# Sample Preparation Kit for mRNA Poly(A) Tail Length Detection

**Cat.No.:HYJ512**

## Product Description

Poly(A) tail length distribution is a critical quality attribute for mRNA-based vaccines, for it is necessary at the 3' end of the mRNA molecule to protect the molecule from degradation and to facilitate successful protein translation. The Sample Preparation Kit for mRNA Poly(A) Tail Length Detection enables efficient and reproducible processing of mRNA samples for accurate detection and assessment of the poly(A) tail length distribution. This kit includes pre-formulated 10× RNase T1 Mix, resin, spin column, wash buffer and elution buffer, enabling the preparation of LC-MS-ready samples within 1 hours.

## Components

| Components | 10 T | 50 T |
|---|---|---|
| 10×RNase T1 Mix | 200 µL | 5×200µL |
| mRNA for positive control (1µg/µL) | 100 µL | 5×100µL |
| Wash buffer 1 | 9×1 ml | 45×1 ml |
| Wash buffer 2 | 1 ml | 5×1 ml |
| Elution buffer | 1 ml | 5×1 ml |
| Resin | 200 µL | 5×200µL |
| Spin columns | 10 pcs | 5×10 pcs |

## Storage

Transportation under 0°C. Resin can be stored at 2~ 8 ℃, Spin column can be stored at room temperature, 10×RNaseT1 Mix, Wash buffer and Elution buffer can be stored at -25 ~ -15 ℃.

## Additional Materials Required

Consumables: 20 µL and 200 µL pipette tips, 1.5 mL microcentrifuge tubes. All consumables should be nuclease-free.

Reagent: nuclease-free water.

## Instructions

### 1. Calculation of mRNA Sample Amounts

Molar mass of the target mRNA (g/mol) = mRNA length (X nt)×330

100 pmol of target mRNA to be added (μL)=0.0001×molar mass of the target mRNA (g/mol) / mRNA concentration (μg/μL)

### 2. Digestion

Prepare the reaction at room temperature according to the following table:

| Components | Volume |
|---|---|
| Nuclease-free $H_2O$ | Up to 200 μL |
| 10×RNase T1 Mix | 20 μL |
| mRNA sample | X μL(100pmol) |

Vortex the solution for 3-5 seconds and short centrifuge. Incubate 15 minutes at 95℃.

### 3. Purification and Recovery

1) **Preparing resin：** Shake the bottle of resin to mix thoroughly. Transfer 20 μL resin slurry to the spin column(in a 2mL outer tube) and centrifuge for 15s in a microcentrifuge. Discard the flow-through and place the spin column into the outer tube.
2) **Washing resin:** Add 200μL Wash buffer1 to the spin column, mix thoroughly. Centrifuge for 15s. Discard the flow-through and place the spin column into the outer tube. Repeat the washing step for a total of two washes.
3) **Sample binding to resin:** Transfer the RNase T1 cleavage reaction mixture to the spin column, mix thoroughly. Incubate 5 minutes at room temperature.
4) **Washing off impurities:** Centrifuge the spin column for 15s. Discard the flow-through. Add 200uL wash buffer1 to the spin column, mix thoroughly. Centrifuge for 15s and discard the flow-through. Add 50μL wash buffer 2 to the spin column, mix thoroughly. Centrifuge for 15s and discard the flow-through. Note:The volume of wash buffer 2 is crucial, neither more nor less.
5) **Elution:** Transfer the spin column to a clean 1.5mL microcentrifuge tube. Add 50μL Elution Buffer, mix thoroughly. centrifuge for 15-30s. Discard the spin column and transfer the filter liquor to an LC-MS sample vial or store it at -20℃.

## Notes

1. The sample preparation reaction should be performed in an environment free of RNase contamination. Wearing gloves is advisable. The tips, tubes and water should be nuclease free.
2. Do not freeze resin.
3. The volume of a wash buffer 2 is crucial, neither more nor less.
4. If the signal is low, increase the amount of target mRNA in the reaction system to 200 pmol, others remain the same.
5. When adding 50ul washing buffer 2/elution buffer in step 3.4/3.5, some resin will be attached to the tube wall, which is a normal phenomenon, mixed it up and down as much as possible.
6. The Poly(A) tail length distribution of the positive control is 30A and 70A.
7. Positive control reaction system

| Reagent | Amount |
|---|---|
| Nuclease-free $H_2O$ | 147 μL |
| 10×RNase T1 Mix | 20 μL |
| Positive control mRNA sample | 33 μL(100pmol) |