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Version: 01

mi-Total RNA Miniprep Kit

Cat. No. mi-RNATO250

Purification of small-scale RNA using phenol/chloroform extraction or ethanol precipitation is laborious and time-consuming. ***meta*bion**'s **mi-Total RNA Miniprep Kit** provides a simple and fast method (average prep time 30 min) to extract and isolate total RNA (longer than 200 bases) from cultured cells, animal tissues and bacteria without using phenol/chloroform.

Small RNAs such as 5.8S RNA, 5S RNA and tRNA, which make up 15-20% of the total RNA, are excluded. This system is based on binding of up to 100µg DNA to silica-based membranes in chaotropic salts with average recovery rates of 50-80%.

Downstream Applications

- RT-PCR and real-time PCR
- cDNA synthesis
- Northern, dot and slot blot analyses
- PolyA+ RNA selection
- Differential display
- Primer extension
- RNAse/S1 nuclease protection
- RNA-Sequencing

Product Contents

Cat. No.	mi-RNATO250
Preps	250
RX Buffer	200ml
WF Buffer (RNA)	150ml
WS Buffer (RNA)	45ml
RNAse-free ddH_20	15ml
RNA Mini Column	250
Elution Tube	250
Protocol	1

Storage Conditions

***meta*bion**'s mi-Total RNA Miniprep Kit can be stored at room temperature up to 12 months. If precipitate forms in any buffer or due to low temperatures, incubate at 37°C for 30 minutes to resuspend.

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Protocol

Please read the following notes before starting the procedures. Perform all steps at room temperature!

WARNING: Don´t use strong acids and/or oxidants together with RX buffer (cyanide generation)!

Important Notes

- All buffers need to be mixed well before use!
- Buffers provided in this system contain irritants. Wear appropriate safety apparels such as gloves and lab coat.
- All plasticware and containers should be treated properly to ensure they are RNAse free (see appendix "**VI. Working with RNA – Some Handling Guidelines**").
- All centrifugation steps should be performed at 10,000 x g or 13,000rpm in a microcentrifuge, unless noted otherwise.
- A small amount of genomic DNA will co-purify with the RNA. Therefore, DNAse treatment is required for DNA-free RNA.
- Remove DNAse by phenol/chloroform extraction (see "V. **DNAse Removal of Genomic DNA from eluted total RNA - phenol/chloroform protocol**") or other suitable DNAse removal procedures/reagents.
- Pipet the required volume of RX Buffer into another tube, and add 10µl ß-mercaptoethanol (ß-ME) per 1ml RX buffer before use. Dispense (ß-ME) in a fume hood.
- Complete disruption and homogenization of the sample is essential for total RNA extraction.
- **Add 180ml of 98-100 % ethanol to WS Buffer bottle when first open.**

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Since the binding capacity of the Total RNA Mini Column is 100 µg of total RNA, in order to avoid exceeding the binding capacity, use the sample preparation guide listed below.

Sample	Recommended amount of sample		Yield (µg)
Animal cells	NIH-3T3	1 x 10^6 cells	12
	HeLa	1 x 10^6 cells	15
	COS-7	1 x 10^6 cells	30
	LMH	1 x 10^6 cells	12

Animal tissues	Mouse/rat tissues		Yield (µg)
	Embryo	10 mg	30
	Heart	10 mg	10
	Brain	10 mg	10
	Kidney	10 mg	35
	Liver	10 mg	45
	Spleen	10 mg	35
	Lung	10 mg	10
	Thymus	10 mg	45

Bacteria	*E. coli*	1 x 10^9 cells	65
	B. subtilis	1 x 10^9 cells	40

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I. Animal Tissue Protocol:

1. **Add 350µl RX Buffer (ß-ME added, see "important notes") to 10mg of liquid, nitrogen-frozen or fresh tissue. Disrupt and homogenize the sample by using 20-G syringe.** Add 10µl ß-mercaptoethanol (ß-ME) per 1ml of RX Buffer. If using 20mg of tissue add 700µl of RX Buffer.

2. **Centrifuge the lysate for 5 minutes to spin down insoluble materials and use only the supernatant in the following steps.**

3. **Determine the final volume of the supernatant. Add an equal volume of 70% ethanol to the clear the lysate and mix by vortexing.** If lysate is lost during the preparation, reduce the volume of ethanol accordingly. Do not centrifuge the ethanol added lysate.

4. **Place a RNA Mini Column onto a Collection Tube. Add 700µl of the ethanol-added sample (including any precipitate) into the column. Centrifuge for 30-60 seconds. Discard the flow-through.** Repeat this step for the rest of the sample. If some sample still remains in the column, repeat centrifugation until the complete sample has passed the column.

5. **Wash the column once with 0,5ml WF Buffer by centrifuging for 30-60 seconds. Discard the flow-through.**

6. **Wash the column once with 0,7ml WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.** Add the respective amount of 98-100 % ethanol to WS Buffer bottle when first open (see "important notes").

7. **Centrifuge the column for another 3 minutes to remove residual ethanol.**

8. **Place the column onto a 1.5ml RNAse-free Elution Tube. Add 30-50µl RNase-free ddH_20 (provided) onto the center of the membrane.** For effective elution, make sure that the elution solution is dispensed onto the center of the membrane.

9. **Stand the column for 1 minute. Centrifuge for 1-2 minutes to elute total RNA. Store RNA at -70°C.**

II. Animal Cells Protocol:

1. **Pellet 1 – 5 x 10^6 cells by centrifuging at 300 x g for 5 minutes. Remove the supernatant.**

2. **Disrupt cells by adding 350µl RX Buffer (ß-ME added, see "important notes") to the cell pellet and vortex the sample. Homogenize the sample by using 20-G syringe.** Add 10µl ß-mercaptoethanol (ß-ME) per 1ml of RX Buffer.

3. **Follow the Animal Tissue Protocol (see protocol I on page 5) starting from Step 2.**

III. Animal Cell Cytoplasm Protocol:

1. **Prepare cytoplasm lysate.**

   Prepare cell lysis buffer: (not contained in the kit) 20mM Tris-HCl pH 8.0, 1mM $MgCl_2$, 0,5% NP-40. Keep at 4°C.

   Only fresh cells are used for preparing cytoplasm lysates.

   a) Harvest 5 x 10^6 – 1 x 10^7 cells and centrifuge at 300 x g to pellet the cells.
   b) Add 180µl of cell lysis buffer to the cell pellet, resuspend and lyse cells by gentle pipetting. Incubate the lysate on ice for 5 minutes.
   c) Centrifuge the lysate at 300x g at 4°C for 3 minutes, transfer the supernatant to the new tube and use the supernatant (the lysate) in the following steps.

2. **Add 600µl of RX Buffer (ß-ME added, see "important notes") to the lysate and mix by vortexing.**

   Add 10µl ß-mercaptoethanol (ß-ME) per 1ml of RX Buffer.

3. **Add 450µl of 98-100% ethanol to the sample and mix by vortexing.**

4. **Follow the Animal Tissue Protocol (see protocol I on page 5) starting from Step 4.**

IV. Bacterial Cells Protocol:

1. **Pellet up to 1 x 10^9 bacterial cells by centrifuging at 5000 x g (7500rpm) for 5 minutes. Remove all the supernatant.**

2. **Resuspend cells in 100µl of TE Buffer by vortexing.**

3. **Add lysozyme (no contained in the kit) to a final concentration of 500µg / ml for Gram-negative bacteria; 2mg/ml for Gram-positive bacteria and incubate at room temperature for 10 minutes.**

4. **Add 350µl of RX Buffer (ß-ME added, see "important notes") to the sample and mix by vortexing.**
   Add 10µl ß-mercaptoethanol (ß-ME) per 1ml of Buffer RX.

5. **Centrifuge lysate for 5 minutes to spin down insoluble materials and use only the supernatant in the following steps.**

6. **Add 250µl of 98-100% ethanol to the sample and mix by vortexing.**

7. **Follow the Animal Tissue Protocol (see protocol I on page 5) starting from step 4.**

V. DNAse Removal of Genomic DNA from eluted total RNA - phenol/chloroform protocol:

1. **Incubate total RNA with RNAse-free DNAse I (1u per µg of total RNA) in 50mM Tris-HCl (pH 7.5), 10mM $MgCl_2$ and 50µg/ml BSA at 37°C for 15-30 minutes.**

2. **Remove DNAse I by adding an equal volume of phenol:chloroform (1:1) and mix well. Centrifuge for 5 minutes. Transfer the upper aqueous layer to a new 1.5ml tube.**

3. **Add 1/10 volume of 3M sodium acetate (pH 5.2) and 1 volume of ice-cold isopropanol to the solution and mix well. Chill on ice for 30 minutes.**

4. **Centrifuge for 10 minutes at 4°C. Discard the supernatant. Wash pellet twice with 1ml of 70% ethanol and centrifuge again.**

5. **Completely remove supernatant. Air-dry the RNA pellet. Re-dissolve RNA in RNAse-free ddH_2O.**

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VI. Working with RNA – Some Handling Guidelines:

1. **Since RNAses are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without prior elimination of possible RNA contamination. Be extremely careful to avoid unwanted introduction of RNAses into the RNA sample during or after the purification procedure.**

2. **Always wear latex or vinyl gloves to prevent RNAse contamination. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when preparing aliquots for downstream applications.**

3. **For decontamination of plasticware, rinse with 0,1M NaOH, 1mM EDTA followed by RNAse-free water.**

4. **Clean glassware used for RNA work with a detergent, thoroughly rinsed, and oven baked at 240°C for at least 4 hours before use. Autoclaving alone will not fully inactivate many RNAses. Alternatively, glassware can DEPC (diethyl carbonate) treated.**

5. **Apply 0,1% DEPC treatment to all solutions (water, buffer, etc.) used. DEPC is a strong, but not absolute, inhibitor of RNAses. It is commonly used at a concentration of 0,1% to inactivate RNAses on glass- or plasticware. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC.**

6. **Purified RNA should be stored at no higher than -20°C in RNAse-free water. Under these conditions, degradation of RNA is reduced to a minimum.**

7. **The ratio of the readings at 260nm and 280nm (A260/A280) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV spectrum, such as protein.**

8. **Reduce the risk of RNA degradation by using metabion´s RNAse Inhibitor (mi-RNAse Inhibitor; Cat# mi-E5001S and mi-E5001L).**