

USER GUIDE

for

Automated purification of DNA from blood, cells & tissue samples

**KingFisher 96/ KingFisher mL instrument
and
MagExtractor – *Genome* - Kit**

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Description

Purification of DNA from whole blood using Toyobo's MagExtractor – *Genome*- kit (NPK-101, 102) can easily be automated using KingFisher® instruments (Thermo Electron Corporation). 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 100 µl of human blood using KingFisher results 2 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 Toyobo's MagExtractor – *Genome*- Kit Handbook for reagent storage, product use limitations, safety information etc.
- Resuspend Magnetic Beads (DNA Binding Beads) thoroughly before use.

KingFisher 96 protocol

Importing protocols from the web

KingFisher Software protocol for MagExtractor –*Genome*- Kit can be downloaded from the website (www.thermo.com/kingfisher). First you have to **save the file “Toyobo_gDNA_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 **MagExtractor –Genome**- is designed to purify DNA from *in vivo* samples such as whole blood, cultured cells and Tissue.
- Use Thermo deepwell microplate (Catalog No. 95040450), KingFisher deepwell tip comb (Catalog No. 97002534) and KingFisher 96 plate (Catalog No. 97002540) with **MagExtractor –Genome**- protocol.
- Sample pre-treatment can be done as described in **MagExtractor –Genome**- Kit handbook. Short descriptions:

- 1) Whole Blood

- If the amount of whole blood is less than 100 µl add Lysis & Binding Solution until the volume is reached.
 - 2) Culture cells
 - Centrifuge the culture cells (600 rpm, 5minutes) and remove the supernatant
 - 3) Tissue, Mouse tail
 - Dissolving the sample using Lysis & Binding Solution (refer to MagExtractor –Genome- Kit handbook).
 - Dissolving samples using proteinase K digestion (refer to MagExtractor – Genome- Kit handbook).
- Add sample and other reagents by **MagExtractor -Genome-** kit to Thermo deepwell microplate according to table 1 and instructions below.

KingFisher 96 process

Table 1 Pipetting instructions for KingFisher 96 and MagExtractor -Genome- protocol.

Plate type *	Plate n:o	Content	Sample/ Reagent volume
A	1	Sample	100 µl
		Lysis&Binding Solution	850 µl
		Magnetic Beads	40 µl
A	2	Washing Solution	700 µl
A	3	Washing Solution	700 µl
A	4	70 % Ethanol	700 µl
A	5	70 % Ethanol	700 µl
B	6	Elution Buffer	150 µl

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

1. Add 100 µl of Sample, 850 µl of Lysis& Binding Solution and 40 µl of Magnetic Beads to plate 1
2. Add 700 µl of Washing Solution to plate 2.
3. Add 700 µl of Washing Solution to plate 3.
4. Add 700 µl of 70 % Ethanol to plate 4.
5. Add 700 µl of 70 % Ethanol to plate 5.
6. Add 150 µl of Elution Buffer to plate 6.
7. Combine the tip comb and The KingFisher plate. See KingFisher 96 User manual.
8. Select the MagExtractor -Genome- 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. 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.
13. When the last plate is removed text End_of _run will appear. Press STOP to complete the run.

Description of MagExtractor -Genome- protocol with KingFisher 96

1. Sample is incubated with Lysis&Binding Solution and with Magnetic Beads in plate 1 for 8 minutes first.
2. Magnetic beads are washed with Washing Solution in plates 2 and 3.
3. Beads are washed also with 70 % Ethanol in plate 4 and 5.
4. Beads are dried outside plate 5 for 10 minutes.
5. DNA is released to Elution Buffer in plate 6 for 15 minutes.
6. Used beads are discarded into plate 2.

KingFisher mL protocol

Importing protocol from the web

KingFisher mL Software protocol for MagExtractor -*Genome*- Kit can also be downloaded from the website (www.thermo.com/kingfisher). Save the file “**Toyobo_gDNA_KFmL**” to your computer.

Sample preparation

- KingFisher mL DNA protocol **MagExtractor -Genome-** is designed to purify DNA from *in vivo* samples such as whole blood, cultured cells and Tissue.
- Use KingFisher mL tubestrips and tip combs (e.g. Catalog No. 97002141) with **MagExtractor -Genome-** protocol.
- Sample pre-treatment can be done as described in **MagExtractor -Genome-** Kit handbook. Short descriptions can be seen in KingFisher 96 protocol.
- Add sample and other reagents supplied by **MagExtractor -Genome-** kit to KingFisher mL tubestrips according to table 2 and instructions below.

KingFisher mL process

Table 2 Pipetting instructions for KingFisher mL and MagExtractor -*Genome*- protocol.

Tube	Content	Sample/Reagent volume
A	Sample	100 μ l
	Lysis&Binding Solution	850 μ l
	Magnetic beads	40 μ l
B	Washing Buffer	900 μ l
C	70 % Ethanol	900 μ l
D	70 % Ethanol	900 μ l
E	High quality water	100 μ l

1. Place an appropriate number of tube strips needed for the samples (one tube strip per sample) into removable tube strip tray.
2. Add 100 μ l of Sample, 850 μ l of Lysis&Binding Solution and 40 μ l of Magnetic Beads to tube strip **A**.

3. Add 900 μ l of Washing Solution to tube strip **B**.
4. Add 900 μ l of 70 % Ethanol to tube strip **C**.
5. Add 900 μ l of 70 % Ethanol to tube strip **D**.
6. Add 100 μ l of High quality water 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 Toyobo_gDNA_KFmL using arrow keys and by pressing START.
9. Remove the tube strip tray from the KingFisher mL after program has completed.

Description of MagExtractor -*Genome*- protocol with KingFisher mL

1. Sample is incubated first with Lysis&Binding Solution and with Magnetic Beads in tube strip A for 8 minutes.
2. Magnetic beads are washed with Washing Solution in tube strips B.
3. Magnetic beads are washed also with 70 % Ethanol in tube strips C and D.
4. Beads are dried outside tube strip D for 10 minutes.
5. DNA is released to High Quality water in tube strip E for 15 minutes.
6. Used beads are discarded into tube strip B.

Trouble shooting

1. Low yield and no DNA extracted
 - Refer to MagExtractor -*Genome*- Kit manual.
2. Unsuccessful PCR
 - Refer to MagExtractor -*Genome*- Kit manual.
3. The beads don't completely scatter when cleansing and they clog up the chips.
 - Refer to MagExtractor -*Genome*- Kit manual.
4. Unfavorable dispensing of the beads.
 - Refer to MagExtractor -*Genome*- 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)
97002111	KingFisher mL tip comb, 800 pcs
97002121	KingFisher mL tube, 900 pcs (20X45 pcs)
<i>NPK-101, 102</i>	MagExtractor - <i>Genome</i> - Kit

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