USER GUIDE

for

Automated purification of total RNA with

Thermo KingFisher 96 instrument
and
Qiagen MagAttract® Tissue Mini M48 Kit

For purification of total RNA from cultured cells and animal tissue

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Description

Purification of total RNA from cultured cells and animal tissue using Qiagen MagAttract® RNA Tissue M48 kit (Catalog No. 959336) is easily automated using KingFisher® instruments (Thermo Electron Corporation). The KingFisher platforms utilize patented technology in which magnetic rods move particles through purification process. KingFisher 96 can process up to 96 samples per run and it is compatible with deep well plates, PCR plates and KingFisher 96 plates. Specially designed magnetic rods and tips combs protecting the magnets are available for different plate types.

MagAttract technology is based on silica particles which bind RNA in the presence of a chaotropic salt. After binding and washing steps, DNA is eliminated by treatment with RNase-free DNase followed by efficient washing steps. Finally the purified RNA is released in elution buffer. The processing time with KingFisher 96 is approximately xx minutes. With one MagAttract RNA Tissue M48 kit and KingFisher 96 you can purify up to 2 x 96 samples if cultured cells are used as starting material. With tissue samples the respective sample amount is 96.

Typically, total RNA isolation from 1 x 10^6 cells (HEp-2) using KingFisher 96 results about 15 µg high quality total RNA. If tissue is used as starting material, the yield is approximately 50 µg (10 mg of mouse liver). Generally, RNA yields vary according to sample type and condition.

The protocols described here are designed for general use and can be modified according to customer individual needs using KingFisher® Software provided with the instrument.

Important notes

- Protocols **Q_RNA_cell_96_1** and **Q_RNA_tissue_96_1** are compatible with KingFisher software version 2.6.
- Make sure that the appropriate magnet head is attached to the KingFisher 96 processor. If magnet head need to be changed follow the KingFisher 96 User manual instructions.
- See MagAttract RNA Cell Tissue M48 Handbook for reagent storage, product use limitations, safety information etc.
- Resuspend magnetic particles (MagAttract Suspension E) thoroughly before use.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature.
- Buffer MW and Buffer RPE are supplied as a concentrate. Before using for the first time, add ethanol as indicated on the bottle to get a working solution.
- **When isolating RNA form tissue samples**, add 10 µl β-mercaptoethanol (β-ME) per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT is stable at room temperature for 1 month after addition of β-ME.
- Prepare DNase I stock solution as indicated in the MagAttract RNA Cell Mini M48 Handbook page 16.
- **Note!** A pause step is included in purification process for DNase treatment. See detailed instructions in section **DNase treatment step.**

Reagents needed but not provided

- Ethanol (96-100%)
- β-mercaptoethanol (if tissue is used as starting material)

Magnet and plastics requirements

- The isolation procedure is designed to operate with 2 ml deep well and KingFisher 96 plates.

- Thermo Microtiter Deep Well 96 plate (prod. No. 95040450) has been tested and is recommended to be used with KingFisher 96 and MagAttract® RNA Tissue M48 kit
- Be sure that the appropriate magnet head (magnet head for deepwell plate) is installed before starting!
- The requirements for magnet and plastics used with KingFisher and **Q_RNA_cell_96_1** are summarized in table 1.

Table 1. Magnet and plastics requirements

Magnet head	Magnet head for deepwell plate
Tip comb	KingFisher 96 tip comb for deep well magnets
Plate types	Deep well plate (5)* KingFisher 96 plate (2)*

*No. of plate types needed/run

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

Total RNA isolation from cultured cells

Sample preparation

- The protocol **Q_RNA_cell_96_1** is designed to purify total RNA from cultured cells. Up to **1.0 x 10^6** cells can be used as starting material.
- Transfer the suspended cells into suitable centrifuge tube and centrifuge 5 min at 1000 rpm. Discard the supernatant and wash twice with PBS.
- Resuspend the cell pellet in 400 µl of RLT buffer and homogenize the sample with desired method (syringe/needle, rotor-stator homogenizer, bead mill etc.)
- Centrifuge the lysate in microtube centrifuge for 3 minutes at maximum speed. Carefully remove the supernatant.

- Fill the deepwell/KingFisher plates with cell lysate, ethanol and other reagents supplied by MagAttract RNA Tissue Mini M48 kit according to table 2 and instructions below.

KingFisher 96 process

Plate type*	Well content	Volume	Plate name in protocol
A	Tip comb	-	Tip plate
B	Cell lysate Ethanol MagAttract Suspension E	400 µl 300 µl 80 µl	Lysis
B	Buffer MW	500 µl	MW wash
B	Buffer RDD RNase-free DNase ***After dispense step…*** Buffer RPE	190 µl 10 µl 700 µl	Digestion
B	Buffer RPE	500 µl	RPE wash 1
B	Buffer RPE	500 µl	RPE wash 2
A	Buffer ME	200 µl	Elution

Table 2. Plate filling instructions for KingFisher 96 and **Q_RNA_cell_96_1** protocol.

1. Fill plates “RPE Wash 1” and “RPE Wash 2” with 500 µl of Buffer RPE.
2. Fill plate “MW Wash” with 500 µl of Buffer MW.
3. Fill plate “Digestion” with 190 µl of Buffer RDD and 10 µl of RNase-free DNase.
4. Fill plate “Elution” with 200 µl of Buffer ME.
5. Add 400 µl of cell lysate, 300 µl of ethanol (96-100%) and 80 µl of MagAttract suspension into plate “Lysis”
6. Combine the tip comb and The KingFisher plate. See KingFisher 96 User manual.
7. Select the **Q_RNA_cell_96_1** protocol 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. In the middle of the run the sound signal will indicate the pause step and addition of Buffer RPE.
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.

DNase treatment step

- A **dispense** step is included in purification process after DNase treatment of the samples in plate “Digestion”.
- A sound signal indicates a time point for extra pipetting step. **“Add 700µl RPE”** text appears on the instrument screen.
- At this point the “Digestion” plate is moved to the loading position. Remove the plate from the instrument and add 700 µl of Buffer RPE.
- After adding the Buffer RPE, put the plate back to the loading position, check the correct orientation! (A1 well to be pointed to upper right corner)
- Press START to continue the protocol.

Q_RNA_cell_96_1 protocol description

1. The tip comb is attached to the tip comb holder.
2. The cell lysate is incubated in deep well plate (plate “Lysis”) with magnetic particles for 7 minutes.
3. The particles are washed in Buffer MW (plate MW Wash).
4. Particles are transferred to plate “Digestion” for DNase treatment (15 minutes). After that, a sound signal indicates time for addition of Buffer RPE (see section DNase treatment step).
5. After addition of Buffer RPE, the protocol continues by pressing START and particles are further incubated for next 5 minutes to allow RNA to rebound to particles after DNase treatment.
6. The particles are washed twice in Buffer RPE.
7. A drying step (10 min) is performed after Buffer RPE washings.
8. RNA is released into buffer ME in plate “Elution”.
9. Particles are discarded to plate “Lysis”.

Total RNA isolation from animal tissue

Sample preparation

- The protocol **Q_RNA_tissue_96_1** is designed to purify total RNA from animal tissue. Up to **10 mg** of tissue can be used as starting material.
- Disrupt the tissue and homogenize the sample in 400 µl of RLT buffer (supplemented with β-ME) with desired method (mortar/pestle, rotor-stator homogenizer, bead mill etc.)
- Centrifuge the lysate at maximum speed in microtube centrifuge for 3 min. Carefully remove the supernatant.
- Fill the deepwell/KingFisher plates with tissue lysate, ethanol and other reagents supplied by MagAttract RNA Tissue Mini M48 kit according to table 3 and instructions below.

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KingFisher 96 process

14. Fill plates "RPE Wash 1" and "RPE Wash 2" with 500 µl of Buffer RPE.
15. Fill plate "MW Wash" with 1000 µl of Buffer MW.
16. Fill plate "Digestion" with 190 µl of Buffer RDD and 10 µl of RNase-free DNase.
17. Fill plate "Elution" with 200 µl of Buffer ME.
18. Add 400 µl of tissue lysate, 300 µl of ethanol (96-100%) and 80 µl of MagAttract suspension E into plate "Lysis"
19. Combine the tip comb and The KingFisher plate. See KingFisher 96 User manual.
20. Select the **Q_RNA_tissue_96_1** protocol using arrow keys and press START button.
21. Load the plates according to protocol request and press START after every plate to confirm the action.
22. **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.
23. The purification protocol will start when the last plate is loaded and START button is pressed.
24. After Buffer MW wash the sound signal will indicate the pause step and addition of Buffer RPE.
25. 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.
26. When the last plate is removed text End_of _run will appear. Press STOP to complete the run.

Plate type*	Well content	Volume	Plate name in protocol
A	Tip comb	-	Tip plate
B	Tissue lysate Ethanol MagAttract Suspension E	400 µl 300 µl 80 µl	Lysis
B	Buffer MW	1000 µl	MW wash
B	Buffer RDD RNase-free DNase ***After dispense step…*** Buffer RPE	190 µl 10 µl 700 µl	Digestion
B	Buffer RPE	500 µl	RPE wash 1
B	Buffer RPE	500 µl	RPE wash 2
A	Buffer ME	200 µl	Elution

Table 2. Plate filling instructions for KingFisher 96 and **Q_RNA_tissue_96_1** protocol.

DNase treatment step

- DNase treatment step for tissue samples is exactly the same as it is for cell samples.
- See page 5 for detailed instructions.

Q_RNA_tissue_96_1 protocol description

- Protocol description for Q_RNA_tissue_96_1 is exactly the same as it is for Q_RNA_cell_96_1.
- See page 5 for detailed information.

Trouble shooting

1. Low RNA yield
   - Check that MagAttract Suspension E is completely resuspended.
   - Before use, check that Buffer RLT does not contain any precipitate by shaking the bottle.
   - Check the amount of starting material. Too high ratio of starting material versus magnetic particles may result inefficient washing steps and elution.
   - Any steps of the protocol (e.g. sample incubation and elution times)

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can be modified with KingFisher® software.

2. The processor is not working properly
   - Refer to KingFisher User Manual

Ordering Information

Product no.	Product Description
5400500	KingFisher 96 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
959336	MagAttract® RNA Tissue M48 kit

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