

USER GUIDE

for

Automated purification of genomic DNA with

KingFisher 96 instrument

and

AGOWA[®] mag Maxi DNA Isolation Kit

For purification of DNA from blood samples

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Description

Purification of genomic DNA from blood samples using *AGOWA[®]mag Maxi DNA Isolation Kit* (Catalog No.40403) can be easily automated using KingFisher[®] instruments (Thermo Electron Corporation). The KingFisher platforms utilize patented technology in which magnetic rods move particles through purification process. KingFisher 96 can process up to 96 samples per run and it is compatible with deep well plates, PCR plates and KingFisher 96 plates. Specially designed magnetic rods and tips combs protecting the magnets are available for different plate types.

AGOWA[®]mag Maxi DNA Isolation Kit is based on coated magnetic particles which bind DNA in the presence of detergents and chaotropic salt. After binding and washing steps, the purified DNA is released in elution buffer. The processing time with KingFisher 96 is approximately 35 minutes. Typically, genomic DNA isolation from 200 µl of EDTA blood with KingFisher 96 results about 4 µg high quality genomic DNA.

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

Important notes

- Make sure that the appropriate magnet head is attached to the KingFisher 96 processor. If magnet head need to be changed follow the KingFisher 96 User manual instructions.
- Protocol **AGOWA_Blood_KF96** is compatible with KingFisher software version 2.6.
- See protocol of *AGOWA[®]mag Maxi DNA Isolation Kit* for reagent storage, product use limitations, safety information etc.
- Resuspend magnetic particles (*AGOWA[®]mag* Particles Suspension BLM) thoroughly before use.

- See [protocol of AGOWA[®]mag Maxi DNA Isolation Kit](#) for preparation of protease solution and Wash Buffer BLM2.

Reagents needed but not provided

- Acetone (96-100%)
- Ethanol (96-100%)

Magnet and plastics requirements

- The isolation procedure is designed to operate with 2 ml deep well and KingFisher 96 plates.
- Thermo Microtiter Deep Well 96 plate (prod. No. 95040450) has been tested and is recommended to be used with KingFisher 96 and *AGOWA[®]mag Maxi DNA isolation Kit*.
- Be sure that the appropriate magnet head (magnet head for deep well plate) is installed before starting!
- The requirements for magnet and plastics used with KingFisher and **AGOWA_Blood_KF96** are summarized in table 1.

Table 1. Magnet and plastics requirements

Magnet head	Magnet head for deep well plate
Tip comb	KingFisher 96 tip comb for deep well magnets
Plate types	Deep well plate (5)* KingFisher 96 plate (2)*

*No. of plate types needed/run

Genomic DNA isolation from blood samples

Sample preparation

- The protocol **AGOWA_Blood_KF96** is designed to purify genomic DNA from blood. Up to **200 µl** of blood can be used as a starting material.
- Pipet 200 µl Lysis buffer BLM into the bottom of the deep well plate.
- Add 20 µl of protease solution and 200 µl of blood sample.
- Mix the samples by pulse-vortexing for 10 s.
- Incubate the sample plate at 55°C for 10 min.
 - Note! Cover the plate during incubation.
- Add 200 µl Ethanol
- Add 20 µl *AGOWA[®]mag* Particles Suspension BLM.
- Fill the deepwell/KingFisher plates with sample lysate and reagents supplied by *AGOWA[®]mag Maxi DNA Isolation Kit* according to table 2 and instructions below.

KingFisher 96 process

Table 2. Plate filling instructions for KingFisher 96 and **AGOWA_Blood_KF96** protocol.

Plate type*	Well content	Volume	Plate name in protocol
A	Tip comb	-	Tip plate
B	Blood lysate Ethanol Particles Suspension BLM.	420 µl 200 µl 20 µl	Lysis
B	Buffer BLM1	500 µl	Wash 1
B	Buffer BLM1	500 µl	Wash 2
B	Buffer BLM2	500 µl	Wash 3
B	Buffer BLM2	500 µl	Wash 4
A	Elution Buffer BLM	200 µl	Elution

*A = KingFisher 96 plate, B = Thermo Microtiter Deep well 96 plate

1. Fill plates "Wash 1" and "Wash 2" with 500 µl of Buffer BLM1.
2. Fill plate "Wash 3" and "Wash 4" with 500 µl of Buffer BLM2.
3. Fill plate "Elution" with 200 µl of Elution Buffer BLM.
4. Add 200 µl of ethanol (96-100%) and 20 µl of Mag particles Suspension BLM into plate containing lysed sample (plate "Lysis").
5. Combine the tip comb and The KingFisher plate. See KingFisher 96 User manual.
6. Select the **AGOWA_Blood_KF96** protocol using arrow keys and press START button.
7. Load the plates according to protocol request and press START after every plate to confirm the action.
8. **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.
9. The purification protocol will start when the last plate is loaded and START button is pressed.
10. 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.
11. When the last plate is removed text End_of_run will appear. Press STOP to complete the run.

AGOWA_Blood_KF96 protocol description

1. The tip comb is attached to the tip comb holder.
2. The blood lysate is incubated in deep well plate (plate "Lysis") with magnetic particles for 4 minutes.
3. The particles are washed in Buffer BLM1 for 2 minutes (plate "Wash 1").
4. The particles are washed in Buffer BLM1 for 1 minute (plate "Wash 2").
5. The particles are washed in Buffer BLM2 for 2 minutes (plate "Wash 3").

6. The particles are washed in Buffer BLM2 for 1 minute (plate "Wash 4").
7. A drying step (10 min) is performed after Buffer BLM2 washings.
8. DNA is released into Elution buffer BLM in plate "Elution".
9. Particles are discarded to plate "Wash 4".

Ordering Information

Product no.	Product Description
5400500	KingFisher 96 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	Thermo Microtiter Deep Well 96 plate
40403	<i>AGOWA[®] mag Maxi DNA Isolation Kit</i>

Contact information

Thermo Electron
Ratastie 2, P.O. Box 100
FIN-01621 Vantaa
Finland
Tel. +358-9-329100
Fax. +358-9-32910415
www.thermo.com

AGOWA GmbH
Dr. Frank Schubert
Glienicker Weg 185
D-12489 Berlin
Germany
Tel. +49 (0) 30 67057238
Fax +49 (0) 30 67057228
Email: service@agowa.de
www.agowa.de