mRNA affinity chromatography purification kit (Large-volume Spin-column method)

Cat.No.:HYJ422

Product Description

The Hyasen mRNA Affinity Chromatography Purification Kit captures mRNA by base-pairing the Oligo dT on the surface of the affinity resin with the Poly A tail of the mRNA. Without the need for purification equipment and cryogenic equipment,mRNA samples with high integrity and obvious NTP removal effect can be obtained quickly and efficiently. The kit contains pre-formulated binders, washes, and resins for sample preparation in 0.5 hours.

Components

Components	5 T	10 T
Sample Binding Buffer	20 mL	40 mL
Equilibration/Wash 1 Buffer	60 ml	120 ml
Wash 2 Buffer	30 ml	60 ml
Resin	10 mL	20 mL
Empty spin columns	5 pcs	10 pcs

Storage

Store at 2-8℃, valid for 1 year. Empty spin columns store at room temperature.

Additional Materials Required

Consumables: Nuclease-free 1.5 mL centrifuge tubes, 20 μL, 200 μL and 1mL pipette tips.

Reagent: Nuclease-free water.

Instructions

1. Prepare

Take out the packing material stored at 2-8 ℃, mix it thoroughly; then pipette 2 mL resin into a 15 mL empty spin column. centrifuge at 4°C centrifuge at 5000 rpm for 30s, and discard the filtrate.

2. Resin washing

Pipette 2 mL of equilibration buffer and add it to a 15 mL empty spin column in step1, shake with a vortex shaker for 5 s or mix 5-10 times with a 1 mL pipette, centrifuge at 4°C centrifuge at 5000 rpm for 30s, and discard the filtrate. Repeat the step.

3. Binding

Add the pretreated sample to the empty spin column of step2, shake with a vortex shaker for 5s or mix 5-10 times with a 1 mL pipette, and place at room temperature for 5-10min.

(Recommended pretreatment of samples: take 150μL of IVT (up to 1.5mg loading) and dilute the samples with RNase-free water at a ratio of 1:10, then add the same amount of sample binding buffer to mix well, and put on ice/4°C for later use.)

4. Washing off impurities

Centrifuge the step3 empty spin column in a 4°C centrifuge at 5000rpm for 30 s, and discard the filtrate. Pipette 2 mL of wash 1 buffer and add it to the hollow spin column, shake it with a vortex shaker for 5s or mix well with a 1mL pipette for 5-10 times, centrifuge at 4°C, centrifuge at 5000 rpm for 30s, and discard the filtrate. Repeat this step. And then Pipette 2 mL of wash 2 buffer and add it to the empty spin column, shake with a vortex shaker for 5s or mix well with a 1mL pipette for 5-10 times, centrifuge at 4°C, centrifuge at 5000 rpm for 30s, and discard the filtrate. Repeat this step.

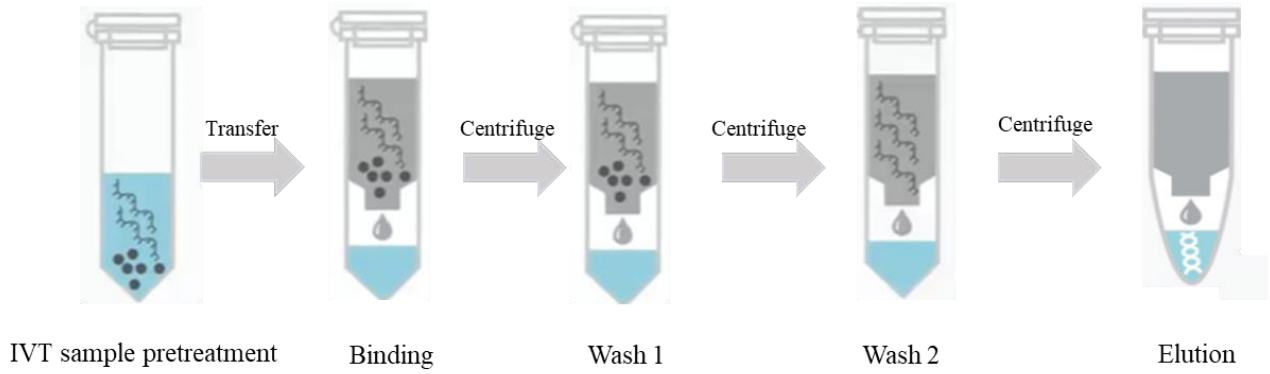
5. Elution

Take out the empty spin column of step4 into a new centrifuge tube, add 1 mL of RNase Free Water, shake with a vortex shaker for 5s or mix well with a 1mL pipette for 5-10 times, centrifuge at 4°C, centrifuge at 5000rpm for 30s, and collect the filtrate, and mark it as E1; Add 2 mL of RNase Free Water again, shake with a vortex shaker for 5 s or mix well with a 1mL pipette for 5-10 times, centrifuge at 4°C, centrifuge at 5000 rpm for 30s, collect the filtrate, and mark it as E2; Finally, 1 mL of RNase Free Water was added, vortex shaker shook for 5 s or mix well with a 1mL pipette for 5-10 times, centrifuge at 4°C, centrifuge at 5000 rpm for 30s, and the filtrate was collected and labeled as E3. The concentrations of E1, E2, and E3 were detected, and the eluted samples were pooled according to the concentration test results.

(The elution volume can also be adjusted according to the needs to fully elution the product).

6. Sample storage

The eluted samples are concentrated for subsequent LNP encapsulation, and can also be immediately used for CE, SEC_HPLC and other analytical applications, or stored at -80°C.



Notes

1. Do not freeze the resin.
2. During the operation, try not to dry the resin for more than 10min, and try to keep it in a nuclease-free environment for the experiment.
3. During elution, it was found that for mRNA samples below 800nt fragments, it was possible that the concentration of E1 was very low, and E1 could be discarded and E2-E3 merged. For mRNA samples larger than 800nt, it is recommended to test the concentrations of E1, E2, and E3 first, and then combine them as needed.
4. If the concentration of E3 detection is high, it is recommended to increase the elution volume and number of elution.
5. This kit is for research use only.