USER GUIDE

for

Automated purification of total RNA from cultured mammalian cells, animal tissues and plant tissues with

KingFisher instrument
and
MagMAX™ -96 Total RNA Isolation Kit

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Description

Purification of total and viral RNA from cultured mammalian cells, animal tissues and plant tissues using Ambion MagMAX™-96 Total RNA Isolation KIT (Catalog No. 1830) 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 total RNA isolation of cultured mammalian cells, animal tissues and plant tissues using KingFisher results with 5-20 pg RNA per mammalian cell, ≤ 5 mg RNA per animal tissue and ≤5-10 mg RNA per plant tissue. 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 Total 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 Total" 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**.
  - **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.

Reagent preparation

- ***Completion of Wash Solutions I and II, RNA Rebinding Solution and Lysis/Binding Solution***
  1) Add 6 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.
  2) 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.
  3) Add 6 ml 100% isopropanol to the bottle labeled RNA Rebinding Concentrate and mix well. The resulting mixture is called **RNA Rebinding Solution** in these instructions.
  4) **Lysis/Binding Solution** is prepared before each measurement. One reaction takes 100 µl of the Solution and it is prepared like showed in the tables below.

Table 1 Lysis/Binding solution for cultured cells.

Component	per reaction	~100 reactions
Lysis/Biding Solution Concentrate	55 µl	6.1 ml
100 % isopropanol	45 µl	5 ml

Table 2 Lysis/Binding solution for animal tissue samples

Component	per reaction	~100 reactions
Lysis/Biding Solution Concentrate	100 µl	11 ml
β-mercaptoethanol (14.3 M)	0,7 µl	77 µl

Table 3 Lysis/Binding Solution for plant tissue samples

Component	per reaction	~100 reactions
Lysis/Biding Solution Concentrate	90 µl	9.9 ml
Plant RNA Isolation Acid (cat # 9690)	10 µl	1.1 ml

- ***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 4 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 5 Diluted TURBODNase

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

Sample preparation

- KingFisher RNA protocol **MagMAX-96 Total RNA isolation** is designed to purify RNA from cultured mammalian cells, animal tissues and plant tissues.
- Sample homogenization can be done as described in MagMAX-96 Total RNA Isolation handbook. Short descriptions:

1)Homogenization of cultured mammalian cells:
- Lyse up to 2 x 10^6 cells in 120 µl prepared Bead Mix

2)Homogenization of animal tissue samples:
- Homogenize up to 5 mg of tissue in 120 µl prepared Bead Mix
- Add 60 µl 100 % isopropanol

3)Homogenization of plant tissue samples
- Homogenize up to 10 mg tissue in 100 µl prepared Lysis/Binding Solution
- Centrifuge lysate at 1000 g for 10 min at room temperature
- Transfer 50 µl of lysate to the plate, add 35 µl 100 % isopropanol and 20 µl Bead Mix (w/o Lysis/Binding Solution).

- Use KingFisher 200 µl microplate with **MagMAX-96 Total RNA isolation** protocol.

- Add sample and reagents supplied by MagMAX -96 Total RNA isolation kit to KingFisher 200 µl microplate (Cat. No. 97002094) according to table 6 and instructions below.

KingFisher process

Table 6 Pipetting instructions for KingFisher and **MagMax-96 Total 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
	RNA Rebinding 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 Total** protocol by using arrow keys in the front end panel and press START.
9. During Pause-step add 100 µl of RNA Rebinding Solution to row **D**.
10. Remove the plates from KingFisher after program has completed.

MagMAX-96 Total RNA isolation protocol description

1. Sample is incubated with magnetic beads in row A for 5 minutes.
2. Magnetic beads are washed with Wash Solution I and II in rows B and C respectively.
3. Genomic DNA is removed by TURBO DNase treatment in row D for 5 minutes.
4. RNA Rebinding solution is added to row D during pause.
5. RNA is rebound to beads by RNA Rebinding Solution in row D for 3 minutes.
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. DNA Contamination
   - If the sample input was too high, the DNA digestion step may not be effective. To avoid DNA contamination, reduce sample size or include an additional DNase treatment step after RNA isolation.
   - The temperature must be 20-25 °C

2. 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.

3. Tip comb was forgotten
   - Clean the magnetic rods using a soft cloth or tissue paper soaked in mild detergent solution, soap or alcohol.

4. The processor is not working properly
   - Refer to Kingfisher User Manual

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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