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AuPreP Citations

AuPreP™ RNA^m Mini Kit

Cat. #: RM74-104LT

AuPreP™ RNAm Mini Kit provides an economical method to purify total RNA from various samples such as cultured cells, tissues, and bacteria. A simple silica-membrane spin-column method can isolate total RNA without need of performing time-consuming phenol/ chloroform extraction and ethanol precipitation. Total RNA longer than 200 nucleotides are isolated, while small RNA such as 5.8S RNA, 5S RNA, and tRNA, which make up 15-20% of the total RNA, are excluded.

Sample Preparation Guide (Preparation Time: ~30 minutes)

Sample	Recommended amount of sample used		Maximum 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)	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

Kit Contents:

	(50 preps) (Cat. No.RM74-104LT)	(250 preps) (Cat. No.RM74-106LT)
RX Buffer	36 ml	200 ml
WF Buffer	30 ml	150 ml
WS Buffer	15 ml*	45 ml**
Rnase-free ddH_2O	1.5 ml x 2	15 ml
Total RNA Mini Columns (Rnase-free)	50 pieces	250 pieces
Collection Tubes (Rnase-free)	50 pieces	250 pieces
1.5-ml Elution Tubes (Rnase-free)	50 pieces	250 pieces
Protocol	1	1

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* For (50 preps), add 60 ml of 98-100% ethanol into WS Buffer bottle when first open.

** For (250 preps), add 180 ml of 98-100% ethanol into WS Buffer bottle when first open.

*** Buffers are available for separate purchase. Please refer to the Cat No. Listed above for ordering

Shearing Tube (Rnase-free)	10 pieces/pk

Shearing Tube is not provided in this system. Separate order is required.

Shipping and Storage

All components of AuPreP™ RNA^{m} Mini Kit are stable at room temperature (20-25^{0}C) for one year.

Notes:

Please read the following notes before starting the procedures.

1. All plasticware and containers should be treated properly to make sure RNase-free. Gloves should be worn when handling RNA.
2. Buffers contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn to protect from skin contact.
3. All procedure should be done at room temperature (20-25^{0}C).
4. Pipet a required volume of RX Buffer into another tube and add 10 μl β-mercaptoethanol (β-ME) per 1 ml RX Buffer before use.
5. For (50 preps), add 60 ml of 98-100% ethanol into WS Buffer bottle when first open. For (250 preps), add 180 ml of 98-100% ethanol into WS Buffer bottle when first open. Ethanol is provided by the user.
6. Do not use more than the suggested maximum amount of sample.

Protocol:

Please refer to the Table of Contents on page 4 to choose the appropriate protocol according to the kind of sample used.

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AuPreP

I Animal Tissue Protocol

1. Add 350 µl RX Buffer (β-ME added) to 10 mg of liquid-nitrogen-frozen or fresh tissue. Disrupt and homogenize the sample by grinding and shearing using 20-G needle syringe or AuPreP™ Shearing Tube.	Add 10 µl β-mercaptoethanol (β-ME) per 1 ml RX Buffer. If use 20 mg tissue, add 700 µl RX Buffer to ensure complete sample lysis. If AuPreP™ Shearing Tube is used, refer to page 13 for "Application of Shearing Tube".
2. Centrifuge the lysate for 5 minutes to spin down insoluble materials and use only the supernatant in the following steps.	A gelatinous layer of substance may form at the bottom of the tube after centrifugation. Avoid taking it up when take out the supernatant. If this layer is not well pelleted, further centrifuge the tube for a few minutes more.
3. Determine the final volume of the supernatant. Add an equal volume of 70% ethanol to the clear lysate and mix by vortexing.	70% ethanol should be prepared using DEPC-treated ddH_2O.
4. Place a Total RNA Mini Column onto a Collection Tube. Add 700 µl 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 retains in the column, repeat centrifugation until all sample pass the column.
5. Wash the column once with 0.5 ml WF Buffer by centrifuging for 30-60 seconds. Discard the flow-through.	
6. Wash the column once with 0.7 ml WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.	Make sure that ethanol has been added into the WS Buffer bottle when first open.
7. Centrifuge the column for another 3 minutes to remove ethanol residue.	Residual ethanol can affect the quality of RNA and inhibit subsequent enzymatic reactions such as reverse transcriptase reaction. If necessary, centrifuge the column for a few minutes more to remove all the ethanol.
8. Place the column onto a 1.5-ml RNase-free Elution Tube. Add 30-50 µl RNase-free ddH2O (provided) onto the membrane.	For effective elution, make sure that the elution solution is dispensed onto the center of the membrane. Eluting the column twice can result in a higher RNA recovery (refer to AuPreP™ RNAm Hints, No. 4, page 18).

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9. Stand the column for 1 minute, and centrifuge for 1-2 minutes to elute total RNA.

10. Store RNA at -700C

II Animal Cell Protocol

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

   Any residual supernatant present will affect cell lysis by RX Buffer.

2. Disrupt cells by adding 350 µl RX Buffer (β-ME added) to the cell pellet and vortex the sample. Homogenize the sample by using 20-G needle syringe or AuPreP™ Shearing Tube.

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

   Add 700 µl RX Buffer when use 1 x 10^7 cells.

   If AuPreP™ Shearing Tube is used, refer to page 13 for "Application of Shearing Tube".

3. Follow the Animal Tissue Protocol starting from Step 2 on page 9.

III. Animal Cell Cytoplasm Protocol

1. Prepare cytoplasm lysate:

   Only fresh cells are used for preparing cytoplasm lysate.

   Prepare cell lysis buffer (20 mM Tris-HCl, pH 8.0; 1 mM $MgCl_2$; 0.5% NP-40). Keep at 4^0C.

   Total RNA extracted from cytoplasm lysate are of minimum genomic DNA contamination.

   a. Pellet 5 x 10^6 to 1 x 10^7 fresh cells by centrifuging at 300 x *g* for 5 minutes. Remove all the supernatant.

   b. Add 180 µl cell lysis buffer (4^0C) to the cell pellet, resuspend and lyse cells by gentle pipetting. Incubate the lysate on ice for 5 minutes.

   c. Centrifuge the lysate at 300 x *g* at 4^0C for 3 minutes, transfer the supernatant to a new tube and discard the pellet. Use the supernatant (lysate) in the following steps.

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2. Add 600 µl RX Buffer (β-ME added) to the lysate and mix by vortexing.

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

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

4. Follow the Animal Tissue Protocol starting from Step 4 on page 9.

IV. Bacteria Protocol

1. Pellet up to 1 x 10^9 bacterial cells by centrifuging at 5,000 x *g* (7,500 rpm) for 5 minutes. Remove all the supernatant.

2. Resuspend cells in 100 µl TE buffer by vortexing.

3. Add lysozyme to a final concentration of 500 µg /ml for Gram-negative bacteria; 2 mg/ml for Gram-positive bacteria, and incubate at room temperature for 5 to 10 minutes to digest the cell wall.

   Lysozyme is provided by user.

4. Add 350 µl RX Buffer to the sample and mix by vortexing.

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

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

   A gelatinous layer of substance may form at the bottom of the tube after centrifugation. Avoid taking it up when take out the supernatant. If this layer is not well pelleted, further centrifuge the tube for a few minutes more.

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

7. Follow the Animal Tissue Protocol starting from Step 4 on page 9.

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V. Removal of genomic DNA in eluted total RNA

1. Incubate total RNA with RNase-free DNase I (1 unit DNase I per μg RNA) in 50 mM Tris-HCl (pH 7.5), 10 mM $MnCl_2$, and 50 μg/ml BSA at 37 ^{0}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 eppendorf tube.

3. Add $^1/_{10}$ volume of 3 M 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^{0}C. Wash RNA pellet twice with 1 ml of 70% ethanol and recentrifuge.

5. Remove all supernatant. Air dry RNA pellet. Redissolve RNA in RNase-free ddH_2O.

VI. Application of Shearing Tube

Shearing Tube is designed for simple and fast homogenization of tissue and cell lysate. The lysate is loaded into a Shearing Tube sitting in a 2-ml Collection Tube and centrifuge the tube for 1-2 minutes at full speed (10,000 x *g* or 13,000-14,000 rpm) in a microcentrifuge. When collecting homogenized lysate from the Collection Tube, avoid pipetting any debris and pellet formed at the bottom of the tube.

Troubleshooting:

Problem	Possible Reason	Solution
Column is clogged when passing the sample	Sample lysate contains insoluble residues or/and gelatinous substance	After sample lysis, centrifuge the sample at full speed for 5 minutes or more and only use the supernatant.
	Sample lysate is very viscous because too much sample is used	Reduce the sample amount or increase the volume of RX Buffer and ethanol proportionally
Little or no RNA eluted	Insufficient disruption or homogenization	Reduce the amount of starting sample and perform more disruption and homogenization to the sample.
	Column is clogged	Reduce the amount of starting sample and perform more disruption and homogenization. Centrifuge the lysate to remove insoluble materials and use only the supernatant.

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	RNA is not completely eluted because RNase-free ddH_2O does not penetrate into the membrane	Add ddH_2O onto the center of the membrane and stand the column for 5 minutes. If ddH_2O still retains on the membrane, pulse centrifuge the column for a few seconds to drag ddH_2O into the membrane.
	RNA is degraded	Flash freeze fresh samples in liquid nitrogen and store at -80^0C if not used immediately. Improper handling (such as thawing) of the sample or storing the sample at -20^0C will cause RNA degradation.

Problem	Possible Reason	*Solution*
Little or no RNA eluted	RNase contamination	Treat bench surface before use. Use RNase-free solutions, plastic-ware and glassware.
	No ethanol or ethanol of incorrect amount is added to the sample lysate	Determine the final volume of sample lysate obtained. Add ethanol of correct volume and concentration as indicated in the protocol.
DNA contamination	DNA is copurified with RNA	Use RNase-free DNase to treat the eluted RNA sample. DNase can then be removed by phenol/ chloroform extraction (refer to Protocol, page 13). Minimize DNA copurification by extracting total RNA from cytoplasm lysate prepared from fresh cultured cells.
A_{260}/A_{280} ratio of eluted total RNA is low	Used ddH_2O of acidic pH to dilute RNA samples for spectrophotometric analysis	Use 10 mM Tris-HCl of pH 7.5 or TE buffer to dilute the RNA samples (refer to AuPreP™ RNA^m Hints, No. 3, page 18).
	Proteins in the sample are not completely denatured	Too much sample is used. Reduce the sample amount or increase the volume of RX Buffer and ethanol proportionally.

Problem	Possible Reason	*Solution*
A_{260}/A_{280} ratio of eluted total RNA is low	DNA is copurified with RNA	Refer to Solution section of Problem - "DNA contamination".
	Eluted RNA carries contaminants	Wash the column twice with 0.7 ml WS Buffer.
RNA appears smearing and degraded	Sample is stored and handled improperly	Flash freeze fresh samples in liquid nitrogen and store at -80^0C if not used immediately. Improper handling (such as thawing) of the sample or storing the sample at -20^0C will cause RNA degradation.

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	Cell samples were harvested from an old or over-grown culture	RNA of good quality is only expected from a healthy cell or bacterial culture.
	RNase contamination	Treat bench surface before use. Use RNase-free solutions, plastic-ware and glassware.
	Gel electrophoresis is performed in running buffer or tank contaminated with Rnase	Use fresh running buffer prepared from DEPC-treated ddH_2O and properly-cleaned tank for electrophoresis.
Poor performance in downstream applications	Eluted RNA carries ethanol residue	After wash with WS Buffer, do discard the flow-through, and centrifuge the column for another 3 minutes. If necessary, centrifuge the column for a few minutes more to remove all the ethanol.

Reference: Wilfinger, W. W., Mackey, K., and Chomczyski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 22:474-481.

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