mRNA affinity chromatography purification kit (Spin-column method)

Cat.No.:HYJ421

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	20 T
Sample Binding Buffer	4 mL	8 mL	20 mL
Equilibration/Wash 1 Buffer	15 ml	30 ml	60 ml
Wash 2 Buffer	8 ml	15 ml	30 ml
Resin	2 mL	4 mL	8 ml
Empty spin columns	5 pcs	10 pcs	20 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 400 μL resin into a 2 mL empty spin column. centrifuge in a handheld centrifuge for 10 s, and discard the filtrate.

2. **Resin washing**

Pipette 400 μL of equilibration buffer and add it to a 2 mL empty spin column in step1, shake with a vortex shaker for 5 s or mix upside down for 5-10 times, centrifuge with a handheld centrifuge for 10 s, and discard the filtrate. Repeat the step.

3. Binding

Add the pretreated sample to the empty spin column of step 2, shake with a vortex shaker for 5s or mix upside down for 5-10 times, and place at room temperature for 5-10min.

(Recommended pretreatment of samples: take 20μL of IVT (up to 200ug 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 empty spin column of step 3 in a handheld centrifuge for 30s, and discard the filtrate. Pipette 400 μL of wash 1 buffer and add it to the hollow spin column, shake it with a vortex shaker for 5 s or mix it upside down for 5-10 times, centrifuge it with a handheld centrifuge for 15 s, and discard the filtrate. Repeat this step. And then Pipette 400 μL of wash 2 buffer and add it to the empty spin column, shake with a vortex shaker for 5 s or mix upside down for 5-10 times, centrifuge with a handheld centrifuge for 15 s, and discard the filtrate. Repeat this step.

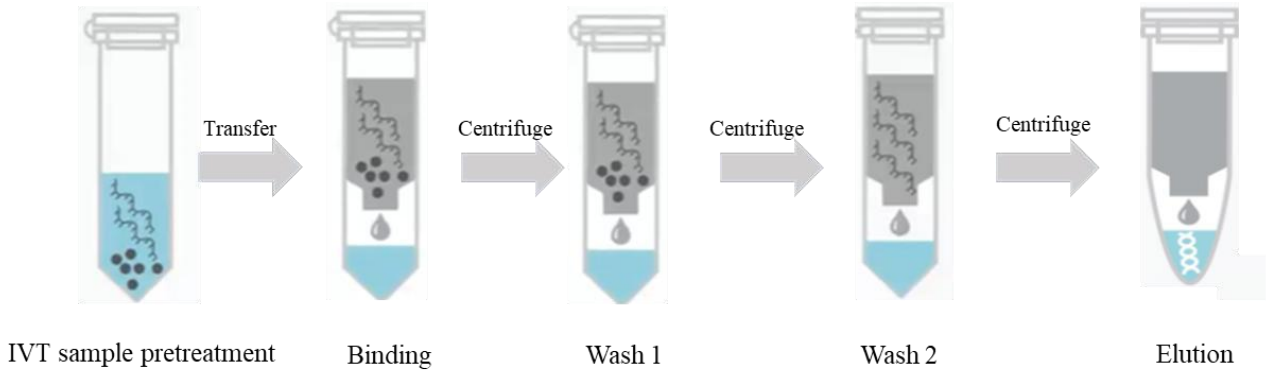
5. Elution

Take out the empty spin column of step4 into a new centrifuge tube, add 200 μL of RNase Free Water, shake with a vortex shaker for 5 s or mix upside down for 5-10 times, centrifuge with a handheld centrifuge for 30 s, collect the filtrate, and mark it as E1; Add 400μL of RNase Free Water again, shake with a vortex shaker for 5 s or mix upside down for 5-10 times, centrifuge with a handheld centrifuge for 30 s, collect the filtrate, and mark it as E2; Finally, 200 μL of RNase Free Water was added, vortex shaker shook for 5 s or upside down to mix 5-10 times, centrifuged on a handheld centrifuge for 30 s, 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 sample was concentrated and used for subsequent LNP encapsulation. It can also be used immediately for CE, SEC_HPLC and other analytical applications, and can also be 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. A low-speed centrifuge is recommended at a speed of 5000rpm or less, as there is a risk of centrifuging packing fragments to the bottom of the EP tube.
4. During elution, the concentration of E1 is found to be low for mRNA samples below 1500 nt fragments, which can be discarded and E2-E3 combined. For mRNA samples larger than 1500 nt, it is recommended to test the concentration of E1, E2, and E3 first, and then combine them as needed.
5. This kit is for research use only