Poly(A) mRNA purification module

Catalog : RK20341

Size : 24 Reactions / 96 Reactions

Product Description

The first Poly(A) mRNA Purification module effectively enriches poly(A) mRNA using poly(T)-oligo attached magnetic beads

Product Components

Prodect Components	24 Reactions	96 Reactions
Oligo $d(T)_{25}$ Capture Beads	480 μL	1920 μL
mRNA Binding Buffer	12 mL	48 mL
Washing Buffer	19.2 mL	76.8 mL
Tris Buffer	1.2 mL	4.8 mL

Storage: 2-8°C;

Do NOT freeze the Oligo $(dT)_{25}$ Capture Beads.

Precautions

1. High-quality RNA is essential for sequencing library construction. The integrity and size distribution of total RNA can be accessed using an Agilent Bioanalyzer to address the RNA integrity number (RIN) score. An RNA sample with a RIN score lower than 7 is NOT recommended in this protocol.
2. To avoid contamination, keep all the reagents and samples in closed tubes on ice and use RNasezap® to clean the workspace.
3. To avoid cross contamination, always carefully add the RNA index primer to the PCR reaction.
4. Prepare fresh 80% Ethanol.

Protocal :

1. Equilibrate mRNA capture beads before starting.

1.1 Resuspend the oligo d(T) capture beads thoroughly by pipetting up and down several times.

1.2 Add 200 μl of mRNA Binding Buffer to 20 μl of the Oligo dT beads, and mix thoroughly by pipetting up and down several times.

1.3 Pellet the beads on a magnetic stand at room temperature (RT) for 2 minutes and carefully remove and discard the supernatant.

1.4 Wash the beads with 200 μl of mRNA Binding Buffer and mix thoroughly by pipetting.

1.5 Pellet the beads on a magnetic stand at RT for 2 minutes and discard the supernatant.

1.6 Add 50 μl of mRNA Binding Buffer to the beads and mix thoroughly by pipetting

2. Dilute 10-1000 ng of total RNA with nuclease-free water to a fifinal volume of 50 μl.

3.Add the diluted RNA to the beads mixture (step 1.6) and mix thoroughly by pipetting.

4. Incubate the reaction tubes in a thermocycler at 65°C for 5 minutes with the heated lid set to ≥ 75°C and then cool to 4°C.

5. Mix thoroughly by pipetting and place at RT for 5 minutes, enhancing the mRNA binding to the beads.

6. Pellet the beads on a magnetic stand at RT for 2

minutes and carefully remove the supernatant.

7. Wash the beads with 200 µl of mRNA Washing Buffer and mix thoroughly by pipetting. Pellet the beads on a magnetic stand at RT for 2 minutes and carefully remove the supernatant.

8. Repeat step 7 for a total of two washes.

9. Resuspend the beads with 50 µl of Tris Buffer and mix thoroughly by pipetting.

10. Incubate at 80°C for 2 minutes with a heated lid set to ≥ 90°C, then hold at 25°C.

11. Add 50 µl of mRNA Binding Buffer to the mixture of capture beads and mix thoroughly by pipetting.

12. Incubate at RT for 5 minutes, allowing the mRNA binding to the beads.

13. Pellet the beads on a magnetic stand at RT for 2 minutes and carefully remove the supernatant.

14. Wash the beads with 200 µl of mRNA Washing Buffer and mix thoroughly by pipetting. Pellet the beads on a magnetic stand at RT for 2 minutes and carefully remove the supernatant.

15. Repeat Step 14 for a total of two washes.

16. Add 10–20 µL (or down to 5 µL) of 10 mM Tris-HCl, pH 7.5 to elute the mRNA.

17. Heat the sample at 65°C to 80°C for 2 minutes and place the tube immediately on the magnet.

18. Transfer the eluted mRNA to a new RNase-free tube.