

USER GUIDE

for

Automated purification of genomic DNA from bacteria with

KingFisher 96/ KingFisher mL

and

chemagic DNA Bacteria Kit

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Description

Purification of DNA from bacteria with chemagen's chemagic DNA Bacteria Kit (cat. no. 190) 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.

Typically, DNA isolation from 200 µl of e.g. overnight bacteria culture 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 chemagen's chemagic DNA Bacteria Kit for reagent storage, product use limitations, safety information etc ([instructions](#)).
- Resuspend Magnetic Beads (DNA Binding Beads) thoroughly before use.

KingFisher 96 protocol

Importing protocols from the web

KingFisher Software protocol for chemagic DNA Bacteria Kit can be downloaded from the website (www.thermo.com/kingfisher). First you have to **save the file "Chem_BactDNA1_Liquid_Culture_KF96" (overnight bacteria culture) and "Chem_BactDNA2_Pellet_KF96" for 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 and reagent preparation

- KingFisher DNA protocol **chemagic DNA Bacteria** is designed to purify DNA from bacteria pellets and cultures.
- Use Thermo deepwell microplate (Catalog No. 95040450), KingFisher deepwell tip comb (Catalog No. 97002534) and KingFisher 96 plate (Catalog No. 97002540) with **chemagic DNA Bacteria** protocol.
- Before starting the protocol, mix one part of resuspended Magnetic particles and 15 parts of Binding Buffer 2. Incubate the mixture 5 minutes at room temperature.
- Add sample and other reagents supplied by chemagic DNA Bacteria Kit to Thermo deepwell microplate according to tables 1 and 2 and instructions below.

KingFisher 96 process – Bacteria Pellets

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

Plate type*	Plate	Content	Sample/ Reagent volume
A	1	Bacteria Pellet	20 µl
		Lysis Buffer 1	200 µl
		RNase A	2 µl
		Magnetic Beads and DNA Binding Buffer 2 (added during Pause step)	320 µl
A	2	Wash Buffer 3	500 µl
A	3	Wash Buffer 4	500 µl
A	4	Wash Buffer 5	1000 µl
B	5	Elution Buffer 6	150 µl

* A= Thermo Deepwell plate, B=KingFisher 96 plate

1. Add 20 µl of Bacteria Pellets, 200 µl of Lysis Buffer1 and 2 µl of RNase A to plate **1**
2. Add 500 µl of Wash Buffer 3 to plate **2**.
3. Add 500 µl of Wash Buffer 4 to plate **3**.
4. Add 1000 µl of Wash Buffer 5 to plate **4**.
5. Add 150 µl of Elution Buffer 6 to plate **5**.
6. Combine the tip comb and The KingFisher plate. See KingFisher 96 User manual.
7. Select the chemagic DNA Bacteria protocol for Bacteria Pellets using arrow keys and press START button.
8. Load the plates according to protocol request and press START after every plate to confirm the action.
9. **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.
10. The purification protocol will start when the last plate is loaded and START button is pressed.
11. Add 320 µl of Magnetic Beads and DNA Binding Buffer 2 mixture during Pause step to plate **1**.

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 chemagic DNA Bacteria protocol (using bacteria pellet as sample) with KingFisher 96

1. Sample (Bacteria Pellet) is incubated with Lysis Buffer 1 and RNase A in plate 1 for 10 minutes in 40°C.
2. Magnetic Beads are added to plate 1 during Pause step.
3. Sample is bound to beads in plate 1 during 5 minutes incubation.
4. Magnetic beads are washed with Wash Buffer 3, 4 and 5 in plates 2, 3 and 4 respectively.
5. Beads are dried outside plate 5 for 10 minutes.
6. DNA is released to Elution Buffer 6 in plate 5 during 5 minutes incubation in 70°C.
7. Beads are discarded into plate 2.

KingFisher 96 process – Overnight bacteria culture

Table 2 Pipetting instructions for KingFisher 96 and chemagic DNA Bacteria protocol.

Plate type *	Plate	Content	Sample/ Reagent volume
A	1	Overnight culture	100 µl
		Lysis Buffer 1	200 µl
		RNase A	2 µl
		Magnetic Beads and DNA Binding Buffer 2 (added during Pause step)	470 µl
A	2	Wash Buffer 3	500 µl
A	3	Wash Buffer 4	500 µl
A	4	Wash Buffer 5	1000 µl
B	5	Elution Buffer 6	150 µl

* A= Thermo Deepwell plate, B=KingFisher 96 plate

1. Add 100 µl of overnight bacteria culture, 200 µl of Lysis Buffer1 and 2 µl of RNase A to plate **1**
2. Add 500 µl of Wash Buffer 3 to plate **2**.
3. Add 500 µl of Wash Buffer 4 to plate **3**.
4. Add 1000 µl of Wash Buffer 5 to plate **4**.
5. Add 150 µl of Elution Buffer 6 to plate **5**.
6. Combine the tip comb and The KingFisher plate. See KingFisher 96 User manual.
7. Select the chemagic DNA Bacteria protocol for overnight bacteria culture using arrow keys and press START button.
8. Load the plates according to protocol request and press START after every plate to confirm the action.
9. **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.
10. The purification protocol will start when the last plate is loaded and START button is pressed.
11. Add 470 µl of Magnetic Beads and DNA Binding Buffer 2 mixture during Pause step to plate **1**.

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 chemagic DNA Bacteria protocol (using overnight bacteria culture as sample) with KingFisher 96

1. Sample (overnight bacteria culture) is incubated with Lysis Buffer 1 and RNase A in plate 1 for 10 minutes in 40°C.
2. Magnetic Beads are added to plate 1 during Pause step.
3. Sample is bound to beads in plate 1 during 5 minutes incubation.
4. Magnetic beads are washed with Wash Buffer 3, 4 and 5 in plates 2, 3 and 4 respectively.
5. Beads are dried outside plate 5 for 10 minutes.
6. DNA is released to Elution Buffer 6 in plate 5 during 5 minutes incubation in 70°C.
7. Beads are discarded into plate 2.

KingFisher mL protocol

Importing protocols from the web

KingFisher Software protocol for chemagic DNA Bacteria Kit can be downloaded from the website (www.thermo.com/kingfisher). First you have to save the file “**Chem_BactDNA1_Liquid_Culture_KFmL**” (overnight bacteria culture) and “**Chem_BactDNA2_Pellet_KFmL**” for 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 and reagent preparation

- KingFisher DNA protocol **chemagic DNA Bacteria** is designed to purify DNA from bacteria pellets and cultures.
- Use KingFisher mL tubestrips and tip combs (e.g. Catalog No. 97002141) with with **chemagic DNA Bacteria** protocol.
- Before starting the protocol, mix one part of resuspended Magnetic particles and 15 parts of Binding Buffer 2. Incubate the mixture 5 minutes at room temperature.
- Add sample and other reagents supplied by chemagic DNA Bacteria Kit to KingFisher mL tubestrips according to tables 3 and 4 and the instructions below.

KingFisher mL process – Bacteria Pellets

Table 3 Pipetting instructions for KingFisher mL and chemagic DNA Bacteria protocol.

Tube	Content	Sample/ Reagent volume
A	Lysate	300 µl
	Binding buffer	450 µl
	Beads	20 µl
B	Washing Buffer 3	500 µl
C	Washing Buffer 4	500 µl
D	Washing Buffer 5	1000 µl
E	Elution Buffer	200 µl

- Place an appropriate number of tube strips needed for the samples (one tube strip per sample) into removable tube strip tray.
- Add 300 µl of Lysate, 450 µl of Binding buffer and 40 µl of Beads to tube strip **A**.
- Add 500 µl of Washing Buffer 3 to tube strips **B**.
- Add 500 µl of Washing Buffer 4 to tube strips **C**.
- Add 1000 µl of Washing Buffer 5 to tube strip **D**.
- Add 200 µl of Elution Buffer to tube strip **E**.
- Insert the tube strip tray to the instrument and insert the tips combs into the slots.
- Close the front lid and start the process by selecting intended protocol Chem_BactDNA2_Pellet_KFmL using arrow keys and by pressing START.
- Remove the tube strip tray from the KingFisher mL after program has completed.
- During Pause step take the tube strip E from the tray and heat it manually in 70°C for 5 minutes.
- After manual heating put the tube strip E back to the tray.
- Beads are postmixed for 1 minute in tube strip E.
- Beads are discarded into tube strip D.

Description of chemagic DNA Bacteria protocol (using bacteria pellet as sample) with KingFisher mL

- Sample is bound to beads in tube strip A during 5 minutes incubation.
- Magnetic beads are washed with Washing Buffer 3, 4 and 5 in tube strips B, C and D respectively.
- DNA is released to Elution Buffer in tube strip E.

KingFisher mL process – Overnight bacteria culture

Table 4 Pipetting instructions for KingFisher 96 and chemagic DNA Bacteria protocol.

Tube	Content	Sample/ Reagent volume
A	Lysate	220 µl
	Binding buffer	300 µl
	Beads	20 µl
B	Washing Buffer 3	500 µl
C	Washing Buffer 4	500 µl
D	Washing Buffer 5	1000 µl
E	Elution Buffer	200 µl

- Place an appropriate number of tube strips needed for the samples (one tube strip per sample) into removable tube strip tray.
 - Add 220 µl of Lysate, 300 µl of Binding buffer and 40 µl of Beads to tube strip **A**.
 - Add 500 µl of Washing Buffer 3 to tube strips **B**.
 - Add 500 µl of Washing Buffer 4 to tube strips **C**.
 - Add 1000 µl of Washing Buffer 5 to tube strip **D**.
 - Add 200 µl of Elution Buffer to tube strip **E**.
 - Insert the tube strip tray to the instrument and insert the tips combs into the slots.
 - Close the front lid and start the process by selecting intended protocol Chem_BactDNA1_Liquid_Culture_KFmL using arrow keys and by pressing START.
 - Remove the tube strip tray from the KingFisher mL after program has completed.
- After manual heating put the tube strip E back to the tray.
 - Beads are postmixed for 1 minute in tube strip E.
 - Beads are discarded into tube strip D.

Trouble shooting

- Any steps of the protocol (e.g. sample incubation and elution times) and the reagent volumes can be modified with KingFisher® software.
- Tip comb was forgotten
 - Clean the magnetic rods using a soft cloth or tissue paper soaked in mild detergent solution, soap or alcohol.
- The processor is not working properly
 - Refer to Kingfisher User Manual

Description of chemagic DNA Bacteria protocol (using overnight bacteria culture as sample) with KingFisher mL

- Sample is bound to beads in tube strip A during 5 minutes incubation.
- Magnetic beads are washed with Washing Buffer 3, 4 and 5 in tube strips B, C and D respectively.
- DNA is released to Elution Buffer in tube strip E.
- During Pause step take the tube strip E from the tray and heat it manually in 70°C for 5 minutes.

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)
190	<i>chemagic DNA Bacteria Kit</i>

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