

InviMag<sup>®</sup> Blood DNA Mini Kit / KingFisher  
96  
for extraction of genomic DNA from up to 200 µl of whole blood  
sample  
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
<b>Lysis Buffer G</b>	1 x 70 ml	2 x 160 ml
<b>Proteinase K</b>	2 x 30 mg for 2 x 1.5 ml working solution	7 x 40 mg for 7 x 2 ml working solution
<b>MAP Solution A</b>	2 x 1.2 ml	11 x 1.0 ml
<b>Binding Buffer T</b>	1 x 40 ml	1 x 160 ml
<b>Elution Buffer D</b>	1 x 15 ml	1 x 60 ml
<b>Wash Buffer</b>	1 x 60 ml (final volume 1 x 200 ml)	6 x 45 ml (final volume 6 x 150 ml)
<b>Microtiter 96 Deep Well Plate (Thermo Electron)</b>	4	20
<b>KingFisher 96 Tip Comb for DW magnets (Thermo Electron)</b>	1	5
<b>Elution Plate</b> KingFisher 96 Plate (200 µl) (Thermo Electctron)	1	5
<b>Receiver 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 140 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 1.5 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 2 ml of ddH<sub>2</sub>O, mix thoroughly and store like described below !</li></ul>

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

## **Protocol: Isolation of genomic DNA from whole blood sample**

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### ***Important Note:***

*The protocol has been optimized for the isolation of genomic DNA from 200 µl of whole blood. For samples which have a smaller volume than 200 µl please fill up to a total volume of 200 µl with 1 x PBS.*

### **1. Sample Lysis**

#### **A. Sample lysis in 1.5 ml reaction tubes**

Transfer 200 µl of whole blood (or a smaller volume of whole blood filled up to 200 µl with 1 x PBS ) into a 1.5 ml reaction tube. Add 200 µl Lysis Buffer A and 15 µl Proteinase K.

#### ***Important Note: Vortex the sample for 10 s !***

Incubate the sample at 56°C for 10 minutes under continuously shaking.

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

#### **B. Sample lysis in a Deep Well Plate**

Transfer 200 µl of whole blood (or a smaller volume of whole blood filled up to 200 µl with 1 x PBS ) into the wells of the Deep Well Plate. Add 200 µl Lysis Buffer A and 15 µl Proteinase K. Seal the Deep Well Plate with a Sealing Foil. Vortex the Deep Well Plate 10 times.

**Incubate the Deep Well Plate at 70°C for 20 minutes (use e.g. hybridization oven or incubator).**

***Important Note: Vortex the Deep Well Plate each 5 minutes for 20 s.***

***Vortexing is essentially for an efficient lysis step. No vortexing will lead to a dramatic reduction of final yield.***

## 2. KingFisher 96 process

***Important: Before starting the purification process with the KingFisher 96 please read carefully the KingFisher 96 user manual!***

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**1. Switch the KingFisher 96 ON**

**2. Fill the plates according to following instructions:**

***(After 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):** Add 400 µl Binding Buffer B6 and 10 µl MAP Solution A into the plate containing lysed samples. Alternatively, transfer samples from 1.5 ml tube to the plate and add Binding Buffer B6 and MAP Solution A as above.

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

**Deep Well Plate 2 (Washing plate\_1):** pipet 500 µl Wash Buffer I into the Plate

**Deep Well Plate 3 (Washing plate\_2):** pipet 500 µl Wash Buffer I into the Plate

**Deep Well Plate 4 (Washing plate\_3):** pipet 800 µl Wash Buffer II into the Plate

**Deep Well Plate 5 (Washing plate\_4):** pipet 800 µl Wash Buffer II into the Plate

**KingFisher 96 plate (Elution plate):** pipet 200 µl Elution Buffer D

**3. Select InviMAG\_Blood\_96 protocol using arrow keys and press START button**

**4. Load the plates according to protocol request and press START after every plate to confirm the action.**

- 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.

**5. The purification protocol will start when the last plate is loaded and START button is pressed.**

**6. After the purification process is completed the plates are removed according to instructions shown in the instrument screen. Press START after each plate removal to confirm the action.**

**7. When the last plate is removed text END\_OF\_RUN will appear. Press STOP to complete the run.**

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

- 1. After finishing the extraction protocol, the Elution Plate contains the extracted genomic DNA. Store the RNA/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  $-20^{\circ}\text{C}$ .**
- 2. If the extracted 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.**

### **Ordering Information (KingFisher 96 and consumables)**

<b>Cat.no</b>	<b>Description</b>
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