

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

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

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

|                                  | <b>15 extractions</b>   | <b>75 extractions</b>   |
|----------------------------------|---|---|
| <b>Extraction Tubes</b>          | 1 x 15  | 1 x 75  |
| <b>MAP Solution A</b>            | 1 x 0.5 ml  | 2 x 1 ml  |
| <b>Binding Solution</b>          | 1 x 8 ml  | 1 x 40 ml   |
| <b>Elution Buffer R</b>          | 1 x 2 ml  | 1 x 15 ml   |
| <b>Wash Buffer R 1</b>           | 1 x 10 ml<br>(final volume 20 ml)   | 2 x 20 ml<br>(final volume 2 x 40 ml)   |
| <b>Wash Buffer R 2</b>           | 1 x 12 ml<br>(final volume 60 ml)   | 1 x 30 ml<br>(final volume 150 ml)  |
| <b>Elution Tubes 1.5 ml</b>      | 1 x 15  | 5 x 15  |
| <b>KingFisher ml Tip Combs</b>   | 1 x 3   | 1 x 15  |
| <b>KingFisher ml Tube Strips</b> | 1 x 15  | 5 x 15  |
| <b>Manual</b>                    | 1   | 1   |
| <b>Initial steps</b>             | <ul style="list-style-type: none"><li>• Add 10 ml of 96 % - 100 % ethanol to the bottle Wash Buffer R1, mix thoroughly and keep the bottle always firmly closed</li><li>• Add 48 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 20 ml of 96 % - 100 % ethanol to the bottle Wash Buffer R1, mix thoroughly and keep the bottle always firmly closed</li><li>• Add 120 ml of 96 % - 100 % ethanol to the bottle Wash Buffer R2 , mix thoroughly and keep the bottle always firmly closed !</li></ul> |

## ***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.*

#### **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.*

#### **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.*

#### **D. Tissue Biopsies**

Transfer 1mg up to – max. 10 mg of the 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 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 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.*

#### **For all sample !**

After lysis transfer the lysed sample into the Tube A of the KingFisher tube strip (for the biopsy-sample, after centrifugation) and add the **400µl of Binding Solution and 20 µl MAP Solution A** (see also below). **Vortex the tube MAP Solution A vigorously before use!**

## **Preliminary Steps to process the sample onto the KingFisher System**

- 1. During the sample lysis prefill the tubes of the KingFisher tube strips with the following Buffers respectively.**

### **KingFisher ml StripTube Setup**

Tube A: will be filled with appr. 450 µl of the lysed sample, 400 µl Binding Solution and 20 µl MAP Solution A after finishing the lysis step 1.

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

Tube B: 800 µl Wash Buffer R1

Tube C: 800 µl Wash Buffer R2

Tube D: 800 µl Wash Buffer R2

Tube E: 120 µl Elution Buffer R

- 2. Place the filled KingFisher tube strips into the KingFisher System on the right position !**

- 3. Place the KingFisher tips onto the magnetic track !**

**After these preliminary steps start the program “InviMAG\_Virus\_KFml” !  
( If you use a disc, load the programm “InviMAG\_Virus\_KFml ” !)**

### ***Important Notes :***

- 1. After finishing the extraction protocol, the Tube E 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°C or –80°C especially for RNA.**
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- 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.**

## **The following extraction steps running automatically on the KingFisher System !**

### **2. Binding of the DNA/RNA**

Automatically sample mixing for 5 minutes. MAP separation. Moving of the MAP into the Tube B.

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### **3. First Washing**

Automatically sample mixing for 50 sec.. MAP separation. Moving of the MAP into the C wells.

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### **4. Second Washing**

Automatically sample mixing for 40 sec.. MAP separation. Moving of the MAP into the D wells.

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### **5. Third Washing and Drying**

Automatically sample mixing for 30 sec.. MAP separation. Drying the MAP outside the Tube for 8 minutes. Moving of the MAP into the E wells.

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### **6. Elution of the DNA/RNA**

Incubation of the MAP into the Tube for 5 minutes by mixing. MAP separation.

The MAP will then automatically be removed into the B wells (disposal).

The extracted DNA/RNA will be now transferred into the 1.5 ml Elution Tubes. Optional, carryover of magnetic particles should be removed by centrifugation of the Elution Tube at max. speed for 1 minute and transfer of the cleared eluate into a new tube.

*Note: The eluate contains DNA and RNA. After extraction place the elution tube on ice. For a long time storage place the nucleic acids at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ .*

**For self programming of the KingFisher ml System ( programm “ InviMAG\_Virus\_KF ml” )**

**1. Bind/Lysis ( Tube A of the strip tube )**

Lysis/Bind parameters; Time: 5 minutes/Speed: slow  
MAP Collection parameters; Collect count: 3

**2. First Wash ( Tube B of the strip tube )**

Wash parameters; Wash time: 50 seconds/Speed: fast dual mix  
MAP Collection parameters; Collect count: 2

**3. Second Wash ( Tube C of the strip tube )**

Wash parameters; Wash time: 40 seconds/Speed: fast dual mix  
MAP Collection parameters; Collect count: 2

**4. Third Wash ( Tube D of the strip tube )**

Wash parameters; Wash time: 30 seconds/Speed: fast dual mix  
MAP Collection parameters; Collect count: 3

**5. Dry ( Outside of Tube D of the strip tube )**

Dry parameters; Dry time: 8 minutes

**6. Elution**

Elution parameters; Elution time: 5 minutes/Speed: medium  
MAP Collection parameters; Collect count: 10  
Remove MAP: Disposal Tube B