

USER GUIDE

for

Automated purification of total RNA with

**KingFisher mL instrument
and
MagAttract[®] Tissue Mini M48 Kit**

For purification of total RNA from animal tissue

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Description

Purification of total RNA from animal tissues 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. With KingFisher mL you can process up to 15 samples per run.

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 mL is approximately 55 minutes. With one MagAttract RNA Tissue M48 kit and KingFisher mL you can purify up to 100 samples.

Typically, total RNA isolation from mouse liver (10 mg) using KingFisher mL results about 20 µg high quality total RNA. 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

- See MagAttract RNA Tissue Mini 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.
- 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.

- 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.
- Prepare DNase I stock solution as indicated in the MagAttract RNA Tissue 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

KingFisher mL protocol

Sample preparation

- The protocol **Q_RNA_tissue_mL_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.) If lot of foam is formed centrifuge the sample for 1 min at 1000 rpm.
- Fill the KingFisher mL tube strips with tissue lysate, ethanol and other reagents supplied by MagAttract RNA Tissue Mini M48 kit according to table 1 and instructions below.

KingFisher mL process

Table 1. Pipetting instructions for KingFisher mL and **Q_RNA_tissue_mL_1** protocol.

Tube	Tube content	Sample/reagent volume
A	Tissue lysate Ethanol MagAttract Suspension E	400 µl 300 µl 80 µl
B	Buffer MW	1000 µl
C	Buffer RDD RNase-free DNase	190 µl 10 µl
D	Buffer RPE	1000 µl
E	Buffer ME	200 µl

1. Place an appropriate number of tube strips needed for the samples (one tube strip per sample) into removable tubestrip tray.
2. Add 1000 µl of Buffer MW into tube **B**.
3. Add 190 µl of Buffer RDD and 10 µl of RNase-free DNase into tube **C**.
4. Add 1000 µl of Buffer RPE into tube **D**.
5. Add 200 µl of Buffer ME into tube **E**.
6. Add 400 µl of tissue lysate, 300 µl of ethanol (96-100%) and 80 µl of MagAttract suspension E into tube **A**.
7. Insert the tubestrip tray to the instrument and insert the tips combs into the slots.
8. Close the front lid and start the process by selecting intended protocol using arrow keys and by pressing START.
9. Remove the tubestrip tray from the KingFisher mL after program has completed.

DNase treatment step

- A **pause** step is included in purification process after DNase treatment of the sample in tube C.
- A sound signal indicates a time point for extra pipetting step. “**Add 700µl Buffer RPE**” text appears on the instrument screen.
- Remove the tubestrip tray from the instrument and add 700 µl of Buffer RPE into **tube C**.
- After adding the Buffer RPE, put the tubestrip tray back to instrument.

- Press START to continue the protocol.

Q_RNA_tissue_mL_1 protocol description

1. Tissue lysate is incubated with magnetic particles for 8 minutes in tube A.
2. Particles are washed with Buffer MW for 3 minutes in tube B.
3. Particles are transferred to tube C for DNase treatment (15 minutes). After that a sound signal indicates time for addition of Buffer RPE (see section DNase treatment step).
4. After addition of Buffer RPE, the protocol continues by pressing START.
5. Particles are incubated in tube C for next 5 minutes.
6. Particles are washed in tubes B and D.
7. A drying step (10 min) is performed after washing in tube D.
8. RNA is released into buffer ME in tube E.
9. Particles are discarded into tube B.

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 that β-ME has been added to the Buffer RLT.
 - 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) can be modified with KingFisher[®] software.
2. Tip comb was forgotten
 - Clean the magnetic rods using a soft cloth or tissue paper soaked in mild detergent solution, soap or alcohol.
3. The processor is not working properly
 - Refer to KingFisher User Manual

Ordering Information

Product no.	Product Description
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 120 (tubes and tips for 120 samples)
959336	MagAttract RNA Tissue Mini M48 Kit

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