mRNA Purification Kit
PRODUCT DATA SHEET

mRNA Purification Kit

Cat No: AKIT-mRNA

Description

The Oligo-dT coated magnetic beads provided by the mRNA purification kit have good dispersion and fast magnetic response. Through hybridization of oligo-dT on the surface of magnetic beads with polyA of mRNA, it is used to rapidly purify high purity and complete mRNA from total RNA extracted from animal tissues, plants or cells, which can be used in various downstream experiments in molecular biology. The kit comes with an optimized prefabricated buffer to provide the best stable conditions for mRNA purification.

For custom sizes, formulations or bulk quantities please contact our customer service department.

Website: www.abvigen.com Phone: +1 929-202-3014 Email: info@abvigenus.com

Kit Composition

Oligo dT coated magnetic beads	10 mL
Binding buffer	50 mL
Washing solution	50 mL
RNase-Free H_2O	50 mL
Magnetic rack	Double row of 16 holes

Operation Process

Corresponding sample

Corresponding sample	**Sample size**
Cultured cell	~ 1×10^7
Animal tissue	~ 50 mg
Plant tissue	~ 100 mg
Total RNA	10 ng ~ 100 μg (RIN value ≥ 7)

1. Prepare before use

1.1 RNase-free 1.5 mL centrifuge tube, pipette and suction head

1.2 Whirlpool oscillator, rotary mixer, metal bath or PCR instrument

2. Magnetic bead pretreatment

Note: The amount of magnetic beads required for sample purification may need to be optimized for optimal use.

2.1 The suspension vortex of the magnetic bead is oscillated for 30 s, so that the magnetic bead is fully oscillated and re-suspended;

2.2 Remove 100 ~ 200 μL magnetic bead suspension into a suitable reaction tube (1.5 mL EP tube or PCR tube); Place the reaction tube on the magnetic separator until the solution becomes clear [The subsequent operation is referred to as "magnetic separation"], remove the supernatant, and remove the reaction tube from the magnetic separator;

2.3 Add 200 μL re-suspended magnetic beads combined with buffer, and use a pipette to slowly blow 5-10 times or swirl for 30 s.

2.4 Magnetic separation to remove supernatant, adding 50 μL binding buffer to re-suspension magnetic beads.

3. Purified mRNA in Total RNA

The following experimental protocol is for the purification of 75 μg Total RNA, which can be adjusted proportionally according to needs.

3.1 Take 50 μL containing 75 μg Total RNA, incubate it at 65°C for 2 min, open the secondary structure of RNA, and put it on ice immediately after completion.

3.2 Add 50 μL total RNA solution to the 50 μL washed magnetic bead, blow and mix well. That is, magnetic bead binding with 1mg of washed and dissolved in 50 μL **binding buffer** per 75 μg of total RNA (step 2).

3.3 Rotate and mix the above mixture at room temperature for 10 ~ 15 min.

3.4 Magnetic separation, let stand for 1 min, remove supernatant.

3.5 Clean the magnetic beads with 200 μL **washing liquid** at room temperature, carefully blow and mix, magnetic separation, and remove possible contaminants. Repeat once.

3.6 10 ~ 20 μL **RNase-Free H_2O** was added and incubated at 65 ~ 75°C for 2 min, and then the supernatant containing mRNA was rapidly transferred to the new RNase-free EP tube.

4. Applied to RNA sequencing library

4.1 Prepare RNA sample: Dilute 10 ng to 100 μg total RNA to 50 μL with **RNase-Free H_2O** and reserve on ice.

Note: Total RNA should be free of DNA, organic solvents (phenol, ethanol, etc.), salt ions (guanidine salt, Mg^{2+}) residue, otherwise it will lead to RNA degradation or mRNA capture efficiency decline

4.2 Perform magnetic bead treatment according to Step 2 magnetic bead pretreatment, and mix 50 µL magnetic bead and 50uL RNA sample with pipette for 6 times.

4.3 Place the sample in the PCR apparatus at 65°C for 5 min, 25°C for 5 min, and 4°C Hold.

4.4 Magnetic separation of the above sample, standing for 1min, remove the supernatant; Clean the magnetic beads with 200 µL **washing liquid**, carefully blow and mix, magnetic separation, and remove the supernatant.

4.5 Add 50 µL **RNase-Free H_2O**, fully suspend the magnetic beads, and place them in a PCR apparatus for 2 min at 65°C and 5 min at 20°C to elutes the mRNA.

4.6 Add 50 µL of **binding buffer**, blow and mix thoroughly, and place at room temperature or on the rotary apparatus for 5 ~ 10 min.

4.7 Magnetic separation, leave for 1 min, remove supernatant.

4.8 Clean the magnetic beads with 200 µL **washing liquid**, carefully blow and mix, magnetic separation, and remove the supernatant.

4.9 If the purified product was used for reverse transcription reaction, 10 ~ 20 µL RNase-free ddH_2O was added, mixed with pipetting device, 65°C for 2 min, magnetic separation for 1 min. After the solution was clarified, the supernatant was carefully absorbed into a new Nuclease-free PCR tube.

4.10 Samples can be placed on ice to continue NGS library construction or other analytical applications (immediate follow-up reaction is recommended), or stored at -85 ~ -65°C.

Notes

1. Experimental operation: When conducting RNA experiments, attention must be paid to inhibiting the action of RNase. Therefore, in addition to preventing RNase from being mixed through the use of utensils and reagents, that is, paying attention to the experimental environment, but also to prevent RNase from being mixed through saliva, sweat, etc., it is recommended to wear a mask and gloves.

2. Equipment: If the experimental equipment allows, please sterilize disposable plastic products with high temperature and high pressure wet heat. In the case of glassware, use dry heat sterilization, or soak in 0.1% DEPC solution at 37°C for 12 h, and then perform high temperature and high pressure humid heat sterilization (121°C, 30 min).

3. RNA quality: Total RNA integrity should be good (RIN value should be ≥ 7), incomplete or degraded total RNA template will lead to the loss of some poly(A)+RNA information.

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