

Protocol Cat. No. K1813



# Product description

mRNA Magnetic Purification kit for RNA library Prep is a magnetic beads kit specifically designed for the purification of mRNA from the total RNA. The mRNA Purification beads in the kit are micrometer-sized paramagnetic microspheres conjugated with Oligo (dT), which can separate and purify mRNA from intact total RNA by binding to mRNA with poly (A) tails. The input amount of total RNA for each reaction in this kit is between 10 ng - 4 µg. The product mRNA of this kit has high purity and low rRNA residue.

# Composition and storage conditions

Size Components	K1813-24 rxns	K1813-96 rxns	Storage
mRNA Purification Beads	1.2 mL	4.8 mL	4°C
mRNA Binding Buffer (2×)	1.2 mL	4.8 mL	4°C
Wash Buffer	15 mL	60 mL	4°C
Tris Buffer	1.2 mL	4.8 mL	4°C
Nuclease-free Water	1 mL	4 mL	4°C
Shipping: Bluc Ice	Shelf life: 12 months		

# Experimental operation

## 1. Experiment preparation

1) **Reagent preparation:** Take the mRNA Magnetic Purification kit for RNA library Prep out of the 4°C refrigerator in advance and allow it to equilibrate to room temperature (about 30 min).

2) Sample preparation: Dilute 10 ng-4 µg total RNA to 50 µL using Nuclease-free H<sub>2</sub>O and place on ice for later use.

#### 2. First binding of mRNA and magnetic beads

1) Invert or pipette to mix the mRNA Purification Beads thoroughly. Then take 50 µL beads and add it to the 50 µL total

RNA sample. Gently pipette up and down several times until beads are homogenous.

2) Run the following program in the PCR instrument:

Temperatu	re	Time
65°C	.0	5 min
25°C	Enterna	5 min
25°C	A province from	Hold

3) Then place the sample on a magnetic rack and incubate at room temperature for about 5 min to separate the mRNA from the total RNA. After that, carefully remove and discard the supernatant.

#### 3. Washing

1) Remove the sample from the magnetic rack, add 200  $\mu$ L of Wash Buffer to resuspend the magnetic beads, and gently

pipette several times to mix thoroughly.

2) Then place the sample on a magnetic rack and incubate at room temperature for about 5 min. After the solution is clear, carefully remove the supernatant.

3) Repeat steps 3.1-3.2 once.

## 4. First time of elution of mRNA samples

1) Remove the tube from the magnetic rack, add 50 µL of Tris Buffer, and gently pipette up and down 6 times.

2) Run the following program in the PCR instrument:

	- Interest
Temperature	Time
80°C	2 min
25°C	+∞

#### 5. Secondary binding of mRNA and magnetic beads

1) Take the sample out of the PCR instrument, add 50  $\mu$ L of mRNA Binding Buffer (2×), and gently pipette up and down 6

times.

2) Incubate at room temperature for 5 min to allow the mRNA to bind to the magnetic beads.

3) Place the sample on the magnetic rack and incubate at room temperature for 5 min. After the solution becomes clear,

carefully remove and discard the supernatant.

#### 6. Sample washing

1) Remove the sample from the magnetic rack, add 200  $\mu$ L of Wash Buffer to resuspend the magnetic beads, and gently pipette up and down 6 times.

2) Place the sample on the magnetic rack and incubate at room temperature for 5 min. After the solution becomes clear, remove and discard all the supernatant.

\*Note: You can first use a 200 µL pipette to remove the liquid, and then switch to a 10 µL pipette to remove the remaining liquid.

#### 7. Follow-up experiments

#### I. If the purified product is used for reverse transcription reaction

1) Remove the sample from the magnetic rack, add 10.5 µL of Nuclease-free water, and pipette to mix thoroughly.

2) Incubate at 80°C for 2 min and then immediately place on a magnetic rack.

3) After the solution becomes clear, carefully transfer 8.5 µL of the supernatant into a new Nuclease-free PCR tube for the subsequent reverse transcription reaction.

#### II. If the purified product is used for the construction of an RNA library

RNA Library construction can be carried out using our RNA Library Prep Kit (Premixed Version) (Cat. No. K1810). Add the corresponding volume of Frag/Prime Buffer according to the instructions for library construction.

## Notes

- Before using the magnetic beads, they need to be balanced to room temperature and thoroughly mixed; otherwise, it may affect the efficiency of sample recovery.
- 2. RNase and nucleic acid contamination must be strictly avoided throughout the operation process.
- 3. This product is for scientific use only!

