GENETIX BRAND

HANDBOOK

Nucleo-pore®

RNASure® Fusion miRNA Mini Kit

☐ **RNASure® Fusion miRNA Mini Kit** NP - 051523 50 Preps

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Nucleo-pore®

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COMPONENTS

Kit contents

Cat. No.	NP-051523	
Components	**Quantity**	**Storage**
Buffer ER	30 ml	4ºC
Buffer Sw1	30 ml	Room temperature (15~25°C)
Buffer RBW	30 ml	
Buffer RNW	110 ml	
RNase-free water	15 ml	
Spin Column Type B (red ring) w/ Collection Tube	50	
Spin Column Type W (blue ring) w/ Collection Tube	50	
Collection Tube 2.0ml	50	
Collection Tube 1.5ml	100	

Material Not Provided

Reagent

- 100 % ethanol, ACS grade or better
- Equipment for homogenizing solid tissue
- Chloroform or 1-bromo-3-chloropropane (BCP)

Disposable material

- RNase-free pipet tips
- Disposable gloves

Equipment

- Equipment for homogenizing solid tissue
- Microcentrifuge for centrifugation at 4°C and at room temperature
- Suitable protector (ex; lab coat, disposable gloves, goggles, etc)

Quality Control

RNASure® Fusion miRNA Kit is manufactured in strictly clean condition, and its degree of cleanness is monitored periodically. For consistency of product, the quality certification process is carried out from lot to lot thoroughly and only the qualified is approved to be delivered.

Storage condition

RNASure® Fusion miRNA Kit except Buffer ER should be stored at room temperature. Buffer ER should be stored at 4°C for optimal performance. All components are stable for 1 year.

User Precautions

Buffer ER contains phenol which is poisonous and guanidine salt which is an irritant. When working with RNASure® Fusion miRNA, use gloves and eye protector to avoid contact with skin or clothing and inhalation of vapor. In case of contact, wash immediately with plenty of water and seek medical advice.

RNASure® Fusion miRNA Mini Kit

Type	Spin
Maximum amount of starting samples	~100 mg or ~ 1x 107 cells
Maximum loading volume	~ 700μl
Minimum elution volume	~ 700μl
Maximum binding capacity	100 μg

Preventing RNase contamination

RNase can be introduced accidentally into a RNA preparation. Wear disposable gloves always, because skin often contains bacteria that can be a source of RNase. Use sterile, disposable plasticwares and automatic pipettes reserved for RNA work to prevent cross-contamination with RNase on shared equipment.

Product Description

RNASure® Fusion miRNA is designed for purification of large and small RNA separately from culture cells or animal tissues, and co-purification in a single tube is also available by modified protocol. This kit utilizes the lysis method at Buffer ER which has a powerful ability of lysis and the purification method based on glassfiber membrane technology.

Samples are homogenized in Buffer ER, a monophasic solution containing phenol and guanidium salt, which rapidly lyse cells and inactivates nucleases. Addition of chloroform brings about a separation of the lysate into aqueous and organic phases. Total RNA locates in the aqueous phase while DNA and protein remain in the interphase and organic phase. Large and small RNA in the aqueous phase is selectively bound to column type B and type W respectively. The column type B selectively adsorbs the RNA larger than 200 nt in length, while the column type W specifically holds the RNA smaller than 200 nt in length. To purify large RNA, the aqueous phase is mixed with ethanol and the mixture is applied to a column type B. After centrifugation, large RNA is bound to membrane and the mixture containing small RNA goes into collection tube through the membrane. The membrane is washed away by two wash buffer (SW1 and RNW) and purified large RNA is eluted from the membrane by RNase-free water. To purify small RNA, the pass-through come from the binding of large RNA is mixed with ethanol and then applied to a column type W. After washing with buffer RBW and RNW, small RNA is eluted by RNase-free water. The procedure of Hybrid-RTM miRNA takes only 30 minutes for complete preparations of pure RNA. The purified RNA is suitable for the isolation of Poly A+ RNA, Northern blotting, dot blotting, in vitro translation, cloning, RT-PCR, RPA and other analytical procedures.

RNASure® Fusion Mini Kit

Protocol for large RNA and small RNA isolation

1. **Homogenize ~ 50 mg tissue samples in 500µl Buffer ER . Homogenize ~ 1 x 107 cells in 500 ul Buffer ER.**

   ### Tissue samples

   Basically, do not use more than 50 mg tissue per 0.5 ml Buffer ER. Exceptionally for adipose tissue, up to 100 mg can be used.

   - Handling fresh tissue

   Immediately after dissection, inactivate RNases by any one of the following treatments.

   * Homogenize in Buffer ER immediately.
   * Freeze rapidly in liquid nitrogen.
   * Submerge in a tissue storage buffer to protect RNA from Rnases.

   ### Cell samples

   Cells grown in Monolayer

   Pour off media, add 500 ul of Buffer ER per 10 cm2 of culture dish area. Pass the cell lysate several times through a pipette. An insufficient amount of Buffer ER may result in contamination of the isolated RNA with DNA.

   Cells grown in suspension

   Pellet cells by centrifugation, then lyse in 500 ul of Buffer ER per

   ~ 1 x 107 cultured cells by repetitive pipetting or vortexing.

   * Do not wash cells before lysing with Buffer ER as this may contribute to mRNA degradation.

2. **Incubate the homogenate for 5 minutes at room temperature.**

   This step allows nucleoprotein complexes to completely dissociate.

   Homogenized samples can be stored at -70°C for at least one month.

3. **(Optional:) Centrifuge at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to a fresh tube.**

   This optional step is required only for homogenate with high contents of proteins, fats, polysaccharides or extracellular materials, such as muscles, fat, tissue, and tuberous parts of plants.

   The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA. Fat tissue samples will form a layer on top of the aqueous phase. It should be removed and discarded.

4. **Add 100 ul of chloroform per 500 ul of Buffer ER Reagent. Shake vigorously for 15 seconds and store for 2 minutes at room temperature.**
   Alternatively, 0.05 ml of BCP (1-bromo-3-chloropropane) can be used in place of chloroform.

5. **Centrifuge at 12,000 x g for 15 minutes at 4°C and transfer the aqueous phase to a fresh tube.**
   The mixture will be separated into three phases; a lower layer, an interphase, and a colorless upper aqueous layer. The upper aqueous layer is about 50% of the volume of Buffer ER used for homogenization.
   Centrifugation at over 8°C may cause some DNA to intrude in the aqueous phase.

6. **Add 1 volume of 50% ethanol to the sample and mix thoroughly by inverting. Do not centrifuge.**

7. **Transfer upto 700 ul of the mixture to a mini spin column (type B, red ring).**

8. **Centrifuge at 10,000 x g for 30 seconds at room temperature. Transfer the column to a new 2 ml collection tube (provided), and store at room temperature. Use the passed-through for small (micro) RNA purification.**
   Make sure that no mixture remains in the column after centrifugation. If the residual mixture has remained, centrifuge again at higher speed until all of the solution has passed through. After this step, large RNA bind to mini spin column (type B, red ring) and small (micro) RNA exist in the flow-through.

   Go on to step 9 for small RNA purification.
   Go on to step 21 for large RNA purification.

   Small (micro) RNA purification (Blue ring column)

9. **Add 1 volume of 100% ethanol to the collection tube including passed-through, and mix well by pipetting. Do not centrifuge.**

10. **Transfer 650 ul of the mixture including any precipitate to a mini spin column (type W, blue ring).**

11. **Centrifuge at 10,000 x g for 30 seconds at room temperature.**
    Discard the flow-through and reinsert the mini spin column back into the same tube.

12. **Repeat step 10 ~ 11 using the remainder of the sample.**

13. **Add 500 ul of buffer RBW to the mini spin column.**

14. **Centrifuge at 10,000 x g for 30 seconds at room temperature.**
    Discard the flow-through and reinsert the mini spin column back into the same tube.

15. **Add 500 ul of buffer RNW to the mini spin column.**

16. **Centrifuge at 10,000 x g for 30 seconds at room temperature.**
    Discard the flow-through and reinsert the mini spin column back into the same tube.

17. **Repeat step 15 ~ 16 once more.**

18. **Centrifuge at 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin column to a new 1.5 ml collection tube (provided).**
    Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of buffer RNW.

19. **Add 50 ul of RNase-free water to the center of the membrane in the mini spin column.**
    According to the expected yield, elution volume can be adjusted.

20. **Centrifuge at 10,000 x g for 1 minute at room temperature.**
    Purified small RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.

Large RNA purification (Red ring column)

21. **Add 500 ul of buffer SW1 to the mini spin column (type B, red ring).**

22. **Centrifuge at 10,000 x g for 30 seconds at room temperature.**
    Discard the pass-through and reinsert the mini spin column back into the same tube.

23. **Add 500 ul of buffer RNW to the mini spin column.**

24. **Centrifuge at 10,000 x g for 30 seconds at room temperature.**
    Discard the pass-through and reinsert the mini spin column back into the same tube.

25. **Repeat step 23 ~ 24 once more.**

26. **Centrifuge at 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin column to a new 1.5 ml collection tube (provided).**
    Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of buffer RNW.

27. **Add 50 ul of RNase-free water to the center of the membrane in the mini spin column.**
    According to the expected yield, elution volume can be adjusted.

28. **Centrifuge at 10,000 x g for 1 minute at room temperature.**
    Purified large RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.

RNASure® Fusion Mini Kit

Supplementary Protocol for Co-purification of total RNA

(Large and Small RNA)

This modified protocol allows co-purification of large and small RNA.
For the purification of total RNA, separated aqueous phase is mixed with ethanol and then the mixture is applied to column type W. Through this simple steps, total RNA is bound to the membrane. After washing steps, total RNA can be eluted by nuclease-free water.

Protocol for simultaneous purification of large RNA and small RNA from cell samples.

1. **Homogenize ~ 50 mg tissue samples in 500 ul Buffer ER. Homogenize ~ 1 x 10^7 cells in 500 ul Buffer ER Reagent.**

   Tissue samples

   Basically, do not use more than 50 mg tissue per 0.5 ml Buffer ER. Exceptionally for adipose tissue, up to 100 mg can be used.
   - *Handling fresh tissue*
   Immediately after dissection, inactivate RNases by any one of the following treatments.
   * Homogenize in Buffer ER immediately.
   * Freeze rapidly in liquid nitrogen.
   * Submerge in a tissue storage buffer to protect RNA from Rnases.

   Cell samples

   Cells grown in Monolayer
   Pour off media, add 500 ul of Buffer ER per 10 cm2 of culture dish area. Pass the cell lysate several times through a pipette. An insufficient amount of BufferER may result in contamination of the isolated RNA with DNA.
   Cells grown in suspension
   Pellet cells by centrifugation, then lyse in 500 μl of Buffer Erper
   ~ 1 x 10^7 cultured cells by repetitive pipetting or vortexing.
   * Do not wash cells before lysing with Buffer ER as this may contribute to mRNA degradation.

2. **Incubate the homogenate for 5 minutes at room temperature.**
   This step allows nucleoprotein complexes to completely dissociate.
   Homogenized samples can be stored at -70°C for at least one month.

3. **(Optional :) Centrifuge at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to a fresh tube.**
   This optional step is required only for homogenate with high contents of proteins, fats, polysaccharides or extracellular materials, such as muscles, fat, tissue, and tuberous parts of plants.
   The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA.
   Fat tissue samples will form a layer on top of the aqueous phase. It should be removed and discarded.

4. **Add 100 ul of chloroform per 500 ul of Buffer ER. Shake vigorously for 15 seconds and store for 2 minutes at room temperature.**
   Alternatively, 0.05 ml of BCP (1-bromo-3-chloropropane) can be used in place of chloroform.

5. **Centrifuge at 12,000 x g for 15 minutes at 4°C and transfer the aqueous phase to a fresh tube.**
   The mixture will be separated into three phases; a lower layer, an interphase, and a colorless upper aqueous layer. The upper aqueous layer is about 50% of the volume of Buffer ER used for homogenization.
   Centrifugation at over 8°C may cause some DNA to intrude in the aqueous phase.

6. **Add 1.5 volume of 100% ethanol to the sample and mix thoroughly by inverting. Do not centrifuge.**

7. **Transfer upto 700 ul of the mixture to a spin column (type W, blue ring).**

8. **Centrifuge at 10,000 x g for 30 seconds at room temperature.**
   Discard the pass-through and reinsert the mini spin column back into the same tube.

9. **Repeat step 7 ~ 8 using the remainder of the sample.**

10. **Add 500 ul of buffer RBW to the spin column.**

11. **Centrifuge at > 10,000 x g for 30 seconds at room temperature.**
    Discard the flow-through and reinsert the spin column back into the same tube.

12. **Add 500 ul of buffer RNW to the mini spin column.**

13. **Centrifuge at > 10,000 x g for 30 seconds at room temperature.**
    Discard the flow-through and reinsert the mini spin column back into the same tube.

14. **Repeat step 12 ~ 13 once more.**

15. **Centrifuge at 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer. Transfer the mini spin column to a new 1.5 ml collection tube (provided).**
    Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of buffer RNW.

16. **Add 50 ul of RNase-free water to the center of the membrane in the mini spin column.**
    According to the expected yield, an appropriate elution volume can be applied on the membrane.

17. **Centrifuge at 10,000 x g for 1 minute at room temperature.**
    Purified small RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.

TROUBLESHOOTING GUIDE

Low yield of RNA

Possible cause
- Poor quality of starting material

Suggestion
- Process the sample immediately after harvest from animal.
  Thaw the frozen sample directly in Buffer ER

Possible cause
- Sample not homogenized completely

Suggestions
- Make sure no particulate matter remains. Be sure to incubate for 5 minutes at room temperature after homogenization.

Possible cause
- Some aqueous phase left

Suggestions
- Perform second extraction with the remaining aqueous phase.

Possible cause
- Incorrect elution conditions

Suggestions
- Add RNase-free water to the center of the mini spin column membrane.

Degradation of RNA

Possible cause
- Sample manipulated too much before the addition of Buffer ER

Suggestion
- Process the sample immediately after harvest from animal.
  For cultured cell, minimize washing steps. Add Buffer ER directly to plates. Do not trypsinize cells.

Possible cause
- Improper storage of RNA

Suggestions
- Store isolated RNA at -70°C, Do not store at -20°C.

Possible cause
- Reagent or disposable is not RNase-free

Suggestions
- Make sure to use RNase free products only.

TROUBLESHOOTING GUIDE

Low $A_{260/280}$ (<1.6)

Possible cause
- Aqueous phase was contaminated with the phenol phase

Suggestion
- Avoid carryover when transferring the aqueous phase to a fresh tube.

Possible cause
- Sample not completely homogenized with Buffer ER

Suggestions
- Use 0.5 ml Buffer ER for up to 50 mg tissue or up to 10^6 cells.

Contamination of DNA

Possible cause
- The interphase was co-transfered by mistake

Suggestion
- Be sure not to transfer any of the interphase (containing DNA) to the aqueous phase.

Possible cause
- Insufficient Buffer ER used

Suggestions
- Use 0.5 ml Buffer ER for 50 mg tissue or 10^7 cells.

Possible cause
- Temperature was too high during centrifugation

Suggestions
- The phase separation should be performed at 4°C to allow optimal separating and removal of genomic DNA from the aqueous phase.

Cells not detached completely from flask after addition of Buffer ER Reagent

Possible cause
- This can be seen with some strongly adherent cells

Suggestion
- After addition of Buffer ER, let cells sit 2 to 3 minutes. Scrape cells with a scraper. Incubate for several minutes. Collect and repeatedly pipette cells over flask surface. Then transfer homogenate to a tube.

TROUBLESHOOTING GUIDE

The yield of miRNA is too low or miRNA do not separate completely

Possible cause

- Incorrect binding step

Suggestion

- Be sure to use the proper concentrations of ethanol at binding step. 50% ethanol should be used for the large RNA preparation step then 100% ethanol should be taken for the small RNA.

Possible cause

- Too much starting sample

Suggestions

- Use 0.5 ml Buffer ER for 50 mg tissue or 10^7 cells.

RNA does not perform well in downstream application

Possible cause

- Residual ethanol remains in eluate

Suggestion

- Centrifuge again to remove any residual ethanol included in buffer RNW from mini spin column membrane (step 14)

ORDERING INFORMATION

Description	Pack Size	Cat. No.
DNASure® Tissue Mini Kit	50 preps	NP-61305
DNASure® Plant Mini Kit	50 preps	NP-79105
DNASure® Plant Mini Kit	250 preps	NP-79107
DNASure® Plant Midi Kit	20 preps	NP-78153
DNASure® Plant Maxi Kit	10 preps	NP-78164
DNASure® Blood Mini Kit	50 preps	NP-61105
DNASure® Blood Mini Kit	250 preps	NP-61107
DNASure® Blood Midi Kit	20 preps	NP-61184
DNASure® Blood Maxi Kit	10 preps	NP-61193
DNASure® Blood FastPure Kit	50 preps	NP-62205
DNASure® Blood FastPure Kit	250 preps	NP-62207
SureSpin® Plasmid Mini Kit	50 preps	NP-37105
SureSpin® Plasmid Mini Kit	250 preps	NP-37107
SureSpin® Plasmid FastPrep Kit	50 preps	NP-47105
SureSpin® Plasmid FastPrep Kit	250 preps	NP-47107
SureSpin® Buffer Set*	1	37107-BS
SurePrep® Plasmid Mini Kit	20 preps	NP-15123
SurePrep® Plasmid Mini Kit	100 preps	NP-15125
SurePrep® Plasmid Midi Kit	20 preps	NP-15143
SurePrep® Plasmid Midi Kit	100 preps	NP-15145
SurePrep® Plasmid Maxi Kit	10 preps	NP-15161
SurePrep® Plasmid Maxi Kit	25 preps	NP-15162
SurePrep® Plasmid Mega Kit	5 preps	NP-15183
SurePrep® Plasmid Giga Kit	5 preps	NP-15191

***SureSpin® Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

****SureSpin® Buffer Set**

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

ORDERING INFORMATION

Description	Pack Size	Cat. No.
Nucleo-pore® Stool DNA Mini Kit	50	NP-7011D
Nucleo-pore® gRNA Blood Kit	50	NP-0201R
Nucleo-pore® gDNA Urine Kit	20	NP-6030D
Nucleo-pore® Yeast Transformation Kit	120	NP-1002T
Nucleo-pore® DNA Methylation Kit	50	NP-6006D
Nucleo-pore® gDNA Clean-up Kit	200	NP-4304D
Nucleo-pore® Bisulphite DNA Clean-up Kit	50	NP-5205D
Nucleo-pore® gDNA Fungal/Bacterial Mini Kit	50	NP-7006D

Product Warranty

RNASure® Fusion miRNA Mini Kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the RNASure® Fusion miRNA Mini Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of RNASure® Fusion miRNA Mini Kit is tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

Product claims are subject to change. Therefore please contact our Technical Support Department for updated information on Genetix products.

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