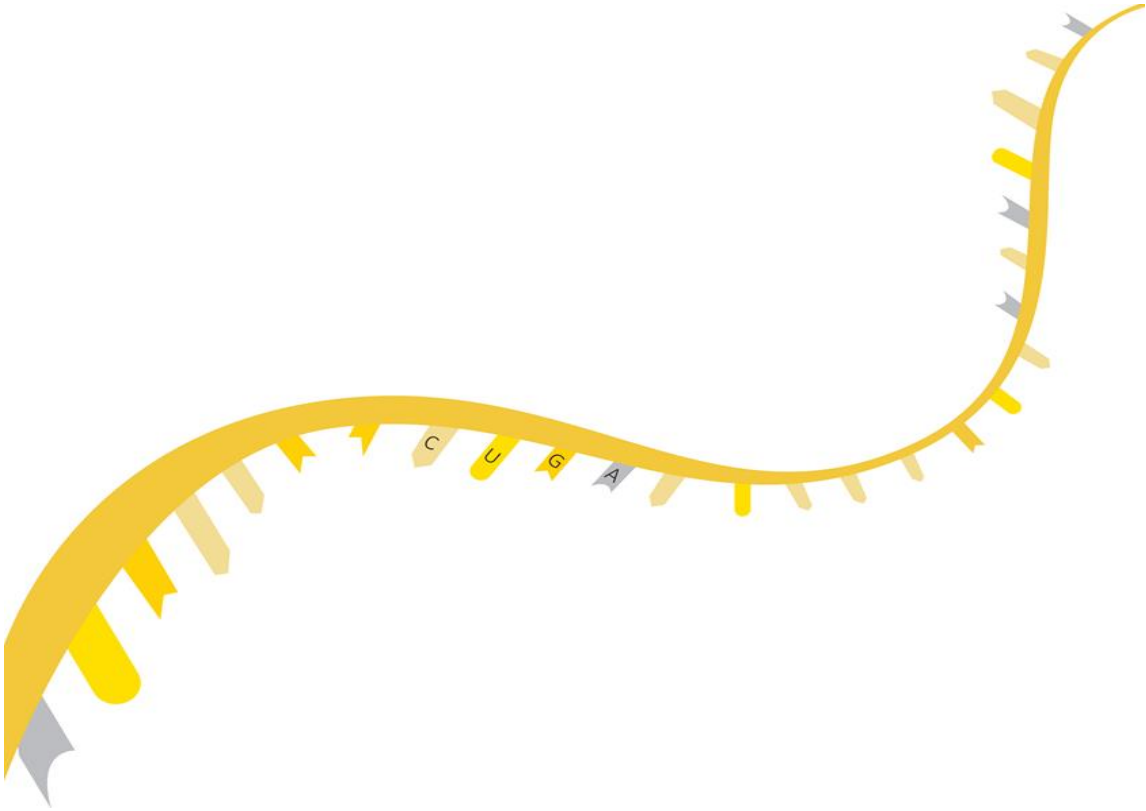
Poly(A) mRNA Capture Module

RK20340



www.abclonal.com.cn

Version: N16D08v1.1

Table of Contents

1. Product Overview

The 2X Oligo $(dT)_{25}$ Capture Beads are magnetic microspheres coupled with Oligo d(T) and applicable to poly(A) RNA isolation from purified total RNAs. This capture method is based on the principle of binding between the poly(A) tail at the 3' end of an mRNA and the Oligo d(T) sequence on the magnetic microsphere. The capture can be finished within 1 hour.

All reagents provided in the kit have undergone rigorous quality control. Each lot of the kits has been verified through library preparation and sequencing to ensure stable performance.

2. Kit Components

Table 1. Kit Components

	Tube Name and Color	8 rxns	24 rxns	96 rxns
○	2X Oligo $(dT)_{25}$ Capture Beads	400 µL	1.2 mL	4.8 mL
○	mRNA Binding Buffer	400 µL	1.2 mL	4.8 mL
○	Washing Buffer	3.2 mL	9.6 mL	38.4 mL
○	Tris Buffer	400 µL	1.2 mL	4.8 mL

○ denotes the color of the tube cap.

3. Storage

Transport with ice bags. Store at 2-8°C. Do not freeze.

4. Additional Materials Required

Nuclease-free PCR tube, magnetic rack, PCR system, etc.

5. Precautions

5.1 Do not store the 2X Oligo $(dT)_{25}$ Capture Beads at -20°C. Otherwise, they will be damaged and can no longer be used. It is recommended to purchase the Poly(A) mRNA Capture Module (ABclonal, RK20340).

5.2 The magnetic beads should be brought to room temperature after being taken out from the 2-8°C environment. Otherwise, their capture efficiency will be affected.

5.3 Each reagent added should be mixed well with magnetic beads.

5.4 The module is applicable to animal, plant, and eukaryote (e.g., fungi) RNA samples. The total initial RNA input is 10 ng-1 μg.

5.5 The RIN of total RNAs should be > 7. If mRNAs show poor integrity or are degraded, the RNA library preparation will be affected. For degraded RNA samples, the rRNA Depletion Module series can be used for mRNA capture.

5.6 In the test, masks and gloves should be worn, and the nuclease-free water should be freshly prepared to avoid contamination.

6. Protocol

6.1 Thaw RNAs on ice, dissolve 10 ng-1 μg of total RNA in 50 μL of nuclease-free water, and place the solution on ice for later use.

6.2 Take 2X Oligo $(dT)_{25}$ Capture Beads out of the 2-8°C environment in advance, let it stand until it reaches room temperature, and vortex-mix them. Add 50 μL of the prepared RNA solution, and mix the solution by pipetting.

6.3 Incubate the mixture in the PCR system (heating lid temperature ≥ 75°C).

Temperature	Time
65°C	5 min
25°C	5 min

6.4 After incubation, Place the centrifuge tube on the magnetic rack for about 2

minutes until the solution becomes clear, and remove and discard the supernatant.

6.5 Take down the centrifuge tube from the magnetic rack, add 200 μL of Washing Buffer, and mix the solution by pipetting. Place the centrifuge tube on the magnetic rack until the solution becomes clear, and remove and discard the supernatant.

6.6 Take down the centrifuge tube from the magnetic rack, add 50 μL of Tris Buffer, mix the solution well by pipetting, and incubate the mixture in the PCR system (heating lid temperature 105°C).

Temperature	Time
80°C	2 min

6.7 After the solution reaches room temperature, add 50 μL of mRNA Binding Buffer, mix the solution well by pipetting, and let the mixture stand at room temperature for 5 minutes.

6.8 Place the centrifuge tubeon the magnetic rack for about 2 minutes until the solution becomes clear, and remove and discard the supernatant.

6.9 Take down the centrifuge tube from the magnetic rack, add 200 μL of Washing Buffer, and mix the solution by pipetting. Place the centrifuge tube on the magnetic rack until the solution becomes clear, and remove and discard the supernatant.

6.10 Microcentrifuge the centrifuge tube after capping, place the tube on the magnetic rack, and remove all the residual supernatant with a 10 μL pipette and discard it.

6.11 Select the appropriate procedure based on the test purpose:

A. For RNA library preparation, add 1X Frag/Elute Buffer with reference to the Instructions for Use of the used library preparation kit (ABclonal, Cat. No. RK20306), and immediately proceed with the library preparation procedures.

B. For reverse transcription or integrity testing, add 10 μL of nuclease-free water into the mRNA elution product, mix the solution well by pipetting, let it stand at

80°C for 2 minutes and then immediately on the magnetic rack for another 5 minutes until the solution becomes clear, pipette 8 μL of the supernatant into a new PCR tube, and instantly place the tube on ice for later use.

C. For other tests, temporarily store the mRNA elution product at -80°C after elution from magnetic beads.

7. FAQs

Table 2. Wrong mRNA Capture Operations and Corrective Actions

Step	Correct Operation	Wrong Operation	Corrective Action
Step 6.6	Add 50 μL of Tris Buffer to re-suspend the beads.	Add 50 μL of mRNA Binding Buffer.	Place the centrifuge tube on the magnetic rack until the solution becomes clear, and remove and discard the supernatant. Take down the centrifuge tube from the magnetic rack, and add Tris Buffer.
Step 6.7	Add 50 μL of mRNA Binding Buffer.	Add 50 μL of Tris Buffer.	Scale up the reaction system by adding 100 μL of mRNA Binding Buffer.
Step 6.7	Add 50 μL of mRNA Binding Buffer, mix the solution well by pipetting, and let the mixture stand at room temperature for 5 minutes.	Add 50 μL of mRNA Binding Buffer, mix the solution well by pipetting, and immediately place the solution onto the magnetic rack.	Mix the solution again by pipetting, and let the mixture stand at room temperature for 5 minutes.
Steps 6.5 and 6.9	Add 200 μL of Washing Buffer.	Add 200μL of mRNA Binding Buffer.	Place the centrifuge tube on the magnetic rack until the solution becomes clear, and

			remove and discard the supernatant. Take down the centrifuge tube from the magnetic rack, and add 200 μL of Washing Buffer.
Step 6.10	Remove all the residual supernatant with a 10 μL pipette and discard it.	Forget to remove and discard the residual supernatant.	If the next step is mRNA fragmentation, the fragmentation system will be scaled up, thereby affecting the mRNA fragment size.

China

www.abclonal.com.cn

Headquarters: Building 5, Precision Medicine Industry Base Project I, Gaokeyuan 3rd Road, Donghu New Technology Development Zone, Jiangxia District, Wuhan, Hubei, China

Shanghai R&D Center: Floor 4, Building 2, Zizhu High-Tech Industrial Development Park, No. 58 Yuanmei Road, Minhang District, Shanghai, China

US R&D Center: 86 Cummings Park Dr, Woburn, MA 01801, United States

Tel.: 400-999-6126

Email: cn.market@abclonal.com