

**InviMag<sup>®</sup> Forensic Kit / KFml**  
for extractions of genomic DNA from forensic samples

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

**Important:** ♦ Store the MAP Solution A at 4 °C  
 Store lyophilized Proteinase K at 2 - 8 °C ;  
 Store diluted Proteinase K at – 20 °C, but repeated freezing and thawing will reduced the activity dramatically. Dividing the Proteinase K into aliquots and storage at – 20°C is recommended.

	15 extractions	75 extractions
<b>Lysis Buffer G</b>	1 x 10 ml	1 x 50 ml
<b>Proteinase K</b>	10 mg for 0.5 ml working solution	5 x 10 mg for 5 x 0.5 ml working solution
<b>MAP Solution A</b>	1 x 0.5 ml	2 x 1 ml
<b>Binding Buffer T</b>	1 x 8 ml	1 x 30 ml
<b>Elution Buffer D</b>	1 x 2 ml	1 x 15 ml
<b>Wash Buffer</b>	1 x 18 ml (final volume 60 ml)	1 x 45 ml (final volume 150 ml)
<b>Receiver Tubes 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 42 ml of 96 % - 100 % ethanol to the bottle Wash Buffer, mix thoroughly and keep the bottle always firmly closed !</li> <li>• Dilute Proteinase K by addition of 0.5 ml of ddH<sub>2</sub>O, mix thoroughly and store like described below !</li> </ul>	<ul style="list-style-type: none"> <li>• Add 105 ml of 96 % - 100 % ethanol to the bottle Wash Buffer , mix thoroughly and keep the bottle always firmly closed !</li> <li>• Dilute Proteinase K by addition of 0.5 ml of ddH<sub>2</sub>O, mix thoroughly and store like described below !</li> </ul>

## ***Protocols: Isolation of DNA from forensic samples***

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### **1. Sample Lysis**

#### **A: Isolation of DNA from Buccal Swabs**

Transfer 600 µl of Lysis Buffer G and 25 µl of Proteinase K into a 1.5 ml Reaction Tube.  
Transfer the swab into the so prepared tube, ***vortex the tube for 5 s*** and incubate the sample at 56°C for 20 minutes under continuously shaking (e.g. by using a thermomixer).

***Important Note: To get maximum yield of DNA 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 reaction tube. It is also possible to do the lysis step with opened cap. The removing of the swab from the reaction tube ahead of time will result in a dramatically reduced final yield !***

After lysis time carefully squeeze out the swab on the wall of the tube and discard the swab.  
Transfer the lysed sample into the Tube A of the KingFisher tube strip and add 300 µl of Binding Buffer T and 20 µl MAP Solution A (see also below).  
(***Vortex the tube MAP Solution A vigorously before use !***).

#### **B: Isolation of DNA from blood stains, saliva stains etc.**

Cut the material containing the stains into small pieces and transfer it into a 1.5 ml Reaction Tube.  
Add 600 µl of Lysis Buffer G and 25 µl of Proteinase K. ***For semen stains at additional 30 µl 1 M DTT (not provided) to the Lysis Buffer and Proteinase K mix. Vortex the tube for 5 s.*** Incubate the sample at 56°C for at least 1 h under continuously shaking (e.g. by using a thermomixer).  
Incubation overnight is also possible. We recommend the overnight lysis at 42°C under continuously shaking.

After lysis time carefully transfer the lysed sample into the Tube A of the KingFisher tube strip (avoid carry over of starting material; optional spin down the starting material)

Add the 300 µl of Binding Buffer T and 20 µl MAP Solution A (see also below).  
(***Vortex the tube MAP Solution A vigorously before use !***).

#### **C: Isolation of DNA from hair roots**

Place a single hair root (or more) into a 1.5 ml Reaction Tube.  
Add 600 µl of Lysis Buffer G and 25 µl of Proteinase K and 30µl 1 M DTT (not provided).  
***Vortex the tube for 5 s.*** Incubate the sample at 56°C for at least 1 h under continuously shaking (e.g. by using a thermomixer). Incubation overnight is also possible. We recommend the overnight lysis at 42°C under continuously shaking.

After lysis time carefully transfer the lysed sample into the Tube A of the KingFisher tube strip (avoid carry over of starting material; optional spin down the starting material)

Add the 300 µl of Binding Buffer T and 20 µl MAP Solution A (see also below).  
(***Vortex the tube MAP Solution A vigorously before use !***).

## **D: Isolation of DNA from cigarette butts**

Remove of a small piece (3 - 5 mm) of the brown filter paper or of a part of the filter and place the material in a 1.5 ml Reaction Tube.

Add 600 µl of Lysis Buffer G and 25 µl of Proteinase K. ***Vortex the tube for 5 s.*** Incubate the sample at 56°C for at least 1 h under continuously shaking (e.g. by using a thermomixer). Incubation overnight is also possible. We recommend the overnight lysis at 42°C under continuously shaking.

After lysis time carefully transfer the lysed sample into the Tube A of the KingFisher tube strip (avoid carry over of starting material; optional spin down the starting material)

Add the 300 µl of Binding Buffer T and 20 µl MAP Solution A (see also below).  
**(Vortex the tube MAP Solution A vigorously before use !).**

## **E: Isolation of DNA from bubble gum**

Cut a part of the bubble gum into small pieces and place the material into a 1.5 Reaction Tube.

Add 600 µl of Lysis Buffer G and 25 µl of Proteinase K. ***Vortex the tube for 5 s.*** Incubate the sample at 56°C for at least 3 h under continuously shaking (e.g. by using a thermomixer). Incubation overnight is also possible. We recommend the overnight lysis at 42°C under continuously shaking.

After lysis time carefully transfer the lysed sample into the Tube A of the KingFisher tube strip (avoid carry over of starting material; optional spin down the starting material)

Add the 300 µl of Binding Buffer T and 20 µl MAP Solution A (see also below).  
**(Vortex the tube MAP Solution A vigorously before use !).**

## **F: Isolation of DNA from stamps and envelopes**

Cut the material into small pieces and transfer it into a 1.5 ml Reaction Tube.

Add 600 µl of Lysis Buffer G and 25 µl of Proteinase K. ***Vortex the tube for 5 s.*** Incubate the sample at 56°C for at least 1 h under continuously shaking (e.g. by using a thermomixer). Incubation overnight is also possible. We recommend the overnight lysis at 42°C under continuously shaking.

After lysis time carefully transfer the lysed sample into the Tube A of the KingFisher tube strip (avoid carry over of starting material; optional spin down the starting material)

Add the 300 µl of Binding Buffer T and 20 µl MAP Solution A (see also below).  
**(Vortex the tube MAP Solution A vigorously before use !).**

## **G: Isolation of DNA from tissue samples**

Place approximately 0.5 – 20 mg of fresh or frozen tissue sample (cut the material into small pieces ) into a 1.5 ml Reaction Tube tube.

Add 600 µl of Lysis Buffer G and 25 µl of Proteinase K. ***Vortex the tube for 5 s.*** Incubate the sample at 56°C for at least 1 h under continuously shaking (e.g. by using a thermomixer). Incubation overnight is also possible. We recommend the overnight lysis at 42°C under continuously shaking.

After lysis time carefully transfer the lysed sample into the Tube A of the KingFisher tube strip (avoid carry over of starting material; optional spin down the starting material)

Add the 300 µl of Binding Buffer T and 20 µl MAP Solution A (see also below).  
**(Vortex the tube MAP Solution A vigorously before use !).**

## **H: Isolation of DNA from nail clippings**

Place the nail clippings into a 1.5 ml Reaction Tube.

Add 600 µl of Lysis Buffer G and 25 µl of Proteinase K and 30 µl 1 M DTT (not provided).

***Vortex the tube for 5 s.*** Incubate the sample at 56°C for at least 1 h under continuously shaking (e.g. by using a thermomixer). Incubation overnight is also possible. We recommend the overnight lysis at 42°C under continuously shaking.

After lysis time carefully transfer the lysed sample into the Tube A of the KingFisher tube strip (avoid carry over of starting material; optional spin down the starting material)

Add the 300 µl of Binding Buffer T and 20 µl MAP Solution A (see also below).  
**(Vortex the tube MAP Solution A vigorously before use !).**

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

1. Using the kit for other kinds of forensic sample, the selection of one of the described protocols is recommended.
2. After lysis, the automatic extraction on the KingFisher ml, is identically for all types of starting materials. So it is possible to extract the DNA from different sample types simultaneously.
3. Optional, for isolation of DNA from forensic samples containing extrem low amounts of DNA it could be helpful to add Carrier RNA to the Binding Step (after lysis ). We recommend to use Carrier RNA (e.g. Poly(A) RNA; Roche Diagnostics; No.108626). Dissolve the RNA in RNase free water to obtain a solution of 1 µg/µl. Divide aliquots and store at –20°C. Do not freeze and thaw the aliquots for more than 3 times. We recommend adding of 1 µl Carrier RNA per sample.

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

*About to finishing the sample lysis prefill the tubes of the KingFisher ml strip tubes with the following Buffers respectively. Please avoid evaporation of the prefilled buffer components by sealing the KingFisher tube strips with a sealing foil or with parafilm !*

### **A. KingFisher ml tube setup**

Tube A: will be filled with appr. 600 µl of the lysed sample, 300 µl Binding Buffer T and 20 µl MAP Solution A after finishing the lysis step 1.

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

Tube B: 800 µl Wash Buffer

Tube C: 800 µl Wash Buffer

Tube D: 120 µl Elution Buffer D

**B. Place the filled KingFisher ml tubes into the KingFisher System on the right position !**

**C. Place the KingFisher tips onto the magnetic track !**

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

### *Important Notes :*

**1. After finishing the extraction protocol, the Tube E contains the extracted DNA.  
Store the DNA under adequate conditions.**

**We recommend to transfer the extracted DNA into the 1.5 ml Receiver Tubes for further storage and freeze the DNA at -20°C.**

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

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

**Binding of the DNA**

Automatically sample mixing for 2 minutes. MAP separation. Moving of the MAP into the Tube B.

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**First Washing**

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

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**Second Washing and Drying**

Automatically sample mixing for 50 sec.. MAP separation. Drying the MAP outright the Tube C for 8 minutes. Moving of the MAP into the Tube D.

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**5. Elution of the DNA**

Incubation of the MAP into the Tube D for 5 minutes by mixing. MAP separation. The MAP will than automatically removed into the Tube B (disposal).

The extracted DNA will be now transfered into 1.5 ml Receiver Tubes.

**Note:** If the DNA contains carryover of some magnetic particles, centrifuge the 1.5 ml tube at maximum speed for 1 minute and pipet the DNA into a new tube.

**For self programming of the KingFisher ml System ( programme “InviMAG\_Forens\_KFml ”.)**

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

Lysis/Bind parameters; Time: 2 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: 50 seconds/Speed: fast dual mix  
MAP Collection parameters; Collect count: 2

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

Dry parameters; Dry time: 8 minutes

**5. Elution**

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