

mRNA isolation with KingFisher mL instrument

Important notes

- **Dynal_mRNA_mL** protocol is intended to use with Dynal Dynabeads mRNA DIRECT Kit (prod. No. 610.11/610.12)
- See Dynabeads mRNA DIRECT handbook for reagent storage, product use limitations, safety information etc.
- Resuspend magnetic particles (Dynabeads Oligo (dT)₂₅) thoroughly before use.
- Heat incubation step (2 min at 65°C) is included in elution stage of the purification protocol. Use e.g. heating block, incubator or water bath to heat the samples.
- The protocol can be modified according to customer individual needs using KingFisher® software provided with the instrument.

Sample preparation

- KingFisher mRNA protocol **Dynal_mRNA_mL** is designed to purify mRNA from animal tissue. Up to **25 mg** of tissue can be used as starting material.
- Disrupt the tissue and homogenize the sample in 1 ml of Lysis/Binding buffer with desired method (mortar/pestle, rotor-stator homogenizer, bead mill etc.).
- If the raw extract is noticeably viscous, force the lysate 3-5- times through 21 gauge needle using a 1-2 ml syringe.
- If lot of foam is formed centrifuge the sample for 1 min at 1000 rpm.
- Add the lysate and other reagents supplied by Dynabeads mRNA DIRECT kit to KingFisher mL tube strips according to Table 1 and instructions below.

KingFisher mL process

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

Tube	Tube content	Sample/reagent volume
A	Cell lysate Dynabeads Oligo (dT) ₂₅	800 µl 200 µl
B	Washing Buffer A	1000 µl
C	Washing Buffer B	500 µl
D	Washing Buffer B	500 µl
E	Elution Buffer	50 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 Washing Buffer A into tube **B**.
3. Add 500 µl of Washing Buffer B into tubes **C** and **D**.
4. Add 50 µl of Elution Buffer into tube **E**.
5. Add 800 µl of cell lysate and 200 µl of Dynabeads Oligo (dT)₂₅ 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. Wait until you hear a sound signal indicating the heat incubation step. "HEATING" text appears on the instrument screen.
9. Remove the tray from the instrument. Cover the tubes (e.g. with sealing tape or parafilm) and place the whole tray into 65°C incubator for 2 min. Alternatively, transfer the samples into

- microcentrifuge tubes for heating in heat block and return the samples to the tube E after incubation.
10. After incubation return the tray back to the instrument and continue the program by pressing START.
 11. Remove the tubestrip tray from the KingFisher mL after the program has completed.

Dynal_mRNA_mL protocol description

1. The cell lysate is incubated with magnetic particles in tube A.
2. The magnetic particles are washed in Washing Buffer A in tube B.
3. The magnetic particles are washed Washing Buffer B in tubes C and D.
4. The particles are released in Elution buffer in tube E.
5. The protocol is paused for heating step. Text "HEATING" appears into the screen.
6. The heating step is performed outside the instrument.
7. After incubation the tray is returned back to the instrument and the protocol continues after pressing START.
8. The particles are collected and discarded into tube C.

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