E.Z.N.A.® PF Micro RNA Kit

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OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

Majority of the current commercial products for isolating miRNA involve organic extraction (most commonly phenol-based) in the procedure. E.Z.N.A.® PF Micro RNA Kit uses an innovative buffer system that completely eliminate the use of phenol extraction. This product provides a rapid and easy method for the isolation of up to 50 µg of small and large-size RNA from cultured eukaryotic cells or bacteria, or from animal, plant, or fungal tissues. Single or multiple samples can be simultaneously processed in less than 30 minutes. Typically, up to 1 x 10^6 eukaryotic cells, 1 x 10^9 bacterial cells, 50 mg animal tissue, or 100 mg plant tissue can be used in a single experiment.

Overview

The E.Z.N.A.® PF Micro RNA Kit combines the reversible binding properties of HiBind® matrix, a silica-based material, with the unique lysis and binding procedure to extract micro and large (>200 nt) RNA from a wide variety of starting materials. A specially formulated high-salt lysis and binding buffer system allows more than 100 µg RNA to bind to the matrix. Cells or tissues are first homogenized with NTL buffer that lyses the cells and inactivates RNases. After adjusting condition with XD Binding Buffer, the lysate is loaded to a filter column to remove cell debris and other contaminants. The flow-through liquid, which contains the miRNA, is mixed with ethanol and loaded onto a HiBind® Micro RNA Column to bind the miRNA. After a few quick wash steps, the miRNA can be eluted from the HiBind® Micro RNA Column with nuclease-free water.

New In this Edition

- The HiBind® Micro RNA Column has been redesigned to increase DNA recovery by reducing the HiBind® matrix retention volume.

Kit Contents

Product	R7036-00	R7036-01	R7036-02
Purifications	5 preps	50 preps	200 preps
HiBind® X-Press Column	5	50	200
HiBind® Micro RNA Column	5	50	200
2 mL Collection Tubes	10	100	400
NTL Lysis Buffer	3 mL	30 mL	120 mL
XD Binding Buffer	5 mL	50 mL	200 mL
miRNA Binding Enhancer	100 µL	1 mL	4 mL
RNA Wash Buffer II	5 mL	12 mL	50 mL
DEPC Water	1 mL	3 mL	10 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® PF Micro RNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature.

Preparing Reagents

Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
R7036-00	20 mL
R7036-01	48 mL
R7036-02	200 mL

Important Notes

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.

- Prepare all materials required before starting the procedure to minimize RNA degradation.

- Carefully apply the sample or solution to the center of the HiBind® Columns. Avoid touching the membrane with pipet tips.

Starting Materials

Although the binding capacity for the HiBind® Micro RNA Columns are approximately 100 µg, the maximum amount of starting material depends on the type of tissue being processed and its corresponding RNA content. It is essential to begin with the correct amount of tissue to get optimal RNA yield and purity with the E.Z.N.A.® PF Micro RNA Kit. For the first time user, we recommend using less than 30 mg of tissue per sample. Depending on the yield and purity obtained, it may be possible to increase the starting material up to 100 mg (maximum amount).

Tissue Homogenization Protocols

Efficient sample disruption and homogenization is essential for successful RNA isolation. Cell wall and plasma membrane disruption is necessary for the release of RNA from the sample and homogenization is necessary to reduce the viscosity of the lysates. Homogenization shears genomic DNA and other high-molecular-weight cell components creating a homogenous lysate. Incomplete homogenization can cause the HiBind® Micro RNA Column to clog resulting in low or no yield.

Liquid Nitrogen Method - Recommended

- Wear appropriate gloves and take great care when working with liquid nitrogen.
- Excise tissue and promptly freeze in a small volume of liquid nitrogen.
- Grind tissue with a ceramic mortar and pestle under approximately 10 mL liquid nitrogen.
- Pour the suspension into a pre-cooled 15 mL polypropylene tube.

  Note: Unless the tube is pre-cooled in liquid nitrogen, the suspension will boil vigorously and may cause loss of tissue.
- Once the liquid nitrogen has completely evaporated, continue to Step 1 of the "miRNA from Cells and Tissue Protocol" on Page 7.

Rotor-stator Homogenizers

Rotor-stator homogenizers effectively homogenize most tissues. The process usually takes less than a minute depending on the tissue. Many rotor-stator homogenizers operate with differently sized probes or generators that allow processing of small volumes in microcentrifuge tubes. Such homogenizers are available from:

- Tekmar Inc., Cincinnati, OH (Tissuemizers®)
- BIOSPEC Products, Bartlesville, OK (Tissue-Tearor™)
- Craven Laboratories, Austin, TX.

Syringe Method

High-molecular-weight DNA is responsible for the viscosity of cell lysates and can be shredded by passing the sample through a 19-21 gauge needle several times.

E.Z.N.A.® PF Micro RNA Kit Protocol

Materials and Equipment to be Supplied by User:

- 100% Ethanol
- 2-Mercaptoethanol
- RNase-free filter pipette tips
- 1.5 or 2 mL nuclease-free microcentrifuge tubes
- Microcentrifuge capable of 13,000 x *g* and 4°C
- Ice Bucket

Before Starting:

- Prepare RNA Wash Buffer II according to the instructions in the "Preparing Reagents" section on Page 4.
- Prepare an Ice Bucket
- Prepare Lysis Mixture according to the following table (per sample):

Components	Volume
NTL Lysis Buffer	0.5 mL
miRNA Binding Enhancer	15 µL
2-Mercaptoethanol	10 µL

1. Lyse cells or tissue with 0.5 mL Lysis Mixture (see above preparation table) following one of the steps below:

   A. For cultured cells grown in monolayer (fibroblasts, endothelial cells, etc.) lyse the cells directly in the culture vessel as follows:
      1. Aspirate and discard the culture medium.
      2. Add 0.5 mL Lysis Mixture directly to the cells making sure to cover the entire surface of the vessel to ensure complete lysis.
      3. Transfer the lysate to a clean 2 mL microcentrifuge tube.
      4. Proceed to Step 2.

      Note: This method is preferable to trypsinization followed by washing because it minimizes RNA degradation by nuclease contamination.

B. For cells grown in suspension cultures:
   1. Pellet cells at no greater than 1,500 rpm (400 x *g*) for 5 minutes.
   2. Discard the supernatant.
   3. Add 0.5 mL Lysis Mixture.
   4. Vortex or pipette up and down to lyse the cells.
   5. Transfer the lysate to a clean 2 mL microcentrifuge tube.
   6. Proceed to Step 2.

C. For tissue samples, determine the size of the samples and homogenize by using one of the methods discussed on Page 6. Unless using liquid nitrogen, homogenize samples directly in 0.5 mL Lysis Mixture and proceed to Step 2.

D. Bacterial cells: Collect bacteria grown to log-phase by centrifugation, add 50 µL Lysozyme (15 mg/mL in TE) and incubate 10 minutes at 37°C. Add 450 µL Lysis Mixture and vortex for 1 minute. Proceed to Step 2.

2. Incubate at room temperature for 3-5 minutes.

3. Add 250 µL XD Binding Buffer. Cap the tubes securely and vortex vigorously for 15 seconds.

4. Incubate on ice for 10 minutes.

5. Centrifuge at 13,000 x *g* at 4°C for 15 minutes.

6. Insert a HiBind® X-Press Column into a 2 mL Collection Tube provided with this kit.

7. Transfer entire supernatant from Step 5 to the HiBind® X-Press Column.

8. Centrifuge at 13,000 x *g* at room temperature for 1 minute.

9. Measure the volume of the filtrate collected and transfer the filtrate to a new 2 mL microcentrifuge tube.

10. Add 1.2 volumes of ethanol to the filtrate. Vortex 20 seconds to mix thoroughly.

11. Insert a HiBind® Micro RNA Column into a clean 2 mL Collection Tube provided with this kit.

12. Transfer 700 µL of the mixture from Step 10 to the HiBind® Micro RNA Column.

13. Centrifuge at 13,000 x *g* for 1 minute. Discard the filtrate and reuse the Collection Tube.

14. Repeat Steps 12-13 until all the remaining sample has been transferred to the HiBind® Micro RNA Column.

15. Add 500 µL 100% ethanol to the HiBind® Micro RNA Column.

16. Centrifuge at 13,000 x *g* for 1 minute. Discard the filtrate and reuse the Collection Tube.

17. Add 500 µL XD Binding Buffer to the HiBind® Micro RNA Column.

18. Centrifuge at 13,000 x *g* for 1 minute. Discard the filtrate and reuse the Collection Tube.

19. Add 500 µL RNA Wash Buffer II to the HiBind® Micro RNA Column.

    Note: RNA Wash Buffer II must be diluted with ethanol before use. Please see Page 4 for instructions.

20. Centrifuge at 13,000 x *g* for 1 minute. Discard the filtrate and reuse the Collection Tube.

21. Repeat Steps 19-20 for a second RNA Wash Buffer II wash step.

22. Centrifuge at 13,000 x *g* for 2 minutes to completely dry the HiBind® matrix.

    Note: It is important to dry the HiBind® Micro RNA matrix before elution. Residual ethanol may interfere with downstream applications.

23. Transfer the HiBind® Micro RNA Column to a clean 1.5 or 2 mL microcentrifuge tube.

24. Add 15-30 µL DEPC Water.

    Note: Make sure to add DEPC Water directly onto the HiBind® Micro RNA Column matrix.

25. Incubate at room temperature for 2 minutes.

26. Centrifuge at 13,000 x *g* for 1 minute. Store eluted RNA at -70°C.

    Note: Any combination of the following steps can be used to help increase RNA yield.
    - Preheat the DEPC Water to 70°C before elution.
    - Increase the incubation time to 5-10 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh DEPC Water (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **800-832-8896**.

Problem	Cause	Solution
Little or no RNA eluted	RNA remains on the column	• Repeat the elution step. • Preheat DEPC Water to 70°C prior to elution. • Increase the incubation time to 10 minutes.
	Column is overloaded	• Reduce the amount of starting material.
Clogged column	Incomplete homogenization	• Completely homogenize sample. • Increase centrifugation time. • Reduce the amount of starting material.
Degraded RNA	Source	• Quickly freeze starting material in liquid nitrogen. • Do not store tissue culture cells prior to extraction unless they are lysed first. • Follow the protocol closely and work quickly.
	RNase contamination	• Ensure not to introduce RNase during the procedure. • Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	• Ensure RNA Wash Buffer II has been diluted with 100% ethanol as indicated in the "Preparing Reagents" section on Page 4. • RNA Wash Buffer II must be stored and used at room temperature. • Repeat the RNA Wash Buffer II wash step.
DNA contamination		• Digest the RNA with RNase-free DNase and inactivate at 75°C for 5 minutes.
Low absorbance ratios	RNA diluted in acidic buffer or Water	• DEPC Water is acidic and can dramatically lower A_{260} values. • Use TE buffer to dilute RNA prior to spectrophotometric analysis.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Products	Part Number
DEPC Water, 100 mL	PR032
RNA-Solv® Reagent	R6830
RNase-free DNase Set	E1091
2 mL DNase/RNase-free Microcentrifuge Tubes, 500/pk, 10 pk/cs	SSI-1310-00