

InviMag[®] Blood DNA Mini Kit / KingFisher mL

for extraction of genomic DNA from up to 200 µl of whole blood using
KingFisher mL 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.

	15 extractions	75 extractions
Lysis Buffer A	5 ml	20 ml
Proteinase K	10 mg for 0.5 ml working solution	5 x 10 mg for 5 x 0.5 ml working solution
MAP Solution A	1 x 0,25 ml	1 x 1 ml
Binding Buffer B6	1 x 8 ml	1 x 40 ml
Elution Buffer D	2 x 2 ml	1 x 20 ml
Wash Buffer I	1 x 7.5 ml (final volume 15 ml)	2 x 30 ml (final volume 60 ml)
Wash Buffer II	1 x 18 ml (final volume 60 ml)	1 x 45 ml (final volume 150 ml)
Receiver Tubes Tubes (1.5 ml)	1 x 15	5 x 15
KingFisher mL Tip Combs (Thermo Electron)	1 x 3	1 x 15
KingFisher mL Tube Strips (Thermo Electron)	1 x 15	5 x 15
Manual	1	1
Initial steps	<ul style="list-style-type: none"> • Add 7,5 ml of 96 % - 100 % ethanol to the bottle Wash Buffer I, mix thoroughly and keep the bottle always firmly closed • Add 42 ml of 96 % - 100 % ethanol to the bottle Wash Buffer II, mix thoroughly and keep the bottle always firmly closed ! • Dilute Proteinase K by addition of 500 µl of ddH₂O, mix thoroughly and store like described below ! 	<ul style="list-style-type: none"> • Add 30 ml of 96 % - 100 % ethanol to the bottle Wash Buffer I, mix thoroughly and keep the bottle always firmly closed • Add 105 ml of 96 % - 100 % ethanol to the bottle Wash Buffer II , mix thoroughly and keep the bottle always firmly closed ! • Dilute Proteinase K by addition of 500 µl of ddH₂O, 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.

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 \geq 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	– <i>Microsoft Windows 2000</i> : Service Pack 4 (or greater) – <i>Microsoft Windows XP Professional</i> : 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>.

Protocol: Isolation of genomic DNA from up to 200 µl of whole blood

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

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 20 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 transfer the lysed sample into the Tube A of the KingFisher tube strip and add the 400µl of Binding Buffer B6 and 10 µl MAP Solution A (see also below).

(Vortex the tube MAP Solution A vigorously before use !).

2. KingFisher mL process

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

After finishing the sample lysis fill 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. Place an appropriate number of tube strips needed for the samples (one tube strip per sample) into removable tube strip tray.**

Note! Resuspend the magnetic particles (MAP solution A) thoroughly before use!

- B. Tube A: Add 400 µl lysed sample, 400 µl Binding buffer B6 and 10 µl MAP solution A**
- C. Tube B: Add 800 µl Wash buffer I**
- D. Tube C: Add 800 µl Wash buffer II**
- E. Tube D: Add 800 µl Wash buffer II**
- F. Tube E: Add 200 µl Elution Buffer D**
- G. Insert the tubestrip tray to the instrument and insert the tip combs into the slots.**
- H. Close the front lid and start the process by selecting protocol InviMAG_Blood_mL using arrow keys and press START.**

InviMAG_Blood_mL protocol description

1. Sample lysate is incubated with magnetic particles for 3 minutes in tube A.
2. Particles are washed with Wash Buffer I for 90 sec in tube B.
3. Particles are washed with Wash Buffer II for 40 sec in tube C.
4. Particles are washed with Wash Buffer II for 30 sec in tube D.
5. A drying step (8 min) is performed after washing step in tube D.
6. DNA is released into Elution Buffer D in tube E.
7. Particles are discarded into tube D.

Ordering Information (KingFisher mL and consumables)

Cat.no	Description
5400050	KingFisher mL, Magnetic Particle Processor, 100-240 V, 50/60 Hz
97002111	KingFisher mL tip comb, 800 pcs
97002121	KingFisher mL tube, 900 pcs (20x45 pcs)
97002131	KingFisher mL Combi 60 (tubes and tip combs for 60 samples)
97002141	KingFisher mL Combi 240 (tubes and tip combs for 240 samples)

