InviMag® Forensic Kit / KingFisher 96

for extractions of genomic DNA from forensic samples using
KingFisher 96 instrument (Thermo Electron)

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.

	96 extractions	**5 x 96 extractions**
Lysis Buffer G	1 x 70 ml	2 x 160 ml
Proteinase K	2 x 30 mg for 2 x 1.5 ml working solution	7 x 40 mg for 7 x 2 ml working solution
MAP Solution A	2 x 1.2 ml	11 x 1.0 ml
Binding Buffer T	1 x 40 ml	1 x 160 ml
Elution Buffer D	1 x 15 ml	1 x 60 ml
Wash Buffer	1 x 60 ml (final volume 1 x 200 ml)	6 x 45 ml (final volume 6 x 150 ml)
Microtiter 96 Deep Well Plate (Thermo Electron)	4	20
KingFisher 96 Tip Comb for DW magnets (Thermo Electron)	1	5
Elution Plate KingFisher 96 Plate (200 µl) (Thermo Electctron)	1	5
Receiver Tubes (1.5 ml)	2 x 50	10 x 50
Manual	1	1
Initial steps	• Add 140 ml of 96 % - 100 % ethanol to the bottle Wash Buffer, mix thoroughly and keep the bottle always firmly closed ! • Dilute Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store like described below !	• Add 105 ml of 96 % - 100 % ethanol to the bottle Wash Buffer , mix thoroughly and keep the bottle always firmly closed ! • Dilute Proteinase K by addition of 2 ml of ddH_2O, mix thoroughly and store like described below !

KingFisher software 2.6.2

KingFisher Software 2.6.2 is used to create protocols for the *KingFisher*, *KingFisher mL* and *KingFisher 96* instruments. Once a protocol has been created, the user can either transfer the protocol into the KingFisher instrument memory or run the protocol directly from the software. Directly run protocols are not stored in the instrument memory.
Note! When creating the protocol using KingFisher 96 and Microtiter Deep Well plates (Thermo Electron) it is essential to use KingFisher software 2.6 or 2.6.2 for protocol development as these software versions include correct adjustments for this plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KingFisher 96 instrument to ensure the best purification result.

Checking the PC requirements

The table below lists the PC requirements for KingFisher Software 2.6.2

PC requirements	
Interface	Serial communication port via an RS-232 full duplex interface
Supported operating systems	– Microsoft Windows 2000 – Microsoft Windows XP Professional
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 700 MHz recommended
Memory	220 MB RAM recommended
Serial ports available	1
Pointing device	Mouse or equivalent is necessary
CD-ROM drive	1
Monitor / color settings	SVGA monitor with at least 1024 x 768 resolution and at least a 16-bit color environment
Service Packs installed	– *Microsoft Windows 2000*: Service Pack 4 (or greater) – *Microsoft Windows XP Professional*: Service Pack 2 (or greater)
Browser	Microsoft Internet Explorer 6.0 (or greater) installed

If you do not have the correct Service Packs installed, you can download them from the Microsoft web pages: http://www.microsoft.com.

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). **Optional, the lysis can be carry out in a Deep Well Plate in a waterbath; vortex the Deep Well Plate each 5 minutes for 10 s (continously shaking increases the lysis efficiency**).

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.

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

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 aditional 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 continously shaking.
Optional, the lysis can be carry out in a Deep Well Plate in a waterbath; vortex the Deep Well Plate each 20 minutes for 10 s (continously shaking increases the lysis efficiency).

After lysis optional spin down the starting material.

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

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 continously shaking. **Optional, the lysis can be carry out in a Deep Well Plate in a waterbath; vortex the Deep Well Plate from time to time (continously shaking increases the lysis efficiency**). After lysis optional spin down the starting material.

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

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 continously shaking. **Optional, the lysis can be carry out in a Deep Well Plate in a waterbath; vortex the Deep Well Plate from time to time (continously shaking increases the lysis efficiency)**.

After lysis optional spin down the starting material.

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

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 continously shaking. **Optional, the lysis can be carry out in a Deep Well Plate in a waterbath; vortex the Deep Well Plate from time to time (continously shaking increases the lysis efficiency)**.

After lysis optional spin down the starting material.

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

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 continously shaking. **Optional, the lysis can be carry out in a Deep Well Plate in a waterbath; vortex the Deep Well Plate from time to time, (continously shaking increases the lysis efficiency)**.

After lysis optional spin down the starting material.

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

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 continously shaking. **Optional, the lysis can be carry out in a Deep Well Plate in a waterbath vortex the Deep Well each 20 minutes for 10 s (continously shaking increases the lysis efficiency)**.

After lysis optional spin down the starting material.

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

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 continously shaking. **Optional, the lysis can be carry out in a Deep Well Plate in a waterbath; vortex the Deep Well Plate from time to time (continously shaking increases the lysis efficiency)**.

After lysis optional spin down the starting material.

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

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 96, 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 96 System

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

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 !)

KingFisher 96 plate (tip comb plate) : place the KingFisher 96 tip comb for DW magnets into the KingFisher 96 plate.

Deep Well Plate 1 (Binding plate): pipet 300 µl Binding Buffer T and 20 µl MAP Solution A into the plate containing lysed samples

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

Deep Well Plate 2 (Washing plate_1) : pipet 800 µl Wash Buffer into the Plate

Deep Well Plate 3 (Washing plate_2): pipet 800 µl Wash Buffer into the Plate

KingFisher 96 Plate (Elution plate): pipet 100 µl Elution Buffer D

3. Choose the program InviMAG_Forensic_96 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.

- Note! Be sure to place the plates in correct orientation: A1 well to be pointed to upper right corner of the plate holder in turntable. A1 row of the plate is then always located in the inner circle of the turntable.

After these preliminary steps start the program !

Important Notes :

1. After finishing the extraction protocol, the Elution Plate contains the extracted DNA. Store the DNA under adequate conditions. We recommend the transfer of the extracted DNA into the 1.5 ml Receiver Tubes. Freeze the DNA at –20°C for a longer storage.

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 1.5 ml tube.

Ordering Information (KingFisher 96 and consumables)

Cat.no	Description
5400500	KingFisher 96 Magnetic Particle Processor, 100-240 V, 50/60 Hz (includes one magnetic head)
24073430	KingFisher 96 Head for Deep Well plate
97002514	KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box
97002524	KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box
97002534	KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box
97002540	KingFisher 96 KF plate (200ul) 48 plates / box
95040450	Microtiter deep well 96 plate, 50 plates/box