Cat.:BP-11-10

Pre-treatment Kit for mRNA Poly A Distribution Detection

V01

Product Description

Poly A tail length is a critical quality attribute for mRNA vaccines and therapeutics, constituting essential validation data required for the regulatory submission of mRNA-based products. Current methods for detecting poly A tail length include LC-MS (Liquid Chromatography-Mass Spectrometry), 3AIM-seq, Nanopore Direct RNA Sequencing, and capillary electrophoresis, among others. LC-MS has emerged as a primary detection method due to its ability to precisely determine poly A tail length and heterogeneity, making it suitable for process development and quality control. The poly A length distribution detection pre-treatment kit developed by Baorui Biotech is designed to efficiently and rapidly obtain the poly A tail of mRNA, accommodating sequences with different characteristics and various types of poly A tail designs. It offers product advantages such as high-efficiency purification, simple operation, excellent high sensitivity and specificity, as well as safety and reliability.

Components

	Components	Volume	Storage
Box 1	T Reagent	60 μL	-20±5℃
	10×T Buffer	120 μL	
Box 2	Magnetic Beads	1 mL	2-8℃
	Buffer Ⅰ	6 mL	
	Buffer Ⅱ	20 mL	
	EB Buffer	1 mL×2	

Storage

Box 1 is stored at -20±5℃ and Box 2 at 2-8℃. Do not freeze-thaw the magnetic beads.

Protocol

1. T Reagent Digestion Sample

Recommended digestion system (50 μL):

Components	Volume
T Reagent	5 μL
RNA	50 μg
10×T Buffer	5 μL
RNase-free ddH_2O	To 50 μL

Digestion was performed at 37°C for 2 hours.

2. Recovery of Adsorption Magnetic Beads

2.1 Processing of Digested Samples

Add 50 μL of Buffer I to the digested sample and mix thoroughly.

2.2 Preparation of Magnetic Beads

Remove the magnetic beads in advance and allow them to equilibrate to room temperature. Mix the beads well, then transfer 100 μL of the beads into a nuclease-free microcentrifuge tube. Place the tube on a magnetic rack, magnetically separate the beads, and discard the supernatant. Remove the tube from the magnetic rack.

2.3 Treatment of Magnetic Beads

Add 100 μL of Buffer Ⅰ to the tube from step 2, mix thoroughly, magnetically separate the beads, and discard the supernatant. Repeat this washing step twice.

2.4 Loading

Resuspend the washed beads in 100 μL of Buffer Ⅰ, then add the diluted digested sample from step 1. Mix on a shaker at room temperature for 30 min.

2.5 Washing

Add 200 μL of Buffer Ⅱ to the tube, magnetically separate the beads, and discard the supernatant. Wash the beads a total of four times, then remove the tube from the magnetic rack.

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2.6 Elution

Take 25 μL of pre-warmed (80°C) EB Buffer and mix thoroughly with the magnetic beads. Heat at 80°C for 4 minutes, then magnetically separate and retain the supernatant into a labeled nuclease-free microcentrifuge tube. Repeat this step twice. Finally, combine the supernatants obtained from the two rounds, place the mixture on the magnetic rack again to ensure removal of any residual magnetic beads.

Tips: The presence of magnetic beads in the prepared sample can damage detection instruments and chromatography columns. To prevent this, avoid touching the beads when aspirating the supernatant. The volume of the prepared sample is sufficient for subsequent applications.

Notes

1. Store the components of the kit as required: T Reagent, 10×T Buffer should be stored at -20°C; all other reagents should be stored at 4°C. Note that the magnetic beads should not be subjected to freeze-thaw cycles.
2. The magnetic beads, reagents, and other materials provided in this kit have undergone extensive screening and validation. To ensure processing efficacy, do not arbitrarily substitute the materials supplied with the kit, as this may compromise experimental results.
3. Samples processed with this kit should be analyzed promptly. Storage time should ideally be less than one week.
4. Observe appropriate personal protective measures during the experiment.
5. Please mix thoroughly before use. Avoid repeated freezing and thawing.