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Blood DNA Isolation Kit (Magnetic Bead System) – Supplementary Protocol

Product # 59800 & 62600

Using KingFisher Flex with 96 deep-well magnetic head

Component Required

Component (Either One)	Product Number
Blood DNA Isolation Kit (Magnetic Bead System)	59800
Blood DNA Isolation 96-Well Kit (Magnetic Bead System)	62600

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 96 – 100 % Ethanol
- Micropipettors
- Microcentrifuge tube
- 70% ethanol (prepare fresh)
- Lysozyme (40 mg/mL) (For blood containing Gram positive bacterial pathogens)
- KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head (Cat. 5400630)
- KingFisher™ Flex Deep Well heating block (24075430)
- KingFisher™ deep-well 96 plate (95040450 or 95040460)
- KingFisher™ Flex 96 heating block (24075420) – *optional*
- KingFisher™ 96 KF plate (97002540) – *optional*
- KingFisher™ 96 tip comb for deep-well magnets (97002534)

Notes prior to use:

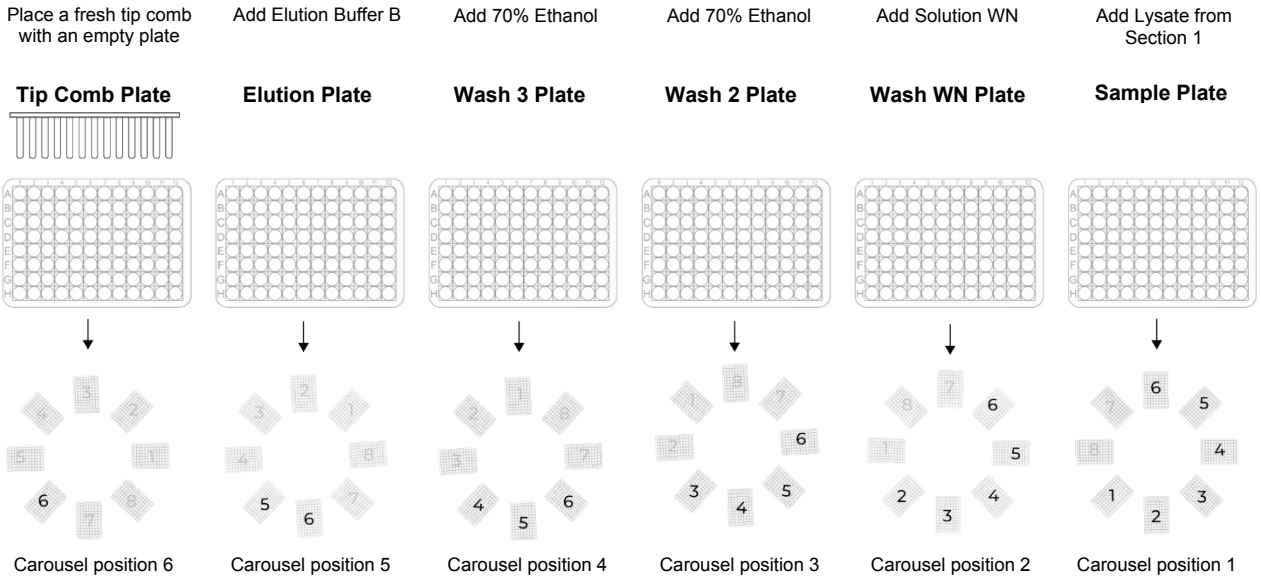
- The procedure provides information and steps to follow to extract DNA using automated methods using KingFisher™ Flex platform. KingFisher instruments come with heat blocks and the scripts provided include the incubation at required temperature.
- **Section 1** describes automated purification of DNA from blood without gram-positive bacteria while **Section 2** describes automated purification of DNA from blood with gram-positive bacteria.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- **Always** vortex the **Magnetic Beads A** and **Proteinase K** before use.
- For best results, the use of whole blood collected into tubes containing an anticoagulant is highly recommended.
- Both fresh and frozen anticoagulated blood may be used with this procedure. Ensure that frozen blood is thawed at room temperature prior to starting the protocol.
- **For blood containing Gram positive bacterial pathogens**, prepare a 40 mg/mL stock solution (approximately 1.7 x 10^7 units/mL) of lysozyme as per supplier's instructions
- Prepare a working concentration of the **Solution WN** by adding 24 mL of 96 - 100 % ethanol (not provided) to the supplied bottle containing the concentrated **Solution WN**. This will give a final volume of 42 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- The isolation can be performed by using the following combination of heating blocks and plates

Instrument	Heat Block	Plate type	Elution Plate type
KingFisher Flex	Deep Well heating block*	deep-well 96 plate (95040450 or 95040460)	deep-well 96 plate (95040450 or 95040460)
	96 heating block		deep-well 96 plate (95040450 or 95040460) or 96 KF plate (97002540)

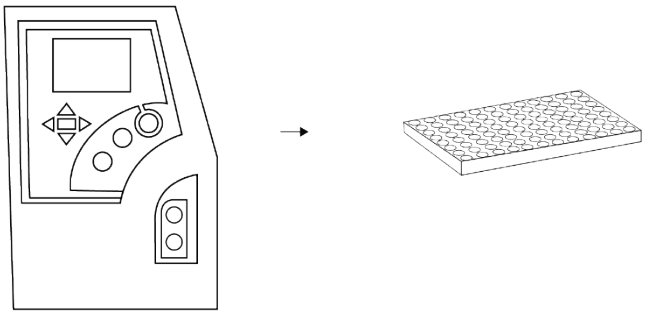
**Deep Well heating block is not compatible with 96 KF plate. Selecting the correct heating block and plates is important as elution requires a heating step. Using incompatible plates can lead to reduced DNA yield.*

Flow Chart

Procedure for using Norgen's Blood DNA Isolation Kit (Magnetic Bead System) – KingFisher™ Flex 96



Run Instrument



Place Sample at position 1 and continue to run program.

Run Complete. Take Elution plate and store DNA.

Table 1. Set-up for Blood DNA Extraction

Prepare plates as per the volumes mentioned in the table below. Detailed description of step-wise process can be found in **Section 1 and 2.**

Table 1a. Setup of plates while using KingFisher™ Flex with a 96 Deep-well Head and a deep well heating block.

Set-up while using Deep well heating block with 96 Deep-Well Head*				
Component	**Plate ID**	**Location on Carousel**	**Plate Type**	**Amount**
Lysate	Sample	1	deep-well	840 µL
Solution WN	Wash WN	2	deep-well	500 µL
70% Ethanol	Wash 2	3	deep-well	500 µL
70% Ethanol	Wash 3	4	deep-well	500 µL
Elution Buffer B	Elution	5	deep-well	100 µL
Tip Comb	Tip Comb	6	Place a 96-well Deep-well Tip Comb in a deep-well plate	

**Refer to the table (1a or 1b) based on the heating block and plates being used. Deep Well heating block is not compatible with 96 KF plate. Selecting the correct heating block and plates is important as elution requires a heating step. Using incompatible plates can lead to reduced DNA yield.*

Table 1b. Setup of plates while using KingFisher™ Flex with a 96 Deep-well Head and a 96 heating block.

Set-up while using 96 heating block with 96 Deep-Well Head*				
Component	**Plate ID**	**Location on Carousel**	**Plate Type**	**Amount**
Lysate	Sample	1	deep-well	840 µL
Solution WN	Wash WN	2	deep-well	500 µL
70% Ethanol	Wash 2	3	deep-well	500 µL
70% Ethanol	Wash 3	4	deep-well	500 µL
Elution Buffer B	Elution	5	KF plate	100 µL
Tip Comb	Tip Comb	6	Place a 96-well Deep-well Tip Comb in a deep-well plate	

**Refer to the table (1a or 1b) based on the heating block and plates being used. Deep Well heating block is not compatible with 96 KF plate. Selecting the correct heating block and plates is important as elution requires a heating step. Using incompatible plates can lead to reduced DNA yield.*

Table 2. KingFisher™ Flex scripts to be used while working with KingFisher™ 96 Deep-Well Head (Cat. 5400630).

Heating Block	Elution Plate	Gram Positive	KingFisher Script
96 Deep-Well (24075430)	Deep-Well 96 plate (95040450 or 95040460)	No	BLDNA_96_KF_DW
		Yes	BLDNA_96_GP_KF_DW
96 KF (24075420)	96 KF plate (97002540)	No	BLDNA_96_KF
		Yes	BLDNA_96_GP_KF

Section 1. Blood DNA Isolation without gram-positive bacteria

a) Setup all the plates according to **Table 1a or 1b.** Place a clean tip comb in an empty plate **(Tip Comb plate).**
b) Add 100 µL of **Elution Buffer B** to the **Elution plate.**
c) Add 500 µL **Solution WN** (added with ethanol) to **Wash WN plate**.
d) Add 500 µL **70% ethanol** to **Wash plate 2** and **Wash plate 3.**
e) Transfer 200 µL of blood sample to **Sample plate**. If the volume of blood is less than 200 µL, simply bring up the volume to 200 µL using 1X PBS.
f) Add 20 µL of **Proteinase K** and 300 µL of **Lysis Buffer B** to the well with blood sample.

Alternatively, prepare a mixture of 20 µL of **Proteinase K** and 300 µL of **Lysis Buffer B** to the sample. We recommend a 10% overage while making mixture for multiple samples, to compensate for pipetting errors. Make sure to mix by inverting the bulk before adding it to the lysate (do not vortex). It is recommended to prepare the bulk fresh every time a new DNA isolation is performed.

g) After setting up, run the program **BLDNA_96_KF or BLDNA_96_KF_DW** based on Table 2.
h) Place the plates as promoted by the KingFisher™ Flex instrument.
i) Prepare a mixture of 500 µL **96-100% ethanol** and 40 µL **Magnetic Beads A** for the number of samples extracted. (For example, if DNA is to be extracted from 10 samples, mix 5 mL of **96-100% ethanol** with 400 µL **Magnetic Beads A)** A fresh bulk mixture can be made every time RNA isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.
j) After a few minutes of incubation, the instrument will pause and allow the user to add mixture prepared in **step i**. Open the instrument carousel door and remove **Sample plate** from the instrument and add 540 µL of **96-100% ethanol** and **Magnetic Beads A** mixture that was prepared in **step i**. Make sure to vortex this mixture before adding to the sample, to maintain a homogenous mixture.
k) Put the plate back into the instrument and follow the instrument prompt. The instrument will continue to perform the extraction.
l) The instrument will prompt after the procedure is complete. Remove the **Elution plate** from the instrument and the DNA is now ready for further downstream processing.
m) The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at –20°C for long-term storage.

Section 2: Blood DNA Isolation with gram-positive bacteria

a) Setup all the plates according to **Table 1a or 1b.** Place a clean tip comb in an empty plate **(Tip Comb plate).**
b) Add 100 µL of **Elution Buffer B** to the **Elution plate.**
c) Add 500 µL **Solution WN** (added with ethanol) to **Wash WN plate**.
d) Add 500 µL **70% ethanol** to **Wash plate 2** and **Wash plate 3.**
e) Transfer 200 µL of blood sample to **Sample plate**. If the volume of blood is less than 200 µL, simply bring up the volume to 200 µL using 1X PBS.
f) Prepare a 40 mg/mL stock solution (approximately 1.7 x 10^7 units/mL) of lysozyme as per supplier's instructions and add 20 µL of **Lysozyme** to **Sample plate**.
g) After setting up, run the program **BLDNA_96_GP_KF or BLDNA_96_GP_KF_DW** based on Table 2.
h) Place the plates as promoted by the KingFisher™ Flex instrument.
i) After an hour of incubation, the instrument will pause and allow the user to continue with next steps. Open the instrument carousel door and remove the plate from the instrument and add 20 µL of **Proteinase K** and 300 µL of **Lysis Buffer B** to the well with blood sample.
Alternatively, prepare a mixture of 20 µL of **Proteinase K** and 300 µL of **Lysis Buffer B** to the sample. We recommend a 10% overage while making mixture for multiple samples, to compensate for pipetting errors. Make sure to mix by inverting the bulk before adding it to the lysate (do not vortex). It is recommended to prepare the bulk fresh every time a new DNA isolation is performed.
j) Put the plate back into the instrument and follow the instrument prompt. The instrument will continue to perform the extraction.
k) Prepare a mixture of 500 µL **96-100% ethanol** and 40 µL **Magnetic Beads A** for the number of samples extracted. (For example, if DNA is to be extracted from 10 samples, mix 5 mL of **96-100% ethanol** with 400 µL **Magnetic Beads A)** A fresh bulk mixture can be made every time RNA isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.
l) After a few minutes of incubation, the instrument will pause and allow the user to add mixture prepared in **step k**. Open the instrument carousel door and remove the plate from the instrument and add the prepared mixture from **step k** to **Sample plate**. Make sure to vortex this mixture before adding to the sample, to maintain a homogenous mixture.
m) Put the plate back into the instrument and follow the instrument prompt. The instrument will continue to perform the extraction.

n) The instrument will prompt after the procedure is complete. Remove the **Elution plate** from the instrument and the DNA is now ready for further downstream processing. The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at –20°C for long-term storage.

Technical Support

Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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