

USER GUIDE

for

Automated purification of poly-A-mRNA with

KingFisher/KingFisher mL instruments
and

MagAttract[®] Direct mRNA M48 Kit

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Description

Purification of poly-A-RNA using Qiagen MagAttract® Direct mRNA M48 kit (Catalog No. 957236) 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, whereas KingFisher mL can handle larger volumes, up to 1 ml, and 15 samples per run. With MagAttract® Direct mRNA M48 kit you can purify approximately 250 samples with KingFisher and 100 samples with KingFisher mL. The purification time for KingFisher is 16 and for KingFisher mL 18 minutes.

Typically, mRNA isolation from 0.5×10^6 cells (HEp-2) using KingFisher results 0.5-1 µg mRNA. With KingFisher mL, isolation from 2×10^6 cells (HEp-2) gives yield of 3 µg mRNA. Generally, mRNA 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 Direct mRNA M48 Handbook for reagent storage, product use limitations, safety information etc.
- Resuspend magnetic particles (MagAttract Suspension C) thoroughly before use.
- Before use, check that buffer MRL does not contain a white precipitate by shaking the bottle. If necessary, incubate for 15 minute at 37°C with occasional shaking to dissolve the precipitate.

KingFisher protocol

Sample preparation

- KingFisher mRNA protocol **Q_mRNA_1** is designed to purify mRNA from cultured cells. Up to 0.5×10^6 cells can be used as starting material.
- Use KingFisher 100 µl microplate with **Q_mRNA_1** protocol.
- 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 200 µl of MRL buffer and homogenize the sample by pipetting up and down several times or by passing them few times through a 23-gauge needle. If lot of foam forms centrifuge the sample for 1 min at 1000 rpm.
- Add cell lysate and other reagents supplied by MagAttract Direct mRNA M48 kit to KingFisher 100 µl microplate according to table 1 and instructions below.

KingFisher process

Table 1. Pipetting instructions for KingFisher and **Q_mRNA_1** protocol.

Row	Content	Sample/Reagent volume
A	Cell lysate	100 µl
B	Cell lysate	100 µl
C	MagAttract Suspension C Buffer MRL	30 µl 50 µl
D	Buffer MRW1	100 µl
E	Buffer MRW1	100 µl
F	Buffer MRW2	100 µl
G	Buffer MRW2	100 µl
H	Buffer MRE	50 µl

1. Add 50 µl of Buffer MRL and 30 µl of MagAttract suspension C to row **C**.
2. Add 100 µl of Buffer MRW1 to rows **D** and **E**.
3. Add 100 µl of Buffer MRW2 to rows **F** and **G**.
4. Add 50 µl of Buffer MRE to row **H**.

5. Add 100 µl of cell lysate to rows **A** and **B**.
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 **Q_mRNA_1** protocol by using arrow keys in the front end panel and press START.
9. Remove the plates from KingFisher after program has completed.

Q_mRNA_1 protocol description

1. Magnetic particles are transferred from row C to row A.
2. Cell lysate is incubated with magnetic particles in rows A and B.
3. Magnetic particles are washed with Buffer MRW1 in rows D and E.
4. Magnetic particles are washed with Buffer MRW2 in rows F and G.
5. mRNA is released to Buffer MRE in row H.
6. Beads are discarded into well E.

KingFisher mL protocol

Sample preparation

- KingFisher mRNA protocol **Q_mRNA_mL_1** is designed to purify mRNA from cultured cells. Up to **2 x 10⁶** 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 740 µl of Buffer MRL and homogenize by passing the cell pellet few times through a 23-gauge needle. If lot of foam forms centrifuge the sample for 1 min at 1000 rpm.
- Add cell lysate and other reagents supplied by MagAttract Direct mRNA M48 kit to KingFisher mL tube strips according to table 2 and instructions below.

KingFisher mL process

Table 2. Pipetting instructions for KingFisher mL and **Q_mRNA_mL_1** protocol.

Tube	Tube content	Sample/reagent volume
A	Cell lysate MagAttract Suspension C	740 µl 60 µl
B	Buffer MRW1	1000 µl
C	Buffer MRW2	600 µl
D	Buffer MRW2	600 µl
E	Buffer MRE	100 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 MRW1 into tube **B**.
3. Add 600 µl of Buffer MRW2 into tubes **C** and **D**.
4. Add 100 µl of Buffer MRE into tube **E**.
5. Add 740 µl of cell lysate and 60 µl of MagAttract Suspension C into tube **A**.
6. Insert the tubestrip tray to the instrument and insert the tips combs into the slots.
7. Close the front lid and start the process by selecting intended protocol using arrow keys and by pressing START.
8. Remove the tubestrip tray from the KingFisher mL after program has completed.

Q_mRNA_mL_1 protocol description

1. The cell lysate is incubated with magnetic particles in tube A.
2. The magnetic particles are washed in Buffer MRW1 in tube B.
3. The magnetic particles are washed in Buffer MRW2 in tubes C and D.
4. mRNA is released in Buffer MRE in tube E.
5. Beads are discarded into tube C.

Trouble shooting

1. Low mRNA yield
 - Check that MagAttract Suspension C is completely resuspended
 - Before use, check that Buffer MRL does not contain white 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) 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 00	KingFisher, 110V -240V, Magnetic particle processor
97002090	KingFisher plastics 100 µl 8-pack, 8 plates + 8 tip combs/box
97002094	KingFisher plastics 200 µl 8-pack, 8 plates + 8 tip combs/box
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)
957236	MagAttract Direct mRNA M48 Kit

Contact information



Ratastie 2, P.O. Box 100
FIN-01621 Vantaa
Finland
Tel. +358-9-329100
Fax. +358-9-32910415
www.thermo.com