

# Instructions for Use

## Life Science Kits & Assays



**innuPREP RNA Virus PLUS Kit – KFFLX**

**Order No.:**

845-KF-5396096 96 reactions

845-KF-5396480 480 reactions

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**Publication No.:** HB\_KF-5396\_e\_180904

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It needs not necessarily agree with future versions. Subject to change!

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# 1 Introduction

## 1.1 Intended use

The innuPREP RNA Virus PLUS Kit - KFFLX has been designed for isolation of viral RNA from different kinds of starting material. The extraction procedure is based on a new kind of chemistry (patent pending). The procedure combines lysis of starting material with subsequent binding of viral RNA on surface modified magnetic particles. After washing steps the viral RNA is eluted from the magnetic particles by using water. The extraction process is running in two steps (1. automated sample lysis followed by 2. automated nucleic acid extraction).

The extraction procedure takes place on the magnetic particle processor KingFisher FLEX and allows the parallel extraction of up to 96 samples. Extraction chemistry and extraction protocol are optimized to get maximum yield of high quality RNA.



### **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.

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## 1.2 Notes on the use of this manual

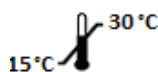
For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:



**REF**  
Catalogue number



**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. 5).
- Working steps are numbered.

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## 2 Safety precautions

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**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.

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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.

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**For single use only!**

This kit is made for single use only!

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**Attention!**

Don't eat or drink components of the kit!

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

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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.

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.

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**Attention!**

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

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**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.

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For more information, please ask for the Safety Data Sheet (SDS):



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### 3 Storage conditions

Store MAG Suspension at 4 °C to 8 °C! Store lyophilized Carrier Mix at -22 °C to -18 °C.

All other components of the innuPREP RNA Virus PLUS Kit - KFFLX should be stored dry, at room temperature (15 °C – 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 solve these precipitates by careful warming. For further information see table kit components (→ "Kit components", p. 8).

### 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 RNA Virus PLUS Kit - KFFLX were tested by recovery of IC RNA spiked in serum in serial dilutions and subsequent real-time PCR.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the innuPREP RNA Virus PLUS Kit - KFFLX 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 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**

The kit is for research use only!

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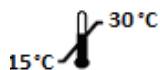
## 6 Kit components



### Important



Store the MAG Suspension at 4 °C – 8 °C.



Store lyophilized Carrier Mix at – 22 °C to – 18 °C.



### Storage conditions

All other components are stored at room temperature.

	 <b>96</b>	 <b>480</b>
<b>REF</b>	845-KF-5396096	845-KF-5396480
<b>MAG Suspension</b>	5,5 ml	3 x 9 ml
<b>Carrier Mix</b>	1x lyophilized powder	5x lyophilized powder
<b>RNase-free Water</b>	1x 2 ml	5x 2 ml
<b>Lysis Solution RL</b>	60 ml	2x 150 ml
<b>Binding Solution RBS</b>	50 ml	250 ml
<b>RNase-free Water</b>	15 ml	3 x 25 ml
<b>Washing Solution HS (conc.)</b>	30 ml (final vol. 60 ml)	2 x 70 ml (final vol. 2x 140 ml)
<b>Washing Solution LS (conc.)</b>	36 ml (final vol. 180 ml)	180 ml (final vol. 900 ml)
<b>Washing Solution D (ready-to-use)</b>	60 ml	250 ml
<b>KF96 Tip Comb with DW Plate</b>	1	5
<b>KF96 DW Plate</b>	5	25
<b>KF 96 Elution Plate</b>	1	5
<b>Manual</b>	1	1

	 96	 480
<b>REF</b>	845-KF-5396096	845-KF-5396480
<b>Initial steps</b>	<p><u>Washing Solution HS:</u></p> <ul style="list-style-type: none"> <li>• Add 30 ml of 96 – 99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed!</li> </ul> <p><u>Washing Solution LS:</u></p> <ul style="list-style-type: none"> <li>• Add 144 ml of 96 – 99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!</li> </ul> <p><u>Carrier Mix:</u></p> <ul style="list-style-type: none"> <li>• Add 1.25 ml RNase-free Water to each tube Carrier Mix, mix thoroughly by pipetting up and down!</li> </ul>	<p><u>Washing Solution HS:</u></p> <ul style="list-style-type: none"> <li>• Add 70 ml of 96 – 99.8 % ethanol to each bottle of Washing Solution HS, mix thoroughly and keep the bottle always firmly closed!</li> </ul> <p><u>Washing Solution LS:</u></p> <ul style="list-style-type: none"> <li>• Add 720 ml of 96 – 99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!</li> </ul> <p><u>Carrier Mix:</u></p> <ul style="list-style-type: none"> <li>• Add 1.25 ml RNase-free Water to each tube Carrier Mix, mix thoroughly by pipetting up and down!</li> </ul>

## 7 Recommended steps before starting

- Ensure that the Washing Solution HS and Washing Solution LS have been prepared according to the instruction (→ "Kit components", p. 8).
- Ensure that the Carrier Mix and Lysis Solution RL / Carrier Mix have been prepared according to the instruction (→ "Carrier Mix" p. 10)
- 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
- 96 – 99.8 % ethanol
  - Note:** Use only absolute/pure ethanol, NO methylated or denatured alcohol!
- PBS, optional for isolation of viral RNA from stool samples
- Physiological saline, optional (0.9 % NaCl for Influenza A testing)

## 9 Carrier Mix

### 9.1 Storage conditions and handling

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

- Add dissolved Carrier Mix to Lysis Solution RL immediately
- Unused Carrier Mix should be kept frozen at -20 °C
- Do not freeze and thaw the Carrier Mix more than 3 times
- Mixture of Lysis Solution RL and Carrier Mix is stable for 7 days at 4 °C
- Internal control RNA or DNA can be detected by real-time PCR using the corresponding assays, as shown below:

Name	Amount	Order-no.
Detection of IC RNA innuDETECT Internal Control DNA Assay	100 rxn	845-ID-0006100
Detection of IC DNA innuDETECT Internal Control RNA Assay	100 rxn	845-ID-0007100
Detection of IC RNA and IC DNA innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100

### 9.2 Preparation of Lysis Solution RL / Carrier Mix

1. Add 1.25 ml RNase-free Water to each tube Carrier Mix.
2. Mix thoroughly by pipetting up and down!
3. After the preparation of Carrier Mix stock solution prepare the mixture of Lysis Solution RL/Carrier Mix as described in the following table:

Reagent	8 samples	48 samples	96 samples	n x samples
<b>Lysis Solution RL</b>	3 ml	18 ml	36 ml	360 µl x sample
<b>Carrier Mix</b>	100 µl	600 µl	1.2 ml	12 µl x sample

**Note:** If customized Extraction Controls should be used, please add these components to the Lysis Solution RL / Carrier Mix!

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## 10 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<sub>2</sub>O.
- 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.



### Important Note

The extraction protocol is based on two automated runs:

Step I: Sample lysis on King Fisher FLEX

Step II: Extraction of viral RNA on KingFisher FLEX

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## 11 Step I: Lysis protocols for isolation of viral RNA from different starting materials



### Note

If Extraction Controls as well as Carrier Nucleic Acids are needed, please add these components to the initial lysis reaction (Lysis Solution RL + Carrier Nucleic Acid and/or Extraction Control).

Please refer also to chapter "Carrier Mix" on page 10.

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### 11.1 Protocol 1: Isolation of viral RNA from cell-free body (serum, plasma, cerebrospinal fluid, liquor)

Label one 96 DW Plate (Deep Well Plate) with "Binding Plate" and transfer **300 µl Lysis Solution RL/Carrier Mix** into the wells. Add **200 µl** of the **sample** to each well containing Lysis Solution RL/Carrier Mix.

Follow the manual with chapter 11.6 "Settings of KingFisher FLEX and automated lysis run" on page 16.

### 11.2 Protocol 2: Isolation of viral RNA from cell culture supernatants

Label one 96 DW Plate (Deep Well Plate) with "Binding Plate" and transfer **300 µl Lysis Solution RL/Carrier Mix** into the wells. Add **200 µl** of the **cell culture supernatant (cell culture medium)** to each well containing Lysis Solution RL/Carrier Mix.

Follow the manual with chapter 11.6 "Settings of KingFisher FLEX and automated lysis run" on page 16.

### 11.3 Protocol 3: Isolation of viral RNA from swab samples (Influenza A testing)

#### **Alternative 1:**

1. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer **300 µl Lysis Solution RL/Carrier Mix** into the wells.
2. Place the swabs into 1.5 ml reaction tubes containing **500 µl physiological saline** (0.9 % NaCl), incubate short and shake the swab vigorously inside the solution, squeeze it at the wall of the tube and remove the swab.
3. Transfer **200 µl** of the **physiological saline** to each well of the “Binding Plate” containing Lysis Solution RL/Carrier Mix.

Follow the manual with chapter 11.6 “Settings of KingFisher FLEX and automated lysis run” on page 16.

#### **Alternative 2:**

1. Transfer **550 µl Lysis Solution RL/Carrier Mix** into the 1.5 ml reaction tubes.
2. Place the swabs into the 1.5 ml reaction tubes containing Lysis Solution RL/Carrier Mix, incubate short and shake the swab vigorously inside the solution, squeeze it at the wall of the tube and remove the swab.
3. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer **approx. 500 µl of the sample** into the wells.

Follow the manual with chapter 11.6 “Settings of KingFisher FLEX and automated lysis run” on page 16.

## 11.4 Protocol 4: Isolation of viral RNA from tissue biopsies

### Alternative 1:

1. Transfer **500 µl Lysis Solution RL/Carrier Mix** into a 1.5 ml reaction tube and add approx. **1 – 5 mg** of the **tissue biopsy**.
2. Close the cap and vortex the 1.5 ml reaction tube for 10 sec.
3. Place the 1.5 ml reaction tube into a thermal mixer and incubate under continuous shaking for 30 minutes at room temperature. Lysis time can be increased up to lysis of starting material is complete (60 min).

**Note:** Alternative the 1.5 ml reaction tube can be mixed by vortexing during the lysis (each 5 min for 5 sec). A longer lysis time can lead to a reduced yield and quality of some viral RNA's

4. After lysis centrifuge the 1.5 ml reaction tube at max. speed for 1 minute to spin down unlysed material.
5. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer **approx. 500 µl of the lysed sample** into the wells.

Follow the manual with chapter 11.6 “Settings of KingFisher FLEX and automated lysis run” on page 16.

### Alternative 2:

1. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer **approx. 300 µl Lysis Solution RL/Carrier Mix** into the wells.
2. Transfer **300 µl Lysis Solution RL/Carrier Mix** into a 1.5 ml reaction tube and add approx. **10 – 20 mg** of the **tissue biopsy**.
3. Close the cap and vortex the 1.5 ml reaction tube for 10 sec.
4. Place the 1.5 ml reaction tube into a thermal mixer and incubate under continuous shaking for 30 minutes at room temperature. Lysis time can be increased up to lysis of starting material is complete (60 min).

**Note:** Alternative the 1.5 ml reaction tube can be mixed by vortexing during the lysis (each 5 min for 5 sec). A longer lysis time can lead to a reduced yield and quality of some viral RNA's

5. After lysis centrifuge the 1.5 ml reaction tube at max. speed for 1 minute to spin down unlysed material.
6. Transfer **200 µl of the lysed sample** to each well of the “Binding Plate” containing Lysis Solution RL/Carrier Mix.



Follow the manual with chapter 11.6 “Settings of KingFisher FLEX and automated lysis run” on page 16.

## **11.5 Protocol 5: Isolation of viral RNA stool samples (tested for Norovirus extraction)**

### **Alternative 1:**

1. Transfer about **0.05 – 0.1 g** of the **stool sample** into a 1.5 ml reaction tube and add **250 µl PBS** (not included in scope of delivery).
2. Vortex the sample for 5 sec and centrifuge it at max. speed for 3 min.
3. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer **300 µl Lysis Solution RL/Carrier Mix** into the wells and add the **clarified supernatant (max. 250 µl)** of the stool sample from step 2 to each well of the “Binding Plate” containing Lysis Solution RL/Carrier Mix.

Follow the manual with chapter 11.6 “Settings of KingFisher FLEX and automated lysis run” on page 16.

### **Alternative 2.**

In some cases the initial feecal sample is mixed with special ELISA Buffer for subsequent ELISA detection of Norovirus.

1. Transfer **250 µl of the sample** into a 1.5 ml reaction tube and centrifuge the tube at maximum speed for 3 minutes.
2. Label one 96 DW Plate (Deep Well Plate) with “Binding Plate” and transfer **300 µl Lysis Solution RL/Carrier Mix** into the wells and add the **clarified supernatant (max. 250 µl)** of the stool sample from step 1 to each well of the “Binding Plate” containing Lysis Solution RL/Carrier Mix.

Follow the manual with chapter 11.6 “Settings of KingFisher FLEX and automated lysis run” on page 16.

## 11.6 Settings of KingFisher FLEX and automated lysis run

1. Label the KF 96 Tip Comb with 96 DW Plate with “Tip Comb”
2. Switch on KingFisher FLEX
3. Select the protocol “RNA SAMPLE LYSIS”
4. Follow the instructions shown on the display and load the Tip Comb and 96 DW Plate successively
  - Tip Comb
  - Binding Plate
5. Start the automated sample lysis.



### Note

After sample lysis protocol plate “Tip Comb” and “Binding Plate” will further be used.

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## 12 Step II: Extraction of viral nucleic acids

### 12.1 Pre-filling of DW Plates and 96 Plate



#### Note

During sample lysis label the DW Plates and pre-fill all needed buffers into the wells of the DW Plates and the 96 Plate as described below!

#### DW Plate

##### **Binding Plate**

#### Buffer

After lysis protocol remove the “Binding Plate” and plate “Tip Comb” from the KingFisher FLEX.

Add 450 µl of Binding Solution RBS and 50 µl MAG Suspension to each well containing the sample and Lysis Solution RL/Carrier Mix.

#### **Note!**

It is important to mix the MAG Suspension by vigorous shaking or vortexing before use (approx. 30 sec)!

**Washing Plate 1** 500 µl Washing Solution HS

**Washing Plate 2** 800 µl Washing Solution LS

**Washing Plate 3** 800 µl Washing Solution LS

**Washing Plate 4** 500 µl Washing Solution D

#### 96 Plate

##### **Elution Plate**

#### Buffer

120 µl RNase-free water

## 12.2 Settings of KingFisher FLEX and automated extraction run

1. Switch on KingFisher Flex
2. Select protocol “INNU\_VirusRNA\_P\_KFFLX”
3. Follow the instructions shown on the display and load the Tip Comb, 96 DW Plates and 96 Plate successively
  - Tip Comb (re-used from Step I)
  - Elution Plate
  - Washing Plate 4
  - Washing Plate 3
  - Washing Plate 2
  - Washing Plate 1
  - Binding Plate (containing lysed sample, Binding Solution RBS and MAG Suspension)
4. Start the automated extraction.



### Note

1. After finishing the extraction protocol, the 96 Plate (Elution Plate) contains the extracted RNA. Store the RNA under adequate conditions. We recommend to store the extracted RNA at  $-80^{\circ}\text{C}$ .
  2. If the RNA contains carryover of magnetic particles, place the plate on a magnet or centrifuge the plate at maximum speed for 3 minutes and pipette the supernatant with RNA into a new 96 Plate.
-

## 13 Related Products

<b>Name</b>	<b>Amount</b>	<b>Order No.</b>
<b>Detection of IC DNA</b>		
innuDETECT Internal Control DNA Assay	100 rxn	845-ID-0006100
<b>Detection of IC RNA</b>		
innuDETECT Internal Control RNA Assay	100 rxn	845-ID-0007100
<b>Detection of IC DNA and IC RNA</b>		
innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100
<b>Products for Reverse Transcription/qPCR</b>		
innuSCRIPT Reverse Transcriptase [25 U/μl]	50 rxn (1.250 U)	845-RT-5000050
	200 rxn (5.000 U)	845-RT-5000200
innuSCRIPT One Step RT-PCR SyGreen Kit	100 rxn	845-RT-6000100
	200 rxn	845-RT-6000200
innuSCRIPT One Step RT-PCR Probe Kit	100 rxn	845-RT-7000100
	200 rxn	845-RT-7000200
<b>Products for PCR &amp; Gel Electrophoresis</b>		
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 <sub>2</sub> - Solution	3x 1.5 ml	845-AS-1000015
50 mM MgCl <sub>2</sub> - Solution	3x 1.5 ml	845-AS-1010015
PCR-grade H <sub>2</sub> O	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
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

<b>Name</b>	<b>Amount</b>	<b>Order No.</b>
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
<b>Products for qPCR</b>		
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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Pictures: Analytik Jena AG  
Subject to changes in design and scope of delivery as well as further technical development!