USER GUIDE

EcoQprep™ mRNA Kit

Cat. No. K-3708

BIONEER
Innovation • Value • Discovery

EcoQprep™ mRNA Kit

Kit for the extraction of mRNA from cultured cells, plant tissue, or animal tissue

User Guide

K-3708

Σ 50

Version No.: 0 (2025-08-18)

Please read all the information in booklet before using the unit

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Intended Use

EcoQprep™ mRNA Kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.

Safety Warning and Precaution

Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information, please consult the appropriate Material Safety Data Sheet (MSDS).

Warranty and Liability

All BIONEER products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

Quality Management System ISO 9001 Certified

Every aspect of our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards.

Trademark

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Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Contents

Product Information

Components

Components	Cat. No.	Amount*	Storage
MRD Buffer	KB-2151	30 mL x 1 ea	Store at room temperature (15-35°C) After opening, refrigerate at 2-8°C
MRW1 Buffer	KB-3141	40 mL x 1 ea	
MRW2 Buffer	KB-4051	50 mL x 1 ea	
MRW3 Buffer	KB-5111	50 mL x 1 ea	
EM Buffer	KB-6051	25 mL x 1 ea	
Magnetic Nano Bead-Oligo dT (10 mg/mL)	KB-7041	3 mL x 1 ea	
1.5 mL Tube	KA-1120	50 ea x 1 pack	
One-Page Protocol	-	1 ea	

* Mini – 50 rxn

Storage

The kit will maintain performance for at least two years when stored under standard conditions. All components of the kit should be stored at room temperature (15-35°C). After opening, it is recommended to store the buffer at 2-8°C to ensure prolonged stability.

Specifications

Starting material & RNA extraction efficiency

The following table provides a general reference for the approximate total RNA yield and expected purity from various sample types, from which 1-5% is typically mRNA.

Product specifications of scale

Scale	Mini
Expected Total RNA Yield (1-5% is mRNA)	~ 100 µg
Minimum elution volume	15 µL
Preparation time	~ 10 min

Product details by sample type

Sample	Starting amount	Total RNA yield (1-5% is mRNA)	Expected purity*
Cultured cells	10^4-10^8 cells	15-20 µg	A_{260}/A_{280} > 2.0
Liver	25-50 mg	10-60 µg	
Spleen	100 mg	30-60 µg	
Plant tissues	100 mg	70-80 µg	

*** Note:** Measured values may vary depending on the sample type.

Precautions

- RNA is highly susceptible to degradation by exogenous RNase that may be introduced during the handling steps, all the steps must be conducted under sterile, RNase-free condition.
- RNase-free reagents, pipette tips, and tubes must be used with gloved hands while handling them.

Introduction

Product Description

The EcoQprep™ mRNA Kit is designed for the selective isolation and purification of highly pure polyadenylated mRNA from mammalian cells, animal tissues, and plant tissues.
This kit utilizes magnetic nanobeads functionalized with oligo dT, which specifically hybridize to the poly(A) tail of mRNA, enabling efficient recovery of mRNA from the total RNA population. In this process, ribosomal RNA (rRNA) is effectively removed without the need for repetitive DNase treatments, thereby minimizing rRNA-derived background signals and improving analytical accuracy in qPCR-based gene expression profiling and transcriptome studies.

When used with the EcoQprep™ Magnetic Separation Rack, mRNA can be isolated quickly and conveniently without centrifugation. The kit is also compatible with the ExiPrep™ 96 Lite (Cat. No. A-5250) system for automated processing of up to 96 samples simultaneously.

The purified mRNA is directly applicable to a variety of downstream molecular biology applications, including RT-PCR, RT-qPCR, cDNA synthesis, and gene cloning.

Principle

The EcoQprep™ mRNA Kit utilizes magnetic nanobeads conjugated with oligo dT to selectively isolate polyadenylated mRNA from total RNA samples. The oligo dT specifically hybridizes to the poly(A) tail of mRNA, allowing selective capture of mRNA on the bead surface.

• Hybridization step

The binding buffer provided in the kit is optimized to promote stable hybridization of mRNA to the oligo dT on the magnetic nanobeads.

• Washing step

Genomic DNA, ribosomal RNA (rRNA), and cell debris do not bind to the beads and are effectively removed with the washing buffer.

• Elution step

The purified mRNA is released from the beads using the elution buffer or RNase-free water, yielding mRNA with high purity and recovery.

* **Note**: Some sample types require a pre-treatment step.

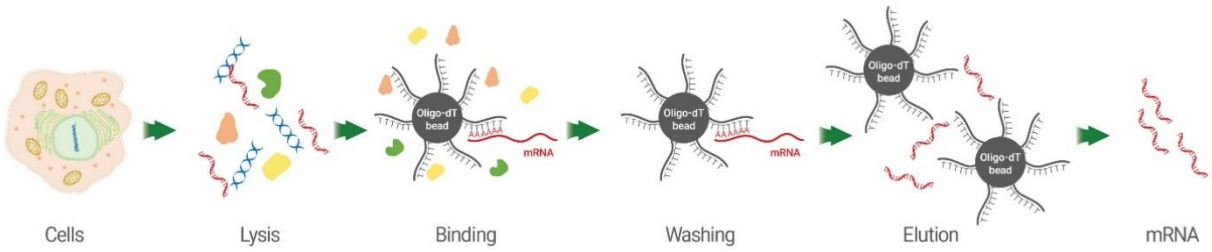


Figure 1. mRNA purification using Magnetic Nano Bead-Oligo dT.

Features & Benefits

- **High Purity &Yield**: Selectively isolates polyadenylated mRNA and efficiently removes genomic DNA and ribosomal RNA, delivering highly pure results.
- **Fast & Convenient Workflow**: Enables mRNA isolation in less than 10 minutes without centrifugation when used with the EcoQprep™ Magnetic Separation Rack.
- **Broad Sample Applicability**: Suitable for a wide range of sample types, including mammalian cells, animal tissues, and plant tissues.
- **Automation-Compatible**: Applicable to the *ExiPrep*™ 96 Lite system for fully automated extraction of up to 96 samples simultaneously.

Magnetic Nano Bead-Oligo dT

Magnetic nanobeads were developed to overcome the limitations of conventional resin-based purification methods and to enable automated, high-efficiency mRNA purification. The bead surface is functionalized with oligo(dT) ligands that specifically bind to the poly(A) tail of mRNA, allowing selective isolation. Owing to their magnetic properties, the beads can be rapidly and conveniently separated and washed using an external magnetic field, eliminating the need for centrifugation.

Specification

Magnetic Nano Beads-Oligo dT	
Matrix	Silica-coated Fe_3O_4
Average size	400 nm
Ligand	- Oligo dT
Working Temperature	0-100°C
Storage	Store at room temperature (15-35°C). After opening, refrigerate at 2-8°C.

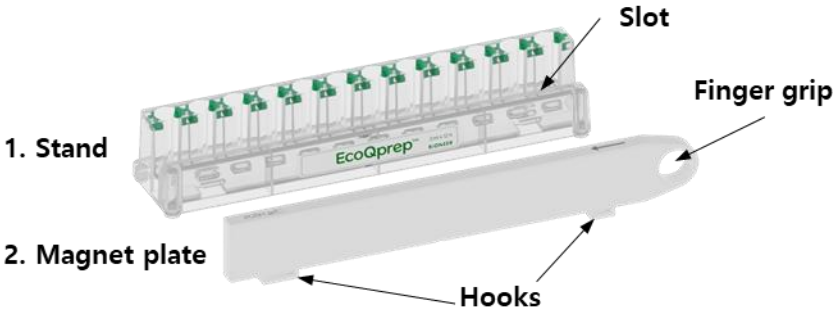
Features & Benefits

- **Rapid**: Fast binding guarantees high throughput automation.
- **Effectiveness**: Large surface area enables more sensitive assay.
- **Specificity**: Globular structure increases specificity by decreasing non-specific binding.

EcoQprep™ Magnetic Separation Rack

EcoQprep™ Magnetic Separation Rack (Cat. No. TM-1012) is designed for a fast and easy separation of the Magnetic Nano Beads. BIONEER offers various racks of different sizes for 1.5 or 2 mL tube (Cat. No. TM-1012), 15 mL tube (Cat. No. TM-1021), 50 mL tube (Cat. No. TM-1031). These racks consisting of different size allow user to choose the product according to their needs.

Components of EcoQprep™ Magnetic Separation Rack

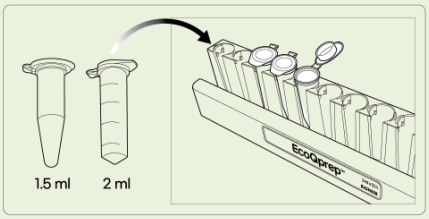
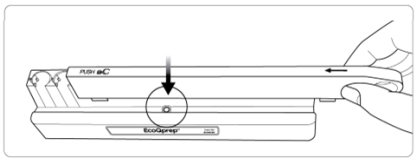


- Stand: Holds up to 12 tubes with a non-slip design.
- Magnet plate: Detachable from the stand's slot and contains a magnet.

Features & Benefits of EcoQprep™ Magnetic Separation Rack

- Rapid: Isolates plasmid DNA, genomic DNA, and/or RNA quickly and economically.
- Convenient: Simply disposes waste solution just by flipping the rack without centrifugation or pipetting as the silicon fixture secures the tube.

The followings are recommended when using the EcoQprep™ Magnetic Separation Rack.

Precautions for Use	
	Confirm the orientation of the tube and the Rack.
	Ensure that the front hook of the Magnet plate overlaps more than half of the Stand.

1. Attach the Magnet plate

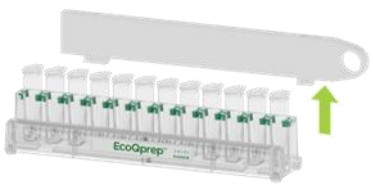
① Align the Magnet plate half of the Stand.

② Hold the finger grip of the Magnet plate and push.

2. Detach the Magnet plate

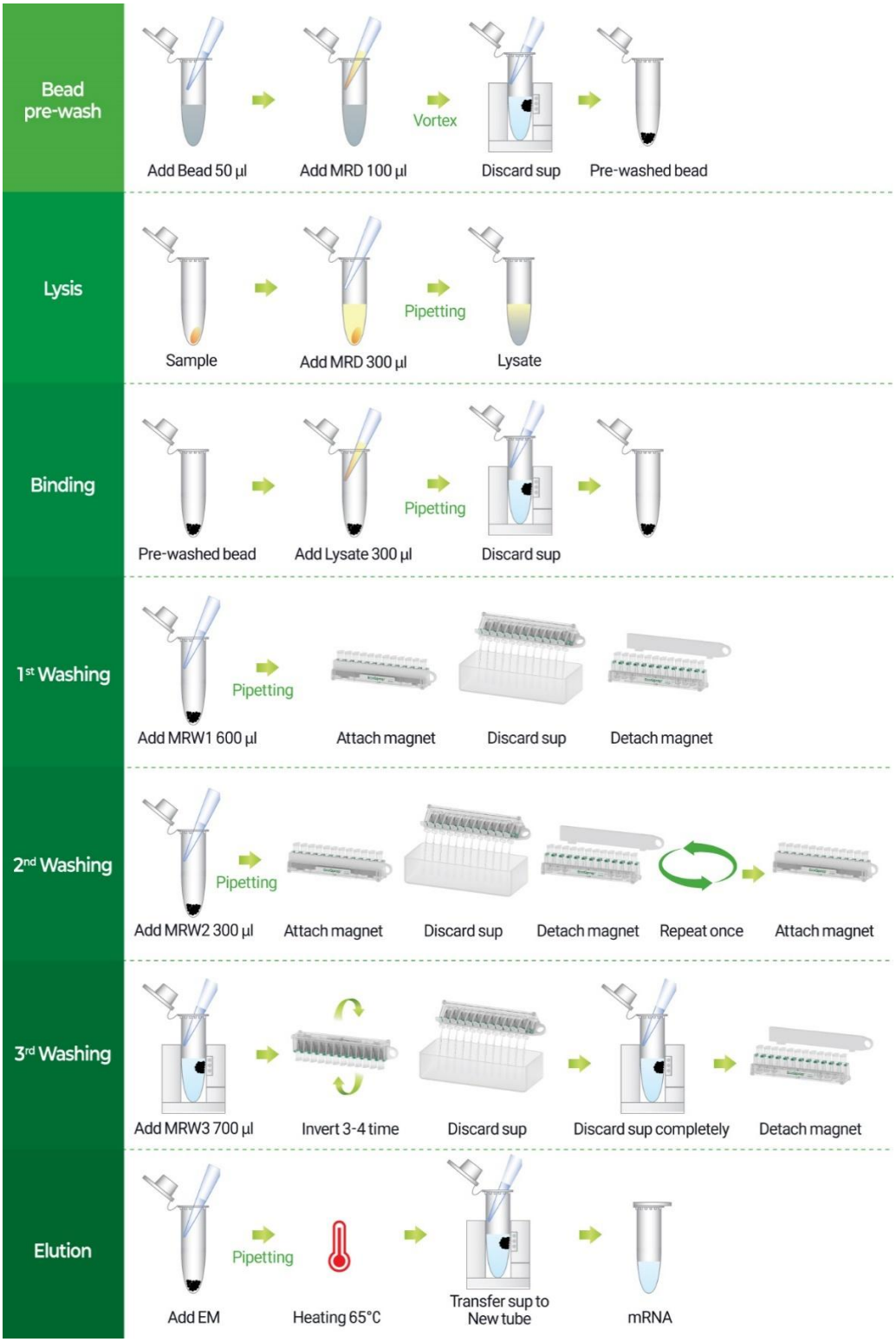
① Hold the finger grip of the Magnet plate and pull.

② Lift the Magnet plate



Experimental Procedures

mRNA Extraction Procedure Overview



Sample Preparation

Several factors such as harvesting method and storage of starting samples can influence the yield and purity of mRNA. All specimens must be stored in a freezer or used immediately after collection. It is recommended to put the sample as soon as possible on ice and avoid repeated freezing and thawing.

- **Cultured cells**

For mRNA extraction, number of cells should be more than 1 x 10^4 cells, which is calculated with a cell counter. Cultured cells can easily be harvested by centrifugation. However, it might be difficult to extract mRNA if cultured cells are too clustered. In this case, trypsin can be used to detach each cell from the cluster. It is recommended to keep the samples on ice until use.

- **Tissues**

Several factors such as harvesting method and storage of starting samples can influence the yield and purity of mRNA. Freshly collected sample must be stored in a freezer or used immediately after collection. If immediate use of the plant tissue sample is not planned, store it in liquid nitrogen or at -70 ~ -80°C. Avoid repeated freezing and thawing.

Before Start

Before proceeding, please check the following:

1. Prepare of MRD Buffer

Add 1 µL of β-mercaptoethanol (99% Purity; Molecular grade; 14.3 M) per 1 mL of MRD Buffer (final concentration of β-mercaptoethanol: 14.3 mM). For optimal antioxidant activity, prepare the MRD Buffer freshly just before use, and long-term storage is not recommended.

2. Pre-wash of Magnetic Nano Bead-Oligo dT

It is highly recommended to pre-wash Magnetic Nano Bead-Oligo dT before use to improve binding efficiency.

1) With the magnet plate detached, insert new 1.5 mL microcentrifuge tubes into the EcoQprep™ Magnetic Separation Rack.
2) Dispense the required volume of beads (e.g., 50 µL per reaction) into each tube.
3) Add 100 µL of MRD Buffer to each tube, and briefly vortex to mix.
4) Attach the magnet plate to the rack, allow the beads to separate, and carefully remove the supernatant using pipette.
5) The washed beads are now ready for the binding step of mRNA Isolation.

3. g-force can be calculated as follows: rcf = 1.12 x r x $(rpm/1,000)^2$

Note: Where 'rcf' is the relative centrifugal force (in g), 'r' is the radius of the rotor in centimeters, and rpm is the speed of the centrifuge in revolutions per minute.

mRNA Isolation from various samples

mRNA Isolation		
Step	**Buffer**	**Amount used**
Sample preparing	Cultured cells	~ 1 x 10^6 cells
	Animal tissues	~ 10 mg
	Plant tissue	~ 20 mg
Lysis	MRD Buffer (mixed with β-mercaptoethanol)	300 µL
Binding	Magnetic Nano Bead-Oligo dT	50 µL
Washing	MRW1 Buffer	600 µL
	MRW2 Buffer	300 µL * 2
	MRW3 Buffer	700 µL
Elution	EM Buffer	15 – 20 µL

Protocol Ⅰ : mRNA Isolation from Cultured Cells

1. Cell harvesting

1) Harvest cells according to step A. or B.

A. Suspension cell culture:

Harvest cultured cells (~1 x 10^6 cells) by centrifugation at 300 x g for 5 minutes to pellet cells. Discard the supernatant carefully without disturbing a cell pellet.

B. Monolayer cell culture:

There are two different methods to collect cells grown in monolayer culture.

① Direct cell harvesting on the culture dishes:

Completely discard the cell culture medium.

* **Note**: Completely remove the cell culture medium to prevent inhibition of mRNA extraction

② Cell harvesting with trypsin:

Remove the cell culture medium and wash the cell monolayer with DPBS. Add 0.1-0.25% trypsin to the washed cell monolayer to detach the cells. After the cells detach, add cell culture medium. Transfer the cells to an RNase-free tube (not provided) and centrifuge at 300 x g for 5 minutes. Discard the supernatant carefully.

2. Lysis

1) Add 300 µL of MRD Buffer, pre-mixed with β-mercaptoethanol, to the cell pellet and mix thoroughly by pipetting.

* **Note**: Prepare MRD Buffer freshly before use by adding 1 µL of β-mercaptoethanol per 1 mL of buffer.

3. Binding

1) Prepare 50 µL of pre-washed Magnetic Nano Bead-Oligo dT solution.

*** Note:** Pre-wash the beads prior to the experiment (See page 10 for details.)

2) Add the entire lysate obtained in Step 2. Lysis to the tube containing pre-washed bead, then mix thoroughly by pipetting until the beads are fully resuspended.

*** (Optional):** In order to increase mRNA yield, incubate at room temperature for 5 minutes on a rotator to allow the poly(A) tail of mRNA to hybridize to the beads. In case of low efficiency, keep the lysate (unbound) from step 3-3) and reuse it for re-binding step to increase yield.

3) Place the tube and attach the Magnet plate to the Stand of EcoQprep™ Magnetic Separation Rack and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

Figure 2. Attachment of the Magnet plate.

4) Completely aspirate the lysate (unbound) a using pipette and choose to either retain it for rebinding or discard.

*** Note:** Completely remove any residual solution by aspiration to ensure efficient washing and obtain high-purity mRNA.

5) Detach the Magnet plate from EcoQprep™ Magnetic Separation Rack.

Figure 3. Detachment of the magnet plate.

4. 1st Washing

1) Add 600 µL of MRW1 Buffer. Mix by pipetting until the beads are fully resuspended.

2) Place the tube and attach the Magnet plate to the Stand of EcoQprep™ Magnetic Separation Rack and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

3) Discard the supernatant carefully without removing the tube from EcoQprep™ Magnetic Separation Rack. Completely remove any residual buffer using a pipette.

*** Note:** Avoid physical impact during blotting, as striking the tube may cause bead detachment.

*** Note:** When discarding the supernatant, tilt the rack toward the tube opening to prevent liquid from spilling onto the rack.

Figure 4. How to discard the supernatant.

4) Detach the Magnet plate.

5. 2nd Washing

1) Add 300 µL of MRW2 Buffer. Mix by pipetting until the beads are fully resuspended.

2) Proceed with step 4-2) to 4-3) to discard the supernatant. Completely remove any residual buffer using a pipette.

3) Detach the Magnet plate.

4) Repeat step 5-1) to 5-2) once more for additional washing.

*** Note:** Do not detach the Magnet plate from the rack after discarding the final supernatant.

6. 3rd Washing

1) Add 700 µL of MRW3 Buffer to the opposite side of the beads without disturbing them. During this step, the bead in the tube must be captured by Magnet plate.

*** Note:** Do not pipette MRW3 Buffer directly onto the beads. Avoid vortexing, vigorous shaking or tapping the tubes. These actions can release nucleic acid from the beads, potentially leading to a lower mRNA yield.

2) Gently invert the rack attached the magnet 3-4 times.

3) Discard the supernatant and completely remove any residual buffer at the bottom of the tube using a pipette.

Figure 5. Third wash step to rinse the beads.

4) Detach the Magnet plate.

7. Elution

1) Add 15-20 µL of EM Buffer to each tube and resuspend by pipetting.

2) Incubate tubes at 65°C for at least 2 minutes, and then tapping thoroughly.

3) Attach the Magnet plate and carefully transfer the supernatant containing mRNA to a new RNase-free tube.

*** Note:** Transfer the eluent to a new tube immediately after elution to prevent mRNA from re-binding to the beads and reducing yield.

4) Do not discard the used beads; the lysate (unbound) retained from step 3-3) can be used for re-binding using the used beads.

5) Please refer to page 17 for removing rRNA contamination, and to page 18 for reuse of Magnetic Nano Bead-Oligo dT.

Protocol Ⅱ: mRNA Isolation from Animal and Plant Tissues

1. Lysis & homogenization.

1) Sample Preparation

A. **Animal tissue**: Place fresh or thawed animal tissue into an appropriate volume of MRD Buffer (≥ 300 µL per 10 mg tissue) pre-mixed with β-mercaptoethanol and homogenize using a homogenizer.

B. **Plant tissue**: Rapidly freeze fresh or thawed plant tissue in liquid nitrogen, then grind thoroughly using a mortar and pestle. Allow the liquid nitrogen to completely evaporate, then immediately add MRD Buffer (≥ 300 µL per 20 mg tissue, pre-mixed with β-mercaptoethanol) before the tissue thaws, and mix thoroughly by pipetting.

* **Note**: Prepare MRD Buffer freshly before use by adding 1 µL of β-mercaptoethanol per 1 mL of buffer. Prevent samples from thawing during preparation. Perform grinding or homogenization as quickly as possible, and avoid repeated freeze–thaw cycles.

2) Centrifuge at full speed for 3 minutes and carefully transfer the supernatant to a new tube with a pipette.

3) Proceed immediately to **step 3. Binding** of "**Protocol Ⅰ: mRNA Isolation from Cultured Cells**" on **page 13**.

Protocol Ⅲ: Removal of rRNA Contamination

This protocol is designed to improve the purity of mRNA by re-binding the eluted mRNA to Magnetic Nano Bead-Oligo dT after the initial isolation step, thereby removing non-specific RNA contaminants.

1. Preparation for Rebinding

1) Immediately transfer the eluted mRNA to a new RNase-free tube and keep it on ice to minimize degradation.

2) Keep the used beads in the original tube for the rebinding step.

2. Bead Washing

1) Add 200 µL of MRW2 Buffer and resuspend the beads thoroughly by pipetting.

2) Place the tube on the EcoQprep™ Magnetic Separation Rack, attach the magnet plate to the stand, and gently invert the rack 3–4 times until the beads bind tightly to the magnet.

3) Discard the supernatant carefully without removing the tube from EcoQprep™ Magnetic Separation Rack.

4) Detach the magnet plate from EcoQprep™ Magnetic Separation Rack.

5) Repeat this step2. Bead Washing once more.

3. Rebinding of mRNA

1) Dilute the eluted mRNA by adding 4 volumes of MRD Buffer.

* **Note**: Prepare MRD Buffer freshly before use by adding 1 µL of β-mercaptoethanol per mL.

2) Add the diluted mRNA solution to the washed beads prepared in step 2.

3) Remove the supernatant by repeating steps 2-2) to 2-3).

4) Proceed immediately to **step 4. 1st Washing** of "**Protocol Ⅰ: mRNA Isolation from Cultured Cells**" on **page 14**.

Protocol Ⅳ: Reuse of Magnetic Nano Bead-Oligo dT

Magnetic Nano Bead-Oligo dT can be reused for multiple isolations from the same sample after mRNA elution, allowing stable reuse up to four times by efficiently removing residual mRNA bound to the oligo dT. However, if a decline in mRNA binding or elution efficiency is observed during reuse, it is recommended to discontinue further use.

1. Removal of residual mRNA

1) To resuspend the bead pellet, add 100 µL of one of the following:

A. EM Buffer

B. RNase-free water

C. For strong elution, prepare a buffer containing 8 M Urea, 10 mM Tris-HCl (pH 7.5), and 0.01% Tween 20.

*** Note:** It is recommended to freshly prepare this buffer for each experiment.

2) Incubate at 75°C for 3-5 minutes to dissociate mRNA from the beads.

3) Mix thoroughly by vortexing or pipetting.

4) Place the tube on the EcoQprep™ Magnetic Separation Rack, attach the magnet plate to the stand, and gently invert the rack 3–4 times until the beads bind tightly to the magnet.

5) Discard the supernatant carefully without removing the tube from EcoQprep™ Magnetic Separation Rack.

6) Detach the magnet plate from EcoQprep™ Magnetic Separation Rack.

2. Washing

1) Add 200 µL of the same solution used in step 1-1) and resuspend the beads thoroughly by vortexing.

2) Remove the supernatant by repeating steps 1-4) to 1-6).

3) Repeat the wash once more using the same buffer.

4) Wash the beads twice with 100 µL of MRD Buffer, each time resuspending by vortexing and removing the supernatant as described in steps 1-4) to 1-6).

* **Note**: Prepare MRD Buffer freshly before use by adding 1 µL of β-mercaptoethanol per 1 mL of buffer.

3. Storage

1) For storage before use, keep the beads in EM Buffer. Optionally, 0.05% Tween 20 can be added to enhance stability.

2) Store under refrigerated condition (2-8°C) in an RNase-free environment.

Troubleshooting

Problem	Comments
Low mRNA yield	• **Buffers or other reagents may have been exposed to conditions that reduce their effectiveness.** Store the reagents at room temperature (15-35°C) at all times. Once opened, it is recommended to store the reagents at 2-8°C. Keep all reagent bottles tightly closed after use to preserve pH and stability and avoid contamination.
	• **Excess amount of starting sample may have been used for mRNA isolation.** Appropriate amount of starting sample should be used for efficient RNA extraction. If lysis is incomplete due to an excessive sample amount, add more MRD Buffer to improve lysis efficiency. Alternatively, increasing the bead volume to 100 µL can enhance binding efficiency.
	• **Cell culture medium may have been incomplete.** Remove the cell culture medium as much as possible. Any leftover in the medium can lead to an inhibition of RNA extraction
	• **Insufficient lysed during lysis step may lead to low RNA yield.** Shake or mix thoroughly by pipetting during lysis step. Repeat the homogenization step for tissues.
	• **Insufficient Hybridization in binding step.** After lysis of sample, incubate the lysate and bead mixture at least for 5 minutes and ensure proper mixing by rotating.
	• **Improving efficiency by re-binding** To improve mRNA yield, reuse the retained lysate (unbound) with the used beads and repeat the binding

	process of mRNA Isolation protocol.
	• **Elution may have been incomplete.** Please expand the incubation time up at elution step (up to 5 min). In addition, make sure that Magnetic Nano Beads are suspended completely in the eluting solution during incubation.
	• **Re-binding of mRNA to beads.** If the beads and mRNA eluent are not separated immediately after elution, mRNA may re-bind to the beads, leading to reduced yield. To prevent this, promptly separate the mRNA eluent from the beads after the elution step.
Degraded mRNA	• **There may have been RNase contamination.** Use a heat gun or blow dryer in a clean bench to prevent the RNase contamination in the air. Use RNase-free pipette tips and change the gloves frequently.
	• **The sample may have been stored inappropriately.** Cultured cell samples and tissue samples should be stored at -80°C.
	• **Repeated freezing and thawing may degrade RNA.** Avoid repeated freezing and thawing.
Contaminated with DNA	• **Incomplete removal of sample lysate or wash buffers.** Completely remove all lysate and wash buffers during magnetic separation.
	• **Insufficient washing.** Make sure the beads bound with mRNA are fully resuspended during each washing step.

Appendix

Measurement of absorbance of RNA samples

The A_{260}/A_{280} ratio is a commonly used criterion for nucleic acid purity. Values for pure RNA are usually >2.0. However, the absorbance of nucleic acids at these wavelengths is dependent upon the ionic strength and pH of the medium. The change in the A_{260}/A_{280} ratio is primarily due to the decrease in the absorbance at 280 nm when the ionic strength or pH is increased. We recommend that diluting the RNA with a DEPC-DW for spectrophotometric assays

1. Measure the volume of the total RNA sample.

2. Transfer 1 µL of total RNA sample to a 1.5 mL tube

3. Add 999 µL of DEPC-DW and mix by pipetting

4. Measure A_{260} and A_{280} with DEPC-DW as a reference blank.

5. Calculate RNA yield as follows:
   1 A_{260} unit of RNA = 40 µg/µL
   Total A_{260} = (A_{260} of diluted sample) x (dilution factor)
   Concentration (µg/mL) = (total A_{260}) x (40 µg/µL)
   Yield (µg) = (total sample volume) x (concentration)

6. Calculate the A_{260}/A_{280} ratio. Pure RNA exhibits A_{260}/A_{280} > 2.0

References

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Ordering Information

Description		Cat. No.
EcoQprep™ mRNA Kit	50 reactions	K-3708

Related Products

Description	Cat. No.
EcoQprep™ Magnetic Separation Rack (2 mL)	TM-1012
EcoQprep™ Magnetic Separation Rack (15 mL)	TM-1021
EcoQprep™ Magnetic Separation Rack (50 mL)	TM-1031

Explanation of Symbols

LOT Batch Code	Consult Instructions For Use	RUO Research Use Only	Caution
Do not Re-use	Contains Sufficient for <n> tests	Temperature Limitation	Manufacturer
REF Catalog Number	Use-by Date		

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