
innuPREP Virus RNA Kit	10 rxn	845-KS-4700010
	50 rxn	845-KS-4700050
	250 rxn	845-KS-4700250
innuPREP Proteinase K	6 mg	845-CH-0010006
	30 mg	845-CH-0010030
Products for Reverse Transcription		
innuSCRIPT One Step RT-PCR SyGreen Kit	100 rxn	845-RT-6000100
	200 rxn	845-RT-6000200
innuSCRIPT Reverse Transcriptase [25 U/µl]	50 rxn (1.250 U)	845-RT-5000050
	200 rxn (5.000 U)	845-RT-5000200
Products for PCR & Gel Electrophoresis		
innuTaq DNA Polymerase (5 U/µl)	500 U	845-EZ-1000500
innuTaq RED DNA Polymerase (1 U/µl)	500 U	845-EZ-2000500
innuTaq Hot-A DNA Polymerase (5 U/µl)	500 U	845-EZ-3000500
innuTaq UltraPure DNA Polymerase (5 U/µl)	500 U	845-EZ-6000500
50x inNucleotide Mix (12,5 mM)	2x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4x 0.25 ml	845-AS-1100250
25 mM $MgCl_2$ - Solution	3x 1.5 ml	845-AS-1000015
50 mM $MgCl_2$ - Solution	3x 1.5 ml	845-AS-1010015
PCR-grade H_2O	2.0 ml	845-AS-1800002
	5x 2.0 ml	845-AS-1800010
innuMIX rapidPCR MasterMix	100 rxn	845-AS-1600100
	200 rxn	845-AS-1600200

Name	Amount	Order No.
innuMIX Standard PCR MasterMix	100 rxn	845-AS-1700100
	200 rxn	845-AS-1700200
innuMIX Green PCR MasterMix	100 rxn	845-AS-1400100
	200 rxn	845-AS-1400200
innuSTAR 100 bp DNA Ladder Express	500 µl	845-ST-1010100
	5x 500 µl	845-ST-1010500
innuSTAR 1 kb DNA Ladder Express	500 µl	845-ST-1020100
	5x 500 µl	845-ST-1020500
6x Loading Dye Bromophenol Blue	3x 1.0 ml	845-ST-3010003
	6x 1.0 ml	845-ST-3010006
6x Loading Dye Orange G	3x 1.0 ml	845-ST-4010003
	6x 1.0 ml	845-ST-4010006
Products for qPCR		
innuMIX qPCR MasterMix Probe	100 rxn	845-AS-1200100
	200 rxn	845-AS-1200200
innuMIX qPCR MasterMix SyGreen	100 rxn	845-AS-1300100
	200 rxn	845-AS-1300200

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