BIO 101 Protocol

RNaid® KIT with SPIN™

1107-200	**25 SPIN™ preps, 200 RNA preps**
1107-600	**100 SPIN™ preps, 200 RNA preps**

- **Isolate and Purify RNA in Twenty Minutes from Agarose and Polyacrylamide Gels, from Solutions, and from Unincorporated Radioactive Label.**

- **RNA is Suitable as a Substrate for Multiple Enzymatic Manipulations Including Reverse Transcription, RNase Protection Assays, and *in vitro* Translation.**

Shipping & Storage:

The **RNaid® Kit** with **SPIN™** is shipped at ambient temperature. Recommended storage is room temperature.

"Check with BIO 101... We have you time in mind!"

BIO 101

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RNaid® with SPIN™ Protocol

Kit Components

1107-200 (25 SPIN™ preps, 200 RNA preps)

Name	Volume	Catalog #
RNAMATRIX®	1.5 ml	1007-204
RNAWash Concentrate*	120 ml	1007-203
RNA Binding Salt	60 ml	1007-202
DEPC Treated Water	15 ml	1007-201
10% Acetic Acid	0.5 ml	1007-205
SPIN™ Filters, RNase-free	25	1107-206
Catch Tubes	25	1107-207

**** Add 120 ml of 100% Ethanol and mix well before use.***

1107-600 (100 SPIN™ preps, 200 RNA preps)

Name	Volume	Catalog #
RNAMATRIX®	1.5 ml	1007-204
RNAWash Concentrate*	120 ml	1007-203
RNA Binding Salt	60 ml	1007-202
DEPC Treated Water	15 ml	1007-201
10% Acetic Acid	0.5 ml	1007-205
SPIN™ Filters, RNase-free	100	1107-606
Catch Tubes	100	1107-607

**** Add 120 ml of 100% Ethanol and mix well before use.***

The* RNaid® Kit *with* SPIN™ *and all of its reagents are for research use only. All kit components have been lot qualified for the isolation of undegraded RNA. To avoid contamination of samples and reagents with RNase, follow appropriate laboratory procedures, wear surgical or similar gloves, use sterile vials, pipets, and pipet tips etc. To prepare glass, ceramic, or metal homogenizers, clean and bake at 200°C for 2 hours to remove any contaminating RNase.

Introduction

The **RNaid® Kit** with **SPIN™** contains all of the solutions and reagents, except for ethanol, necessary for the isolation and purification of RNA in twenty minutes from agarose and polyacrylamide gels, from solutions, and from unincorporated radioactive labels (see Protocols). The resulting RNA is suitable as a substrate for multiple enzymatic manipulations including reverse transcription, RNase protection assays, and *in vitro* translation.

A procedure for the isolation of RNA from tissue or cells is provided, however cell lysis reagents are not included. This protocol utilizes guanidine thiocyanate (user-supplied) and acid phenol (user-supplied) extraction (Chomczynski, P., and Sacchi, N., 1987, Anal. Biochem. 162, 156-159) followed by purification on **RNAMATRIX®** (kit-supplied) to yield pure RNA in RNase free water in one hour with no further steps required. BIO 101's **RNaid® PLUS** Kit (catalog #1009-200) is designed for the isolation of RNA from tissue or cells and provides the necessary cell lysis reagents except phenol and chloroform.

Protocols

A. Isolation of RNA from Solution, Agarose or Polyacrylamide Gels
B. Isolation of RNA from Agarose/Formaldehyde Gels
C. Purification of RNA from Transcription Reactions
D. Trouble Shooting Guide

A. Isolation of RNA from Solution, from Agarose or Polyacrylamide Gels Containing 0-6 M Urea

Note: Reagents for agarose/urea gels are not included with the kit.

For an agarose gel in 0.5x TAE buffer containing 6M urea, prepare two solutions.

Solution A: Dissolve 8M urea in water by heating to 60°C. Cool to room temperature and adjust pH to 3.8 with solid citric acid (approximately 0.8g citric acid/100 ml 8M urea; use free acid, not sodium salt).

Solution B: Prepare a 4x agarose solution in 2x TAE buffer, pH 6.0. Melt agarose completely by boiling.

Mix Solution A with 1/4 volume melted Solution B and cast gel. The final concentration is 6M urea and 0.5x TAE at the desired agarose concentration. The gel will solidify within 30 to 60 minutes at 4°C. An agarose concentration of less than 1% may take overnight at 4°C to solidify. The gel will remain clear upon solidification. Load sample and run at 4°C. Heat denature RNA sample before loading by incubation at 60°C for 10 minutes in the presence of 50% formamide, or at 80°C for 10 minutes without formamide.

Isolation of RNA from Solution

1. Add 3 volumes of **RNA Binding Salt** and mix well.

2. Continue with step 3., page 6.

Isolation of RNA from Agarose

1. Excise desired RNA band from ethidium bromide stained gel and determine approximate volume by its weight. Place gel slice in microcentrifuge tube.

2. Add 3 volumes of **RNA Binding Salt** (i.e. to 0.1g gel slice add 0.3 ml of RNA Binding Salt). Mix and incubate at room temperature for 10 minutes to dissolve agarose. Alternatively, place tube in 45-55°C water bath to dissolve agarose more rapidly. Continue with step 3, below.

Isolation of RNA from Polyacrylamide

1. Excise band from ethidium bromide stained gel and determine approximate volume by weight. Place into microcentrifuge vial. If gel concentration is 10% or higher, crush or cut into small pieces. Add 3 volumes of **RNA Binding Salt**. Soak for 20 minutes at 60°C. Remove liquid with small bore pipet tip while avoiding gel pieces, and transfer to new vial.

2. Add 2µl of **10% Acetic Acid** (included with kit) for every 0.5 ml of liquid to change pH to 5.0-5.5 (check with pH paper). This will increase recovery efficiency. Continue with step 3, below.

3. Estimate the amount of RNA expected and add 1µl of **RNAMATRIX®** for every µg of RNA (add a minimum of 5µl of **RNAMATRIX®**). Mix well and allow binding of RNA to the matrix for at least five minutes at room temperature. Mix occasionally to keep **RNAMATRIX®** in suspension during adsorption.

4. Spin for 1 minute in microcentifuge at maximum speed to pellet RNA/**RNAMATRIX®** complex. Remove supernatant and save aside; if supernatant contains residual RNA, more **RNAMATRIX®** can be added for complete recovery. Spin pellet again briefly and remove residual liquid with small bore pipet tip.

5. Add 500µl of **RNA Wash Solution** (remember to add ethanol before first use*) and resuspend pellet completely by mixing with pipet tip. Spin for 1 minute in microcentrifuge at maximum speed and remove supernatant.

**** Add 120 ml of 100% Ethanol and mix well before use.***

6. Repeat washing step 5 one or 2 times. After last wash, spin tube again briefly and remove residual liquid with small bore pipet tip.

7. Resuspend pellet in **DEPC Treated Water**. Use 10-20µl per 5µl **RNAMATRIX®** . Mix thoroughly with pipet tip and elute RNA by incubating at 45-55°C for 5 minutes.

8. Spin tube for 2 minutes and remove supernatant with RNA. If using the **SPIN™** option, transfer suspension to a **SPIN™ Filter** and spin for 1 minute in microcentrifuge. The supernatant containing RNA will be in the catch tube.

Optional: A second elution will yield 5-15% more RNA.

B. Isolation of RNA from Agarose Gels Containing Formaldehyde

Note: Reagents for agarose/formaldehyde gels are not included with the kit.

10x Gel Buffer:
200 mM MOPS, pH 7.0 (adjust with NaOH)
10 mM EDTA
10 mM NaOAc.

Prepare 1.2% agarose gel containing 6.6% formaldehyde and 1x gel buffer. Do not add ethidium bromide to the gel, only to the RNA sample as described below. Run gel at 3-5V/cm in 1x gel buffer with 6.6% formaldehyde at pH 7.0.

Preparation of RNA sample for gel:
10 µl formamide (deionized)
4 µl formaldehyde (37%/12.3M)
2 µl 10x gel buffer
3 µl RNA (up to 20 µg)
1 µl ethidium bromide (400 µg/ml)

Heat at 65°C for 10 minutes before loading in well of agarose gel. Formamide is dense enough to allow the sample to be loaded without adding an additional dense liquid, however, a loading dye mixture can be used if preferred.

Isolation of RNA from Agarose/Formaldehyde Gel

1. Excise RNA band(s) from gel after electrophoresis. Visualize RNA with long wave UV for minimal length of time while cutting gel. Determine approximate volume of gel slice(s) by weight and place slice(s) into microcentrifuge tubes.

2. Adjust the pH of the gel slice to pH 5.0 by adding 2 µl of **10% Acetic Acid** (included with kit) to 1 ml of **RNA Binding Salt** and add 3 volumes to the gel slice (i.e. to 0.1 g gel slice add 0.3 ml **Binding Salt/ Acetic Acid** mixture). The lower pH will optimize the binding efficiency of RNA to the **RNAMATRIX®**. Incubate at 37°C for approximately 10 minutes with occasional mixing to melt agarose.

3. When gel is completely melted, place vial at room temperature and add 1-2 µl of **RNAMATRIX®** per µg of RNA (add a minimum of 5 µl of **RNAMATRIX®**. Mix well and allow RNA to adsorb to the matrix for 10 minutes at room temperature with periodic mixing.

4. Centrifuge for 1 minute in microcentrifuge at maximum speed to pellet the RNA/**RNAMATRIX®** complex. Remove supernatant to new tube and save for possible re-adsorption. Briefly spin again to collect remaining liquid in bottom of the tube. Remove all traces of liquid with a small bore pipet tip.

5. ***Optional (recommended):*** Resuspend pellet in same amount of **RNA Binding Salt** as in step 2 to wash pellet and help remove remaining traces of agarose and formaldehyde. Mix thoroughly with pipet tip. Spin for 1 minute and remove supernatant. Pulse spin and remove traces of liquid with small bore pipet tip.

6. Resuspend pellet in 500µl **RNA**Wash solution (remember to add ethanol before first use*) by mixing with pipet tip. Spin for 1 minute and remove supernatant.

**** Add 120 ml of 100% Ethanol and mix well before use.***

7. Repeat washing step 6 one or two times. Re-spin and remove traces of liquid as described in step 5.

8. Resuspend pellet completely in **DEPC Treated Water** by mixing with pipet tip. Use 10-20µl of water per 5µl **RNAMATRIX®**. Elute RNA by incubating at 80°C for 10 minutes. Spin tube for 2 minutes and remove supernatant with RNA. If using the **SPIN™** option, transfer suspension to a **SPIN™ Filter** and spin for 1 minute in microcentrifuge. The supernatant containing RNA will be in the catch tube.

   Optional: A second elution will yield 5-15% more RNA.

9. Heat eluted RNA to 80°C for 10 minutes to further dissociate residual formaldehyde from RNA. This second heating step will reverse chemical modification of the RNA caused by formaldehyde (Boedtker,

H., 1967, Biochemistry 6, 2718-2727) and will render RNA biologically active as substrate for modifying enzymes. Let cool to room temperature to allow RNA to renature, or place on ice immediately to avoid renaturation. The RNA is now ready for use in enzymatic manipulations.

C. Purification of RNA from Transcription Reactions

Note: Reagents for transcription, template removal, and hydrolysis are not included with the kit.

Transcription Reaction
(commercially available as a kit from several manufacturers)

1. Combine: 5µl 5x transcription buffer
(200 mM Tris-HCl, pH 7.5 at 37°C, 30 mM $MgCl_2$, 50 mM NaCl, 10 mM spermidine)
1µl RNase Inhibitor (1 unit/µl)
1µl 50 mM DTT
1µl each rATP, rGTP, rUTP (10 mM each)
4µl radioactively labeled rCTP (200 µCi)
8µl distilled water
1µl DNA template (1µg/µl)
1µl RNA Polymerase (i.e. T3, T7, or Sp6 Polymerase)
25µl total reaction volume

2. Mix and incubate at 37°C for 1 hour. When completed, remove 1µl of the reaction and determine TCA precipitable counts to calculate % incorporation.

Removal of DNA Template and Hydrolysis of RNA

Note: Depending on the size of the transcribed RNA and the purpose of use, it may be necessary to shorten the transcripts by hydrolysis with sodium hydroxide. After hydrolysis, it is crucial to neutralize the pH before purification of the RNA with* RNAMATRIX®*. If the pH of the sample is alkaline the RNA will not adsorb to the matrix.

3. Add 1µl of RNase-free DNase (10 units/µl). Incubate at 37°C for 10 minutes and place on ice. If probe is to be hydrolyzed, continue with step 4. Otherwise, continue with **Purification of RNA**, step 7., page 10.

4. Add 50µl ETS buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1% SDS), 1.7µl 5M NaCl, and 1µl 1M DTT. Mix.

5. Add 10µl 2N NaOH for hydrolysis of RNA transcripts. Incubate on ice for 30 minutes for smaller transcripts (< 1kb) or for 60 minutes for larger transcripts (> 1kb). As an example, a 1kb transcript can be shortened to 150-220 bases by a 30-40 minute incubation at 4°C.

6. Warm to room temperature, then add 20µl (2x volume) of 1M MES buffer to neutralize pH and stop hydrolysis. Add MES buffer after warming tube to room temperature to prevent precipitation. Volume is approximately 110µl at this point.

Purification of RNA with RNAMATRIX®

7. Add 3 volumes of **RNA Binding Salt** and mix. Do not precipitate or gel purify RNA prior to adding **RNA Binding Salt** and **RNAMATRIX®**. Pre-purifying the "hot" RNA can lead to very tight binding to the **RNAMATRIX®** and will be difficult to elute.

8. Estimate the amount of transcripts and add 1-2µl of **RNAMATRIX®** per µg of RNA; add a minimum of 5µl **RNAMATRIX®**. Mix well and incubate at room temperature for 5 minutes with occasional mixing to allow adsorption of RNA to the matrix.

9. Spin for 1 minute in microcentrifuge at maximum speed and remove and discard supernatant which contains most of the unincorporated label. Follow precautions and regulations for handling and disposing of radioactive materials as specified in Radioactive Materials License.

10. Wash pellet two times with 500µl **RNA Wash Solution** (remember to add ethanol before first use) and resuspend pellet completely by mixing with pipet tip. Spin for 1 minute in microcentrifuge at maximum speed and remove supernatant.

11. Remove residual traces of liquid and elute RNA with appropriate volume of **DEPC-Treated Water** by carefully resuspending pellet and incubating at 50°C for 3 minutes. Spin for 1 minute and remove supernatant containing labeled RNA to new tube. If using the **SPIN™** option, transfer suspension to a **SPIN™ Filter** and spin for 1 minute in microcentrifuge. The supernatant containing RNA will be in the catch tube.

Optional: A second elution will yield 5-15% more RNA.

***Note: If the RNAMATRIX® retains radioactive label, elute a second time by resuspending pellet in RNase-free water and incubating at 80℃ for 3 minutes. Spin and remove supernatant to new tube. If the second elution does not contain any radioactive label and the RNAMATRIX® still retains radioactivity, all RNA has been eluted. Radioactivity retained by the RNAMATRIX® represents reagent decay products which bind to the RNAMATRIX®. If available, use fresh radioactive reagents no older than two weeks from the date of manufacture since the amount of decay products increases with age of radioactive materials (especially ^{32}P-labelled reagents). It is important to purify radioactively labelled RNA using the* RNaid® Kit *with* SPIN™ *immediately after transcription reaction is complete to minimize binding of RNA radiolysis products to the* RNAMATRIX®.**

D. Trouble Shooting Guide

1. **Gel Purification**
2. **Adsorption to RNaid® Matrix™**
3. **Spectrophotometer Readings**

1. Gel Purification

Technical Background: The right choice of gel separation system is extremely important for best results. Criteria are native versus denaturing conditions and agarose versus polyacrylamide gel matrix. Generally, recovery of RNA from agarose gels is more efficient and convenient than from polyacrylamide. However, separation properties of polyacrylamide are usually superior. To improve recovery efficiency from polyacrylamide, extract RNA at elevated temperature (55-65°C) and extended time (20 min). Both formaldehyde/agarose gels as well as TBE/polyacrylamide gels create unfavorable binding conditions for RNA to **RNAMATRIX®** as the pH increases upon dissociation of the gel. Adjustment to more acidic conditions is necessary. In both cases, optimal binding efficiency can be restored by adjusting the pH to an appropriate range using 10% acetic acid (kit-supplied). When using formaldehyde, do not exceed a final concentration of 6.6% in the gel and buffer, and prepare all solutions fresh just before use. Avoid adding ethidium bromide to the formaldehyde gel since it will fluoresce and render the detection of RNA bands extremely difficult. Instead, add ethidium bromide to the RNA sample before loading as described in Section **C**. Always remember to process RNA as quickly as possible, never leave RNA inside gel for long periods of time (> 1hr), remove immediately and store under proper conditions to avoid degradation.

Important Note: Agarose or acrylamide used for separation of RNA should be of highest purity available. The quality of chemicals used for gel buffers is equally critical for good separation results. When preparing urea solutions, always filter-purify through 0.45μm membrane to remove insoluble particles. Pre-treat hardware, such as glassware, gel devices, combs, spacers, etc., with DEPC to remove contaminating RNases. Use sterile, individually wrapped and unopened disposable supplies if possible. Prepare all buffers and gels with **DEPC-Treated Water** ***and autoclave buffers.***

Agarose gel slice does not dissolve completely.

Solution: Follow instructions and incubate agarose at 45-55°C for 10 minutes. If gel has not dissociated, add more RNA Binding Salt and continue incubation at same temperature for 10 minutes.

Liquid cannot be completely removed from polyacrylamide gel elution, or transferred liquid contain small pieces of polyacrylamide.

Solution: Remove gel pieces by forcing liquid trough a 0.2μm membrane attached to a syringe (Acrodisc PF with 0.2μm Supor and 0.8μm Pre-filter works well, supplied sterile by Gelman Sciences). Alternatively, use a large pipet tip with a sterile cotton plug. ***Do not use glass wool***.

The RNA solution eluted from the gel has been checked with pH paper and shows a pH>7.5.

Solution: Use the kit-supplied **10% Acetic Acid** to adjust pH. Add 2μl **10% Acetic Acid** per every 0.5 ml of eluted liquid to change pH to 5.0-6.0 and check with pH paper. This procedure should be used for both TBE/polyacrylamide and formaldehyde/agarose eluates. Once pH has been adjusted to optimal range, continue with RNA purification and add **RNAMATRIX®** to the eluted RNA.

2. Adsorption to RNAMATRIX®

Technical Background: The conditions for binding of RNA to the **RNAMATRIX®** can be varied according to lysis and/or purification method (see Gel Purification section) and desired RNA population or size of RNA molecules.

The binding efficiency is influenced by ionic strength and pH. Adsorption in the presence of guanidine thiocyanate only (lysis solution) selects for larger RNA's. Addition of **RNA Binding Salt** during adsorption causes binding of smaller molecules. Optimal pH range for adsorption of RNA to **RNAMATRIX®** is pH 5.5-7.5; higher pH conditions result in a loss of binding capacity. In general, lower pH (5.5-6.0) tends to increase binding of smaller RNA molecules, slightly higher pH (6.5-7.5) favors larger sizes.

After addition of RNAMATRIX® and mixing, the matrix forms aggregates and does not disperse evenly throughout the sample.

Solution: Aggregation of the **RNAMATRIX®** tends to occur at high concentrations of nucleic acid. Try breaking up aggregates with pipet tip or by rocking tube back and forth quickly. Small aggregates will not effect the procedure.

During RNA wash steps of the pellet, the RNAMATRIX®does not disperse evenly.

Solution: The pellet is as easily dispersed in the **RNA**Wash solution (containing ethanol) as in water. When adding the **RNA**Wash solution to the pellet, use same pipet tip and brake up pellet by stirring. Be careful to expell any trapped matrix from the tip back into the sample. Small aggregates will not affect the procedure.

During adsorption to RNAMATRIX®, not all RNA is bound and removed from solution.

Solution: The amount of **RNAMATRIX®** was not sufficient to adsorb all of the RNA. Transfer supernatant with unbound RNA to new vial, add 5-10µl of new **RNAMATRIX®**. You may also re-use the matrix previously applied to this sample. Save supernatant until after the initially bound RNA is eluted from the matrix, then add supernatant back to the same matrix. Incubate, and follow wash procedures as described. Elute RNA with 5-10µl **DEPC Treated Water** and combine with RNA eluted from the first adsorption process.

3. Spectrophotometer Readings

Technical Background: The amount of nucleic acid in the eluate from **RNAMATRIX®** can be determined by optical density at 260nm. The **RNAMATRIX®** material absorbs over a wide range of the spectrum, including UV. This absorption is consistent and very low over a range from 210nm to

approximately 310nm, absorbing 0.01 O.D. units. Residual matrix material can easily be removed by centrifugation and will not contribute to any absorption. Guanidinium salt, **RNA Binding Salt**, and phenol absorb UV light at wavelengths close to that of nucleic acid and do not allow for reliable O.D. readings. Phenol is effectively removed from solution by extraction with chloroform; guanidinium and binding salts are removed by washings with **RNA𝕎𝕒𝕤𝕙 Solution** as described, allowing for reliable O.D.(260/280) readings of the (second) wash step supernatant.

O.D.(260) measurement of the RNA𝕎𝕒𝕤𝕙 Solution supernatant shows UV absorption.

Solution: Spin vial with pellet of RNA/**RNAMATRIX®** complex for 10 seconds and remove traces of liquid with a small bore pipet tip. Resuspend pellet in 500µl **RNA𝕎𝕒𝕤𝕙** Solution and mix with pipet tip to resuspend the matrix evenly. Spin for 1 minute in microcentrifuge at maximum speed. Remove supernatant and measure OD_{260}. If absorption is still above background, repeat washing procedure. If no absorption is detectable, proceed with elution of the RNA as described.

Eluted RNA is contaminated with residual RNAMATRIX®.

Solution: Small amounts of **RNAMATRIX®** in the RNA sample are inert and will not effect enzymatic reactions. However, **RNAMATRIX®** absorbs UV light and might effect OD_{260} measurements. To remove residual **RNAMATRIX®** from the eluted RNA, re-spin vial in microcentrifuge for 30 seconds at maximum speed and remove RNA solution with small bore pipet tip. Be careful not to touch bottom of tube near pellet area. For consistant and reliable measurement of multiple samples, complete removal of residual **RNAMATRIX®** particles can be accomplished by centrifugal filtration through a **SPIN™ Filter** unit, or by passing RNA sample through a sterile 0.2µm membrane attached to a syringe.

Notes

Product Use Limitation & Warranty

Unless otherwise indicated, this product is for research use only. Purchase of BIO 101 products does not grant rights to reproduce, modify, repackage the products or any derivative thereof to third parties. BIO 101 makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and BIO 101's sole liability hereunder shall be limited to, at our option, product credits, refund of the purchase price of, or the replacement of all material(s) that does not meet our specification. By acceptance of the product, Buyer indemnifies and holds BIO 101 harmless against, and assumes all liability for the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refunds or replacement is conditioned on Buyer notifying BIO 101 within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (days) shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

General Information

BIO 101 is a pioneer in developing kits for molecular biology research. We introduced the **GENECLEAN®** Kits in 1986 and have since been manufacturing products to bring convenience into your research. Our goal is to make your life easier by simplifying the complexities of lab work.

Technical Support and Ordering Information

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