

## **USER GUIDE**

for

**Automated purification of DNA from  
human blood samples with**

**KingFisher 96/ KingFisher mL instrument  
and  
chemagic DNA Blood250 Kit**

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## Description

Purification of DNA from whole blood using Chemagen's chemagic DNA Blood250 kit (T296) can easily be automated using KingFisher® instruments (Thermo Scientific). The KingFisher platforms utilize patented technology where magnetic rods move particles through the processing steps. KingFisher 96 instrument operates on microplates and can process up to 96 samples per run, whereas KingFisher mL can handle 15 samples per run. With both instruments the volumes handled can be up to 1 ml.

Typically, DNA isolation from 250 µl of human blood using KingFisher results 5-10 µg DNA. Generally, DNA yields vary according to sample type and condition.

The protocol described here is designed for general use and can be modified according to customer individual needs using KingFisher® Software provided with the instrument.

## Important notes

- See chemagic DNA Blood250 Kit Handbook for reagent storage, product use limitations, safety information etc.
- Resuspend magnetic beads (RNA Binding Beads) thoroughly before use.

## KingFisher 96 protocol

### Importing protocols from the web

KingFisher Software protocol for chemagic DNA Blood250 Kit can be downloaded from the website ([www.thermo.com/kingfisher](http://www.thermo.com/kingfisher)). First you have to **save the file "Chem\_DNABlood\_KF96" to your computer.**

1. Open KingFisher Software.
2. Select **Protocol** → **Import/Export data**.
3. Click **Read file**.
4. Select the database (\*.KF2) by browsing in the **Open** dialog and click **Open**.
5. Select the protocol(s) you wish to import from the *Protocols in file* list. Use the SHIFT key together with the mouse button to select protocols between two clicked protocols and the CTRL key to select only the clicked protocols.
6. Tick **Update existing** if you wish to overwrite the protocols with identical protocol name(s) in the target database.
7. Click **Import**.

If there are protocols with identical names and you have not ticked the **Update existing** tick box, you will be prompted to change the name of the protocol that is being imported:

- Type in a new name and click **OK**.
  - o **Note:** Check that the name of the protocol does not exceed 17 characters.
- You will receive a message stating whether the database updating procedure was successful or not.

### Sample preparation

- KingFisher DNA protocol **chemagic DNA Blood250** is designed to purify DNA from human whole blood (fresh, non-coagulated and frozen).
- Use KingFisher deepwell microplate (Catalog No. 95040450), KingFisher deepwell tip comb (Catalog No. 97002534) and KingFisher 96 plate (Catalog No. 97002540) with **chemagic DNA Blood250** protocol.
- Add sample and other reagents except Elution Buffer supplied by **chemagic DNA Blood250** kit to KingFisher deepwell microplate according to table 1 and instructions below. Add the Elution Buffer to KingFisher 96 plate

## KingFisher 96 process

**Table 1** Pipetting instructions for KingFisher 96 and chemagic DNA Blood250 protocol.

Plate	Content	Sample/Reagent volume
A	Sample	150 µl
	Lysis Buffer 1	210 µl
	Magnetic beads (added during pause)	30 µl
	Binding Buffer 2 (added during pause)	570 µl
B	Wash Buffer 3	500 µl
C	Wash Buffer 4	500 µl
D	Wash Buffer 5	500 µl
E	Wash Buffer 6	500 µl
F	Elution Buffer	200 µl

1. Add 150 µl of Sample and 210 µl of Lysis Buffer to plate **A**
2. Add 500 µl of Wash Buffer 3 to plate **B**.
3. Add 500 µl of Wash Buffer 4 to plate **C**.
4. Add 500 µl of Wash Buffer 5 to plate **D**.
5. Add 500 µl of Wash Buffer 6 to plate **E**.
6. Add 200 µl of Elution Buffer to plate **F**.
7. Combine the tip comb and The KingFisher plate. See KingFisher 96 User manual.
8. Select the chemagic DNA Blood250 protocol protocol using arrow keys and press START button.
9. Load the plates according to protocol request and press START after every plate to confirm the action.
10. **Note!** Confirm that the plates are placed 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.
11. The purification protocol will start when the last plate is loaded and START button is pressed.
12. During Pause-step add 30 µl of resuspended Magnetic Beads and 570 µl of Binding Buffer 2 to plate **A**.
13. After the purification process is completed the plates are removed according to instructions shown in instrument screen. Press START after each plate removal to confirm the action.

14. When the last plate is removed text End\_of\_run will appear. Press STOP to complete the run.

## Description of Chemagic DNA Blood250 protocol with KingFisher 96

1. Sample is incubated first with Lysis buffer 1 in plate A for 5 minutes. After pause, sample is incubated with magnetic beads and Binding Buffer 2 for 5 minutes. Magnetic bead/DNA complexes are formed.
2. Magnetic beads are washed with Wash Buffer 3, 4, 5 and 6 in plates B, C, D and E respectively.
3. Beads are dried outside plate E for 8 minutes.
4. DNA is released to Elution Buffer in plate F for 10 minutes with heating.
5. Beads are discarded into plate B.

## KingFisher mL protocol

### Importing protocol from the web

KingFisher mL Software protocol for chemagic DNA Blood250 Kit can also be downloaded from the website ([www.thermo.com/kingfisher](http://www.thermo.com/kingfisher)). Save the file “**Chem\_DNABlood\_KFmL**” to your computer.

### Sample preparation

- KingFisher mL DNA protocol **chemagic DNA Blood250** is designed to purify DNA from human whole blood (fresh, non-coagulated and frozen).
- Use KingFisher mL tubestrips and tip combs (Catalog No. 97002141) with **chemagic DNA Blood250** protocol.
- Add sample and other reagents supplied by **chemagic DNA Blood250** kit to KingFisher mL tubestrips according to table 2 and instructions below.

### KingFisher mL process

**Table 2** Pipetting instructions for KingFisher mL and chemagic DNA Blood250 protocol.

Tube	Content	Sample/Reagent volume
A	Sample	150 µl
	Lysis Buffer 1	250 µl
	Magnetic beads (added during pause)	30 µl
	Binding Buffer 2 (added during pause)	570 µl
B	Wash Buffer 3	500 µl
C	Wash Buffer 4	500 µl
D	Wash Buffer 5	500 µl
E	Elution Buffer	200 µl

1. Place an appropriate number of tube strips needed for the samples (one tube strip per sample) into removable tube strip tray.
2. Add 150 µl of Sample and 250 µl of Lysis Buffer to tube strip **A**.
3. Add 500 µl of Wash Buffer 3 to tube strip **B**.
4. Add 500 µl of Wash Buffer 4 to tube strip **C**.
5. Add 500 µl of Wash Buffer 5 to tube strip **D**.

6. Add 200 µl of Elution Buffer to tube strip **E**.
7. Insert the tube strip tray to the instrument and insert the tips combs into the slots.
8. Close the front lid and start the process by selecting intended protocol Chem\_DNABlood\_KFmL using arrow keys and by pressing START.
9. During Pause-step add 30 µl of Magnetic Beads and 570 µl of Binding Buffer 2 to tube strip **A**.
10. Remove the tube strip tray from the KingFisher mL after program has completed.

### Description of Chemagic DNA Blood250 protocol with KingFisher mL

1. Sample is incubated first with Lysis buffer 1 in tube strip A for 5 minutes. After pause, sample is incubated with magnetic beads and Binding Buffer 2 for 5 minutes. Magnetic bead/DNA complexes are formed.
2. Magnetic beads are washed with Wash Buffer 3, 4 and 5 in tube strips B, C and D respectively.
3. Beads are dried outside tube strip D for 8 minutes.
4. Sample is released to Elution Buffer in tube strip E. The sample is then moved manually to eppendorf tube and incubated in a heat block for 10 minutes to release the DNA. The sample is then moved back to tube strip E.
5. Beads are discarded into tube strip A.

## Trouble shooting

1. Low yield
  - Refer to chemagic DNA Blood250 Kit manual.
2.  $A_{260}/A_{280}$  ratio is too high or low
  - Refer to chemagic DNA Blood250 Kit manual.
3. Precipitate in reagent bottle
  - Refer to chemagic DNA Blood250 Kit manual.
4. Degraded DNA
  - Refer to chemagic DNA Blood250 Kit manual.
5. Any steps of the protocol (e.g. sample incubation and elution times) and the reagent volumes can be modified with KingFisher® software.
6. Tip comb was forgotten
  - Clean the magnetic rods using a soft cloth or tissue paper soaked in mild detergent solution, soap or alcohol.
7. The processor is not working properly
  - Refer to Kingfisher User Manual

## Ordering Information

Product no.	Product Description
540 05 00	KingFisher 96, 110V-240V, Magnetic particle processor
24073430	Magnet head for Deep Well plate
97002534	KingFisher 96 tip comb for DW magnets (10 x 10 pcs/box)
97002540	KingFisher 96 plate (200 µl), 48 plates/box
95040450	Deep Well 96 plate, V-bottom, Polypropylene
540 00 50	KingFisher mL, 110-240 V, Magnetic particle processor
97002131	KingFisher mL Combi 60 (tubes and tips for 60 samples)
97002141	KingFisher mL Combi 240 (tubes and tips for 240 samples)
104	<i>chemagic DNA Blood250 Kit</i>

## Contact information

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