Sample Preparation Kit for mRNA Capping Efficiency Detection

Cat.No.:HYJ511

Product Description

Capping efficiency is a critical quality attribute for mRNA-based vaccines, because a cap is necessary at the 5' end of the mRNA molecule to protect the molecule from degradation and to facilitate successful protein translation. The Sample Preparation Kit for mRNA Capping Efficiency Detection enables efficient and reproducible processing of mRNA samples for accurate detection and quantitative assessment of ribose-methylated and ribose-unmethylated cap structures, which can be separated and quantified using LC-MS. This kit includes pre-formulated 4× RNase H Mix, Streptavidin Magnetic Beads, and an optimized protocol, enabling the preparation of LC-MS-ready samples within 1.5 hours.

Components

Components	10 T	50 T
4×RNase H Mix	250 µL	5×250µL
Streptavidin Magnetic Beads	500 µL	5×500µL
Wash Buffer	8×1 ml	40×1 ml
Elution Buffer	300 µL	5×300 µL
mRNA Positive control（1µg/µL）	200 µL	5×200 µL
Biotin Probe Positive Control (10µM)	100 µL	5×100 µL

Storage

Transportation under 0°C; Streptavidin Magnetic Beads and Wash Buffer can be stored at 2~8 °C, and the other components can be stored at -25～-15 °C.

Additional Materials Required

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

Reagent: RNase H cleavage probe, nuclease-free water.

Instructions

1. Probe Design and Synthesis

This kit does not include site-specific RNase H cleavage probe. A 14-30nt probe, reverse complementary to the target 5' end sequence to produce 5' cap fragment, needs to be designed. The probe should contain 4-6 DNA bases near the 5' end or in the middle as the cleavage site, and the remaining positions with 2'-O-methyl-modified RNA bases. The 3' end of the probe should be labeled with Biotin TEG. An example was shown in Fig 1. The synthesized dry powder probe should be centrifuged and then dissolved in nuclease-free water to a final concentration of 10 μM.

2. Calculation of mRNA Sample and Probe 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)

Probe amount: 2 times the molar number of target mRNA (200 pmol)

Probe volume to be added (μL) = 0.01×200pmol×probe concentration (10 μM)

3. RNase H Cleavage Reaction

Thaw the 4×RNase H Mix and the probe on ice. In a 200 μL nuclease-free PCR tube. Assemble the reaction at room temperature in the following order:

Components	Volume
Nuclease-free H_2O	Up to 100 μL
4×RNase H Mix	25 μL
mRNA sample	X μL(100pmol)
10μM Probe	20 μL

Once the solution is prepared, vortex and pulse-spin in microfuge to collect solutions to bottom of tubes. Place the PCR tube in a PCR machine and incubate at 50°C for 15minutes.

4. Purification and Recovery

1) **Prepare magnetic beads:** Shake the bottle of Streptavidin Magnetic Beads to evenly resuspend the beads; then pipette 50 μL into a 1.5 mL microcentrifuge tube. Place the tube in the magnetic separator to separate the beads; when the supernatant becomes clear, gently aspirate the supernatant and discard it.
2) **Bead washing:** Add 200 μL of Wash Buffer, remove tube from magnetic separator, and pipette the entire volume up and down several times to resuspend the beads. Then magnetically separate

the beads, and remove and discard the supernatant. Repeat the washing step for a total of two washes.

3) **Sample binding to beads:** Remove tube from magnetic separator, add the RNase H cleavage reaction mixture to the tube, and pipette the entire volume up and down several times to resuspend the beads. Incubate at room temperature for 30 minutes with constant or periodic mixing (100-150 rpm). Then, place the tube on the magnetic separator to separate the beads. Remove the supernatant using a pipette.
4) **Washing off impurities:** Add 200 μL of Wash Buffer, remove tube from magnetic separator, and pipette the entire volume up and down several times to resuspend the beads. Then magnetically separate the beads, and discard the supernatant. Repeat the washing step once. Add 200 μL of pre-chilled nuclease-free water, then magnetically separate the beads, and discard the supernatant. Repeat the washing step for the last one of the wash steps.
5) **Elution:** Add 30 μL of Elution Buffer, remove tube from magnetic separator, and pipette the entire volume up and down several times to resuspend the beads, and place tube in a metal bath or a temperature-controlled incubator at 80°C for 3 minutes, then put tube at room temperature for 5 minutes. Then magnetically separate the beads, and transfer the supernatant to a new 1.5 mL microcentrifuge tube. Finally, magnetically separate the residual beads, and transfer the supernatant to an LC-MS sample vial. Make sure no beads transferred.

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, dry or centrifuge Streptavidin Magnetic Beads. Freezing, drying or centrifuging will cause the beads to aggregate and lose binding activity.
3. If no signal is detected, increase the amount of target mRNA in the reaction system to 200 pmol and scale up the reaction system proportionally.
4. This kit is for research or production use only and is not intended for direct application in humans.
5. The positive control mRNA contained in this kit was capped by co-transcriptional capping, the theoretical enzyme digestion product is: 5'-m7GpppmAGGAAAUAAGAGAGAAAA-3', and the corresponding relative molecular mass is 6453.92Da.