

USER GUIDE

for

**Automated purification of total and viral RNA from
mammalian whole blood and milk with**

**KingFisher instrument
and
MagMAX[™] -96 Blood RNA Isolation Kit**

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Description

Purification of total and viral RNA from mammalian whole blood and milk using Ambion MagMAX™ -96 Blood RNA Isolation kit (Catalog No. 1837) 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 instrument operates on microplates and can process up to 24 samples per run.

Typically, RNA isolation from 50 µl of human blood using KingFisher results 300-500 ng of intact RNA. Generally, RNA 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 MagMAX-96 Blood RNA Isolation Handbook for reagent storage, product use limitations, safety information etc.
- Resuspend magnetic beads (RNA Binding Beads) thoroughly before use.

KingFisher protocol

Importing protocols from the web

KingFisher Software protocol for MagMAX-96 Blood RNA Isolation Kit can be downloaded from the website (www.thermo.com/kingfisher). First you have to **save the file “MagMAX Blood” to your computer**. Protocols that have been exported from KingFisher Software 2.6, 2.5 or 2.0 can be imported to the database.

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. If it does, change the name.
- You will receive a message stating whether the database updating procedure was successful or not.

Sample preparation

- KingFisher RNA protocol **MagMAX-96 Blood RNA isolation** is designed to purify RNA from mammalian whole blood and milk
- Use KingFisher 200 µl microplate (Catalog No. 97002084) with **MagMAX-96 Blood RNA isolation** protocol.
- Add sample and other reagents supplied by MagMAX -96 Blood RNA isolation kit to KingFisher 200 µl microplate according to table 1 and instructions below.

Reagent preparation

- **Completion of Lysis/Binding Solution and Wash Solutions I and II**
 - 1) Add 16 ml 100% isopropanol to the bottle labeled Lysis/Binding Solution Concentrate and mix well. The Mixture is called Lysis/Binding Solution in these instructions.
 - 2) Add 12 ml 100% isopropanol to the bottle labeled Wash Solution 1 Concentrate and mix well. The resulting mixture is called Wash Solution 1 in these instructions.
 - 3) Add 44 ml 100% ethanol to the bottle labeled Wash Solution 2 Concentrate and mix well. The resulting mixture is

called Wash Solution 2 in these instructions.

• **Preparing Bead Mix**

- 1) Vortex the RNA Binding Beads at moderate speed to form a uniform suspension before pipetting.
- 2) Prepare Bead Mix by combining the volumes of RNA Binding Beads, Lysis/Binding Enhancer and Lysis/Binding Solution (with isopropanol) shown in the table below appropriate for the number of isolation reactions to be performed that day. Mix thoroughly.

Table 1 Bead Mix

Component	per reaction	~100 reactions
RNA Binding Beads	10 µl	1100 µl
Lysis/Binding Enhancer	10 µl	1100 µl
Lysis/Binding solution	100 µl	11 ml

• **Preparing Diluted TURBO DNase**

- 1) At room temperature combine the volumes of MagMAX TURBO DNase Buffer with TURBO DNase shown in the table below appropriate for number of samples being processed. Mix thoroughly.

Table 2 Diluted TURBODNase

Component	per reaction	~100 reactions
MagMAX TURBO DNase Buffer	49 µl	5.4 ml
TURBO DNase	1 µl	110 µl

KingFisher process

Table 3 Pipetting instructions for KingFisher and MagMax-96 Blood RNA isolation protocol.

Row	Content	Sample/Reagent volume
A	Sample	50 µl
	Lysis/Binding Solution with beads	120 µl
B	Wash Solution I	150 µl
C	Wash Solution II	150 µl
D	TURBO DNase	50 µl
	Lysis/Binding Solution (added during pause)	100 µl
E	Wash Solution II	150 µl
F	Wash Solution II	150 µl
G	Elution Buffer	50 µl

1. Add 50 µl of Sample and 120 µl of Lysis/Binding Solution to row **A**
2. Add 150 µl of Wash Solution I to row **B**.
3. Add 150 µl of Wash Solution II to rows **C**, **E** and **F**.
4. Add 50 µl of Diluted TURBO DNase to row **D**.
5. Add 50 µl of Elution Buffer to row **G**.
6. Insert the filled plate to the instrument plate carrier, refer the KingFisher User Manual.
7. Insert the tip combs into the slots and close the front lid.
8. Choose the **MagMAX Blood** protocol by using arrow keys in the front end panel and press START.
9. During Pause-step add 100 µl of Lysis/Binding Solution to row **D**.
10. Remove the plates from KingFisher after program has completed.

- Refer to Kingfisher User Manual

MagMAX-96 Blood RNA isolation protocol description

1. Sample is incubated with magnetic beads in row A for 8 minutes.
2. Magnetic beads are washed with Wash Solution I and II in rows B and C respectively.
3. Beads are dried outside well C.
4. Genomic DNA is removed by TURBO DNase treatment in row D for 10 minutes.
5. RNA is rebound to beads 3 minutes by Lysis/Binding Solution in row D.
6. Magnetic beads are washed with Wash Solution II in rows E and F.
7. Beads are dried outside well F for 2 minutes.
8. RNA is released to Elution Buffer in row G for 3 minutes.
9. Beads are discarded into well B.

Trouble shooting

1. Variation in RNA Yield Between Wells
 - Check that the RNA Binding Beads are fully resuspended before pipetting.
 - Do not over dry the beads before eluting.
 - Any steps of the protocol (e.g. sample incubation and elution times) and the reagent volumes can be modified with KingFisher[®] software.
2. DNA Contamination
 - If the RNA Binding Beads aggregate or fail to disperse after adding TURBO DNase solution the plate or the TURBO DNase solution could be incubated in 37°C for 5 minutes before adding TURBO DNase.
3. Low Viral RNA Detection Sensitivity
 - Include second wash with Wash Solution 1. This wash helps to remove protein and other RT-PCR inhibitors from sample.
4. Tip comb was forgotten
 - Clean the magnetic rods using a soft cloth or tissue paper soaked in mild detergent solution, soap or alcohol.
5. The processor is not working properly

Ordering Information

Product no.	Product Description
540 00 00	KingFisher, 110V-240V, Magnetic particle processor
97002094	KingFisher plastics 200 µl 8-pack, 8 plates + 8 tip combs/box
1837	MagMAX [™] -96 Blood RNA Isolation Kit

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