TIANSeq mRNA Capture Kit

Specifically designed for capturing eukaryotic mRNA

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Cat. no. 4993009/4993010

Kit Contents

Contents	4993009 24 rxn	4993010 96 rxn
Beads Binding Buffer TM	6 ml	24 ml
Beads Washing Buffer TD	12 ml	48 ml
mRNA Capture Beads	240 µl	960 µl
Nuclease-Free ddH_2O	15 ml	15 ml
Handbook	1	1

Storage Conditions:

TIANSeq mRNA Capture Kit can be stored at 2-8°C for one year.

Introduction

TIANSeq mRNA Capture kit is designed to specifically capture messenger RNA (mRNA) by coupling the magnetic beads of Oligo (dT). The non-specific binding RNA can be removed by washing the beads with washing buffer, and the complete mRNA in total RNA of human, mouse, rat and plant can be obtained.

The kit has a good mRNA capture effect for the complete total RNA. The obtained mRNA can be used for high-throughput sequencing, which can significantly improve the proportion of effective data in the sequencing results. In addition, the purified mRNA can also be used for random primer cDNA synthesis or other downstream applications.

Features

Wide range of samples: It is suitable for the capture of mRNA in high-quality (complete) samples. It is suggested that RIN (RNA Integrity Number) of RNA should be more than 7.

Comprehensive data: Complete mRNA information is retained to improve the validity of transcriptome data.

Wide range of application: Suitable for the capture of 100 ng-1 μg total RNA.

Required Reagents

mRNA capture: 8-Tube Strip (0.2 ml); Magnetic Frame (TIANGEN Cat# OSE-MF-04); Nuclease-Free centrifugation tube; Nuclease-Free PCR tube.

PCR detection of mRNA capture efficiency: rRNA Primer Mix; mRNA Primer Mix; FastKing RT Kit (With gDNase) (TIANGEN Cat#4992223/4992224/4992250); SuperReal PreMix Plus (SYBR Green) (TIANGEN Cat#4992214/4992215/4992248)

Important Notes <u>Please read before use.</u>

1. Please pay attention to avoid cross contamination between nucleic acid sample and product during operation.
2. Please operate the experiment in the nucleic acid-free environment. The nucleic acid aerosol pollution in the operating environment should be removed in time.
3. Please use DNase- and RNase-free pipette tips and centrifuge tubes for experiment.
4. Before the experiment, please clean the operating table. It is recommended to use RNase and DNase cleaning reagent to treat the table surface. Make sure there is no contamination of RNase and DNase.

Protocol

1. Treatment of magnetic beads: Take out the mRNA Capture Beads from the 2-8°C refrigerator and equilibrate at room temperature for 5 min. During this period, vortex and mix evenly. Transfer 10 µl mRNA Capture Beads to a 200 µl nuclease-free PCR tube, then place the tube on a magnetic stand for 1 min. Carefully discard the supernatant after the reagent is clarified. Remove the EP tube from the magnetic stand, add 50 µl Beads Binding Buffer TM. Wash the beads by pipetting up and down for 6 times, and then place it on the magnetic stand for 1 min. After the solution is clarified, carefully discard the supernatant, and repeat the washing step once. Resuspend the magnetic beads in 50 µl Bead Binding Buffer TM and store it at room temperature for standby.
2. Dilute 100-1000 ng total RNA to 50 µl with Nuclease-Free ddH_2O in a 200 µl nuclease-free PCR tube, and placed on ice for standby.

   Note: There should be no residual DNA, salt ions (such as Mg^{2+}, guanidine salt) and organic reagents (such as phenol and ethanol) in the total RNA sample, otherwise, unexpected RNA degradation or reduced mRNA capture efficiency may be caused.
3. Prepare mRNA capture reaction system according to the following table:

Components	Volume (µl)
Treated mRNA Capture Beads	50
Total RNA	50
Total volume	100

4. Thoroughly mix the mRNA Capture Beads and RNA reaction solution in step 3 by pipetting up and down for 6 times.
5. Place the reaction solution from step 4 in a thermal cycler (hot lid temperature: 99-105°C). Run the follow program, with a total reaction time of 10 min.

Step	Temperature	Time
1	65°C	2 min
2	20°C	5 min

6. Place the reaction solution from step 5 on a magnetic stand for 1 min to pellet the beads, and discard the supernatant carefully. Remove the tube from the magnetic stand, add 200 µl Beads Washing Buffer TD. Wash the beads by pipetting up and down for several times. Place the

tube on the magnetic stand for 1 min to pellet the beads, and discard the supernatant.

7. Suspend the beads with 50 µl Nuclease-Free ddH_2O, and place in a thermal cycler (hot lid temperature: 99-105°C). Run the follow program, with a total reaction time of 10 min.

Step	Temperature	Time
1	75°C	2 min
2	20°C	5 min

8. Add 50 µl Beads Binding Buffer TM to the reaction reagent from step 7, and mix evenly by pipetting up and down for several times. Incubate at 20°C for 5 min.
9. Place the reaction solution from step 8 on a magnetic stand to pellet the beads. Carefully discard the supernatant after the solution is clarified.
10. Remove the tube from the magnetic stand. Add 200 µl Beads Washing Buffer TD to wash the beads by pipetting up and down for several times. Place the reaction tube on a magnetic stand to pellet the beads, and carefully remove the supernatant.
11. Remove the tube from the magnetic stand, add 6.5 µl Nuclease-Free ddH_2O and pipet up and down for 10 times to mix evenly. Incubate at room temperature for 2 min.

    Note: The elution volume is designed for TIANSeq RNA Library Kit (TIANGEN Cat#4992375/4992376; 4993007/4993008). If other RNA library kits are applied, please choose an appropriate elution volume according to the user manuals.
12. Place the tube on a magnetic stand for 2 min to pellet the beads. Carefully transfer 5 µl supernatant (the volume can be adjusted according to the actual elution volume in step 11 so that the eluent can be fully used) to a new Nuclease-Free PCR tube without disturbing the beads.
13. The eluent can be applied to RNA library construction or other analysis right away, or can be stored at -20°C overnight or at -80°C for 30 days.

Real-Time PCR Detection (Optional)

This kit provides two pairs of quantitative PCR primers, which are rRNA primer mix for 18S rRNA and mRNA primer mix for beta-actin. It is recommended to use the same amount of initial total RNA without mRNA capture treatment as the control (the control needs to be diluted to elution volume with Nuclear-Free ddH_2O) to evaluate the mRNA capture efficiency and rRNA residue ratio.

Two step RT-qPCR example: Use 1 µl RNA product as template to synthesize the first strand cDNA, and then use 2 µl cDNA as template for real-time PCR. It is recommended to use Fastking RT Kit (With gDNase) (TIANGEN Cat#4992223/4992224/4992250) and SuperReal PreMix Plus (SYBR Green) (TIANGEN Cat# 4992214/4992215/4992248).

1. Reverse transcription

1) Prepare the reverse transcription reaction on ice according to the following table:

Components	Volume (µl)
RNase-Free ddH_2O	12
5×gDNA Buffer	2
10×King RT Buffer	2
FQ-RT Primer Mix	2
FastKing RT Enzyme Mix	1
RNA	1
Total volume	20

2) Thoroughly mix the reaction solution by pipetting up and down for several times. Short-spin to collect the drops on the tube wall. Place the tube in a thermal cycler (hot lid temperature: 99-105°C). Incubate at 42°C for 15 min, then incubate at 95°C for 3 min.

3) Take out the tube and short-spin to collect the drops on the tube wall. The cDNA can be used in the downstream quantification test, or stored at -20°C.

2. Real-time PCR (take Bio-Rad CFX96 as an example)

1) Prepare the real-time PCR reaction solution in a Nuclease-Free PCR tube on ice according to the table below:

Components	Volume (μl)
2×SuperReal PreMix Plus	10
50×ROX Reference Dye	0 ▲
rRNA Primer Mix or mRNA Primer Mix (self-prepared)	1
cDNA template	2
RNase-Free ddH_2O	To 20

2) Mix the reaction solution by pipetting up and down for several times. Short-spin to collect the drops on the tube wall.

3) Place the reaction tubes in a real-time thermal cycler, and run the following program:

Phase	Cycle	Temperature	Time	Content	Signal acquisition
Initial denaturation	1×	95°C	15 min	Initial denaturation	No
PCR reaction	40×	95°C	10 sec	Denaturation	No
		60°C	30 sec ▲	Annealing /Extension	Yes
Melting/Dissociation Curve Stage					

▲ The program is designed for Bio-Rad CFX96 Real Time System. If other real-time systems are applied, please refer to the instrument user's manual or the suggestion in SuperReal PreMix Plus (SYBR Green) (TIANGEN Cat# 4992214/4992215/4992248).

3. Please see the reference two step RT-PCR results using mouse RNA as example:

Primer	RNA sample	Ct value
rRNA Primer Mix	mRNA not captured	9.11
	mRNA captured	21.37
mRNA Primer Mix	mRNA not captured	18.08
	mRNA captured	19.62