

**InviMag<sup>®</sup> Virus DNA/RNA Mini Kit / KF96**  
For extractions of viral DNA/RNA from serum, plasma, cell culture  
supernatant, tissue sample and swabs

## **Kit components (storage at room temperature)**

**Important:** ♦ Store the MAP Solution A at 4°C

	<b>96 extractions</b>	<b>5 x 96 extractions</b>
<b>Extraction Tubes</b>	1 x 96	5 x 96
<b>Binding Solution</b>	1 x 45 ml	1 x 220 ml
<b>MAP Solution A</b>	2 x 1.2 ml	10 x 1.2 ml
<b>Wash Buffer R1</b>	1 x 30 ml ( final volume 60 ml )	2 x 80 ml ( final volume 2 x 160 ml )
<b>Wash Buffer R2</b>	1 x 40 ml ( final volume 1 x 200 ml )	4 x 50 ml ( final volume 4 x 250 ml )
<b>Elution Buffer R</b>	1 x 15 ml	1 x 60 ml
<b>Deep Well Plate ( 2 ml)</b>	5	25
<b>KingFisher 96 Tip Comb for DW magnets</b>	1	5
<b>Elution Plate</b> KingFisher 96 KF Plate (200 µl)	1	5
<b>Elution Tubes (1.5 ml)</b>	2 x 50	10 x 50
<b>Manual</b>	1	1
<b>Initial steps</b>	<ul style="list-style-type: none"><li>• Add 30 ml of 96 % - 100 % ethanol to the bottle Wash Buffer R1, mix thoroughly and keep the bottle always firmly closed !</li><li>• Add 160 ml of 96 % - 100 % ethanol to the bottle Wash Buffer R2, mix thoroughly and keep the bottle always firmly closed !</li></ul>	<ul style="list-style-type: none"><li>• Add 80 ml of 96 % - 100 % ethanol to the bottle Wash Buffer R1 , mix thoroughly and keep the bottle always firmly closed !</li><li>• Add 200 ml of 96 % - 100 % ethanol to the bottle Wash Buffer R2, mix thoroughly and keep the bottle always firmly closed !</li></ul>

## **Hard Ware (not included in the Kit )**

Laptop or desktop computer with a minimum of Windows 95 operating systems and serial connection cable to connect the computer to the KingFisher instrument.

## ***Protocol: Isolation of viral RNA and DNA from different types of specimen***

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### **1. Sample Lysis depends on type of specimen**

#### **A. Cell free body fluids (serum, plasma, cerebrospinal fluid, liquor)**

Mix the 200 µl of the sample with 200 µl of ddH<sub>2</sub>O. Transfer the sample into the Extraction Tube. Close the cap and **mix by vortexing for 10 s** . Place the Extraction Tube into a Thermomixer and incubate under continuously shaking for 15 minutes at 65°C.

***Note: Optional we recommended an additional incubation step at 95°C for 10 minutes, which leads to higher sensitivity for some viruses or some strains of viruses e.g. HIV or HCV.***

After lysis time the lysed sample will be transferred carefully into the Well of the Deep Well Plate (how described below ! ).

#### **B. Cell culture supernatants**

Mix the 200 µl of the cell culture supernatant (cell culture media) with 200 µl of ddH<sub>2</sub>O. Transfer the sample into the Extraction Tube. Close the cap and **mix by vortexing for 10 s** . Place the Extraction Tube into a Thermomixer and incubate under continuously shaking for 15 minutes at 65°C.

***Note: Optional we recommended an additional incubation step at 95°C for 10 minutes, which leads to higher sensitivity for some viruses or some strains of viruses e.g. HIV or HCV.***

After lysis time the lysed sample will be transferred carefully into the Well of the Deep Well Plate (how described below ! ).

#### **C. Swabs**

Place the swab into the Extraction Tube and add 400 µl of ddH<sub>2</sub>O. ***Vortex shortly !*** Place the Extraction Tube into a Thermomixer and incubate under continuously shaking for 15 minutes at 65°C.

**Important Note: To get maximum yield of viral nucleic acids it is essential to leave the swab during the complete lysis time into the reaction tube. It is possible to cut the shaft of the swab, so that you can close the cap of the Extraction Tube. It is also possible to do the lysis step with opened cap. The removing of the swab from the Extraction Tube ahead of time will lead to a dramatically reduced final yield !**

**After lysis time carefully squeeze out the swab on the wall of the tube and discard the swab.**

***Note: Optional we recommended an additional incubation step at 95°C for 10 minutes, which leads to higher sensitivity for some viruses or some strains of viruses e.g. HIV or HCV.***

After lysis time the lysed sample will be transferred carefully into the Well of the Deep Well Plate (how described below ! ).

## D. Tissue Biopsies

Transfer 1mg up to max. 10 mg of the tissue biopsy into the Extraction Tube. Add 400 µl of ddH<sub>2</sub>O. Close the cap and **mix by vortexing for 10 s**.

Place the Extraction Tube into a Thermomixer and incubate under continuously shaking for 15 minutes at 65°C. Lysis time can be increased up to 30 min. A longer lysis time could be reduce the final yield and the quality of some viral RNA species. After lysis centrifuge the sample at max. speed for 1 minute to spin down unlysed material and follow exactly the next step.

***Note: Optional we recommened an additional incubation step at 95°C for 10 minutes, which leads to higher sensitivity for some viruses or some strains of viruses e.g. HIV or HCV.***

After lysis time the lysed sample will be transfered carefully into the Well of the Deep Well Plate (how described below !).

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### **Important Notes:**

**After lysis, the automatic extraction on the KingFisher 96, is identically for all types of starting materials. So it is possible to extract the DNA from different sample types simultaneously.**

## 2. Preliminary steps to process the sample onto the KingFisher System

***Important: For working with the KingFisher 96 please read carefully the KingFisher 96 documents !***

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1. Switch the KingFisher 96 ON

2. Prefill the Deep Well Plates with the buffers:

*( About to finishing the sample lysis prefill the Plates with the following Buffers respectively. Please avoid evaporation of the prefilled buffer components by sealing the Deep Well Plates with a sealing foil or with parafilm ! )*

**Deep Well Plate 1:** place the KingFisher 96 tip comb for DW magnets into the Deep Well Plate 1

**Deep Well Plate 2:** pipet 400 µl Binding Solution and 20 µl MAP Solution A and the lysed samples from the Extraction Tube into the Plate !

***It is important to mix the bottle with MAP Solution A by vigorously vortexing !***

**Deep Well Plate 3:** pipet 500 µl Wash Buffer R1 into the Plate

**Deep Well Plate 4:** pipet 800 µl Wash Buffer R2 into the Plate

**Deep Well Plate 5:** pipet 800 µl Wash Buffer R2 into the Plate

**KingFisher 96 KF Plate:** pipet 100 µl Elution Buffer R

3. Choose the program InviMAG\_Virus\_KF on the display of the KingFisher 96 and press the bottom “start”.

4. Place the filled Plates on the right position of the KingFisher 96 surface by following the specification of the KingFisher 96 display.

**After these preliminary steps start the program !**

***Important Notes :***

1. After finishing the extraction protocol, the Elution Plate contains the extracted RNA/DNA. Store the RNA/DNA under adequate conditions.

We recommend to transfer the extracted RNA/DNA into the 1.5 ml Elution Tubes for further storage and the freeze the DNA at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  especially for RNA.

2. If the RNA/DNA contains carryover of magnetic particle, transfer the RNA/DNA into a 1.5 ml reaction tube and centrifuge at maximum speed for 1 minute and pipet the RNA/DNA into a new tube.