

Poly(A) mRNA Capture Module

Capture Module to isolate poly(A) RNA from purified total RNAs

Component	Cat#	M4411.0024	M4411.0096	Colour code of cap
2X Oligo (dT) ₂₅ Capture Beads		1.2mL	4.8mL	
mRNA Binding Buffer		1.2mL	4.8mL	
Washing Buffer		9.6mL	38.4mL	
Tris Buffer		1.2mL	4.8mL	

Description

Genaxxon's 2X Oligo (dT)₂₅ Capture Beads offer a reliable and efficient solution for isolating poly(A) RNA from purified total RNA samples. 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.

With a streamlined protocol, the capture process is completed in under 1 hour, making it ideal for fast and accurate RNA preparations. The kit includes all necessary reagents, each subjected to stringent quality control. Every batch is verified through library preparation and sequencing, guaranteeing consistent, high-performance results in RNA isolation experiments.

This product is for research use only.

Storage and shipment

Shipment: on wet ice. Store at 2-8°C. DO NOT FREEZE.

Product Use Limitations

2X Oligo (dT)₂₅ Capture Beads are developed, designed, and sold for research purposes only. They are not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as a pdf-file or upon request (info@genaxxon.com).

Quality control

Genaxxon bioscience 2X Oligo (dT)₂₅ Capture Beads undergo stringent quality controls and functional testing to ensure optimal stability and repeatability.

Important notes before getting started

The protocol serves as a guideline. Multiple factors such as the sample, protocol, equipment, and operation may vary. Thus, it may be necessary to adjust the parameters of the library preparation procedures. Please read the following notes carefully to obtain a high-quality library. In case of any questions, please contact Genaxxon: info@genaxxon.com.

- Do not freeze the 2X Oligo (dT)₂₅ Capture Beads. Otherwise, they will be damaged and can no longer be used.
- The magnetic beads should be brought to room temperature before starting the lab process. Otherwise, their capture efficiency will be affected.
- Each reagent added should be mixed well with magnetic beads.
- The module is applicable to animal, plant, and eukaryote (e.g., fungi) RNA samples. The total initial RNA input is 10ng-1µg.
- 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.
- During lab work, masks and gloves should be worn, and the nuclease-free water should be freshly prepared to avoid contamination.

Protocol Part

- Thaw RNA on ice, dissolve 10-1000ng of total RNA in 50µL of nuclease-free water and place the solution on ice for later use.
- Vortex 2X Oligo (dT)₂₅ Capture Beads after returning to RT (+15°C to +30°C). Add 50µL of the beads to the RNA solution and mix by pipetting.
- Incubate the mixture in the thermocycler (heating lid temperature ≥ 75°C) according to Table 1.

Table 1: Incubation protocol - step 1

Temperature	Time
65°C	5min
25°C	5min

- After incubation, let the centrifuge tube stand on the magnetic rack for about 2min until the solution becomes clear, and remove and discard the supernatant.
- Add 200µL of Washing Buffer and mix the solution by pipetting. Let the centrifuge tube stand on the magnetic rack until the solution becomes clear and remove and discard the supernatant.
- Remove the PCR tube from the magnetic rack, add 50µL of Tris Buffer, mix the solution well by pipetting, and incubate the mixture in the thermocycler (heating lid temperature 105°C) according to Table 3.

Table 3: Incubation protocol - step 2

Temperature	Time
80°C	2min

- Let the solution cool down to RT, add 50µL of mRNA Binding Buffer, mix the solution well by pipetting, and let the mixture stand at RT (+15°C to +30°C) for 5min.
- Let the centrifuge tube stand on the magnetic rack for about 2min until the solution becomes clear and remove and discard the supernatant.
- Add 200µL of Washing Buffer and mix the solution by pipetting. Let the centrifuge tube stand on the magnetic rack until the solution becomes clear and remove and discard the supernatant.
- Centrifuge instantaneously after capping, place the tube on the magnetic rack, and remove all the residual liquid with a 10µL pipette.
- Select the appropriate procedure based on the test purpose:
 - For RNA library preparation, add 1X Frag/Elute Buffer with reference to the instructions for Use of the used library preparation kit (Genaxxon, M4408), and immediately proceed with the library preparation procedures.
 - For reverse transcription or integrity testing, add 10µL of nuclease-free water into the mRNA elution product,

- mix the solution well by pipetting, let it stand at 80°C for 2min and then immediately on the magnetic rack for another 5min until the solution becomes clear, pipette 8µL of the supernatant into a new PCR tube, and instantly place the tube on ice for later use.
- For other tests, temporarily store the mRNA elution product at -80°C after elution from magnetic beads.