Working with RNA

Wear gloves and use disposable, sterile plasticware!

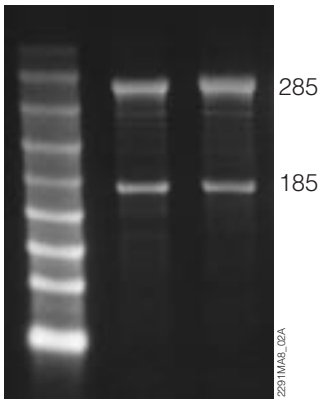
Establish an RNase-Free Environment

When working with RNA, care must be taken to create a ribonuclease-free environment. Ribonucleases or RNases are everywhere! RNases are very stable and difficult to inactivate. To ensure success, it is important to maintain an RNase-free environment starting with RNA purification and continuing through analysis. Below are some tips and techniques to remember when working with RNA:

1. The most common sources of RNase contamination are hands (skin) and bacteria or mold that may be present on airborne dust particles or laboratory glassware. To prevent contamination from these sources, wear gloves at all times and use sterile technique when handling the reagents used for RNA isolation or analysis.
2. Whenever possible, use sterile, disposable plasticware for handling RNA. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
3. Treat non-disposable glassware and plasticware before use to ensure that it is RNase-free. Bake glassware at 250°C overnight. Thoroughly rinse plasticware with 0.1N NaOH/1mM EDTA and then with diethyl pyrocarbonate (DEPC)-treated water. COREX® tubes should be rendered RNase-free by treating them overnight with 0.05% DEPC and not by baking. This will reduce the failure rate of this type of tube during centrifugation.
4. While most sources of fresh, deionized water are free of contaminating RNases, deionized water can be a contributor of RNase activity. If degradation of the target or probe RNA occurs, it may be necessary to test the water source for RNase activity by incubating an RNA sample with the water and checking for degradation by gel electrophoresis.
5. Chemicals for use in RNA isolation and analysis should be reserved for RNA applications and kept separate from chemicals for other uses. Wear gloves when handling the chemicals, and use only baked spatulas and untouched weigh boats or weighing paper.
6. Autoclaving alone is not sufficient to inactivate RNases. Solutions prepared in the lab should be treated by adding DEPC to 0.05% and incubating overnight at room temperature. The treated solutions should be autoclaved for 30 minutes to remove any trace of DEPC. Also, Tris-based buffers cannot be used with DEPC; the DEPC reacts with the free amino group and Tris loses its buffering ability. Purchase Tris that is tested RNase-free and use DEPC-treated or Nuclease-Free Water to make your Tris-buffered solutions.

Some labs have found it useful to set up an "RNA Work Only" area that has dedicated labware, pipets, ice buckets, apparatus, etc.

If the total RNA sample is undegraded, the 28S rRNA subunit band will appear approximately twice as intense as the 18S rRNA subunit band.



Total RNA isolated from mouse (lane 1) and rat (lane 2) brain RNA with the SV Total RNA Isolation System (Cat.# Z3100). RNA Markers (0.28–6.58kb) were run next to 2.5µg of each RNA sample. See more from "Measuring gene expression in mammalian brains" in: eNotes Online at: www.promega.com/enotes/applications/9910a/ap0007.htm

DEPC*

Diethyl pyrocarbonate
Formula Weight: 162.1
Density: 1.2g/ml

0.05% (v/v) Solution: 50µl DEPC per 100ml solution.

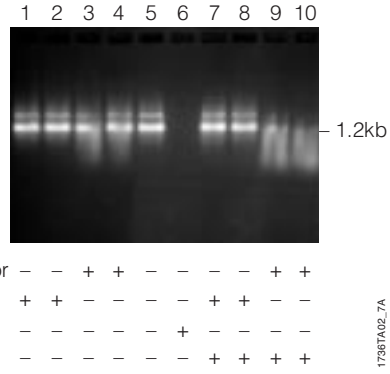
* DEPC is a suspected carcinogen.

See page 4 for gel electrophoresis of RNA

See page 42 for use of RNasin® Inhibitor in direct, single-cell RT-PCR

Maintain RNase-Free Conditions in Reactions

Recombinant RNasin® Ribonuclease Inhibitor helps you maintain an RNase-free environment, preventing RNA degradation by inhibiting some RNases without interfering with downstream applications. Protection of RNA used in reactions is critical for success. RNase inhibitors are great tools for maintaining an RNase-free environment. To be useful, the RNase inhibitor must not interfere with other enzymes in the reaction, and the inhibition must be rapid and specific. One inhibitor that meets these requirements is the Recombinant RNasin® Ribonuclease Inhibitor, the native form of which was first isolated from human placenta (1). The RNasin® Inhibitor has a rapid on-rate ($K_A = 10^{16}M^{-1}$), forming a 1:1 complex with RNase. Other antibody-based inhibitors have a much slower on-rate and may not immediately protect RNA in a reaction (2,3). The RNasin® Inhibitor is also quality tested for latent, hidden RNases already in complex with the protein that may be a problem for inhibitors isolated from other species (4).



Comparison of RNase and latent RNase activity in Recombinant RNasin® Ribonuclease Inhibitor and porcine RNase inhibitor. Two different amounts (100 units or 200 units) of each inhibitor were incubated with 0.1mg/ml of RNA at 37°C for 60 minutes. As indicated in the figure, some samples were preheated to 67°C for 15 minutes (to denature the inhibitor and release any latent RNase) before the addition of the RNA. The reactants were analyzed on a 1.5% agarose gel with ethidium bromide staining. Lanes: lanes 1 and 2, 100 and 200 units, respectively, of Recombinant RNasin® Ribonuclease Inhibitor; lanes 3 and 4, 100 and 200 units, respectively, of porcine RNase inhibitor; lanes 7 and 8, 100 and 200 units, respectively, of Recombinant RNasin® Ribonuclease Inhibitor preheated to 67°C; lanes 9 and 10, 100 and 200 units, respectively, of porcine RNase inhibitor preheated to 67°C; lane 5, RNA with no RNase inhibitor added; lane 6, RNA incubated with RNase in the absence of inhibitor taken from reference 4.

Recombinant RNasin® Ribonuclease Inhibitor

Product information on the web site:
www.promega.com/tbs/9pin251/9pin251.html

Cat.# N2511

See Citations online at:
www.promega.com/citations/

Need a more oxidation-resistant form of RNasin® Inhibitor? Check out the new RNasin® Plus RNase Inhibitor. The new protein has all the benefits of the human RNasin® Inhibitor and can work in a more oxidative environment. Also has applications in reverse transcription reactions above 50°C.


1367CA02_6A

Three-dimensional ribbon model of RNasin® Ribonuclease Inhibitor. Beta sheets are indicated in red, alpha-helical structures in blue and the remainder in green. The model was generated using the RIBBONS program and kindly provided by Drs. B. Kobe and J. Deisenhofer. Reprinted with permission from *Nature*, ©1993, Macmillan Magazines Limited.

RNasin® Plus RNase Inhibitor

Cat.# N2611

Featured in eNotes online @ http://www.promega.com/enotes/applications/ap0049_tabs.htm

Ethidium bromide can detect as little as 5ng of RNA in a band.
Brown, T.A. (1998) In: Molecular Biology LABFAX II: Gene Analysis. 2nd ed. Academic Press, p. 101.

Gel Analysis of RNA

Running RNA on gel is quite different than running DNA on an agarose gel. The RNA needs to be denatured for optimal electrophoresis. Also, because it is single-stranded, RNA does not incorporate stains as easily as DNA. Below is a common method used to analyze RNA on gel.

MOPS Buffer/Formaldehyde Protocol

1. Prepare a 1% agarose/formaldehyde gel (containing 0.5µg/ml ethidium bromide):

   20.0ml 5X MOPS Buffer
   72.0ml DEPC-treated or Nuclease-Free Water
   1.0g Agarose, molecular biology grade

2. Heat to boiling (in a microwave oven) and cool to about 55°C. In a fume hood, add 17.6ml 37% formaldehyde and 5µl of 10mg/ml ethidium bromide to the mixture. Gently mix. **Note:** If you are going to transfer the RNA to a membrane for a Northern blot, you may not want to add the ethidium bromide (5).
3. Pour the gel and allow to cool.
4. Prepare the RNA samples by mixing 1 part RNA sample with two parts RNA Sample Buffer to a total volume of 10–30µl, depending on how much volume you can load onto your gel. Heat the sample to 65°C for 5 minutes and then cool on ice for 2 minutes. Add 2µl of RNA loading buffer.
5. Pre-run the gel for 10 minutes in 1X MOPS buffer prior to loading the samples. Load the RNA samples and run the gel at 4–5V/cm. Continue electrophoresis until the bromophenol blue dye has migrated at least 2/3 to 3/4 of the length of the gel.

MOPS
3-Morpholinopropanesulfonic Acid
MW 209.3.
Maximum solubility in water (0°C): 3.1M
pKa @ 20°C: 7.2
Δ in pKa per °C rise: –0.011
Brown, T.A. (1998) In: Molecular Biology LABFAX II: Gene Analysis. 2nd ed. Academic Press, p. 191.

Recipes

Nearly all of these reagents can be purchased premade (Promega Technical Services can help find a supplier if necessary).

To make them:

Deionized Formamide

Add Dowex® XG8 mixed-bed resin to formamide and stir at room temperature for 1 hour. Filter twice through Whatman® No. 1 filter paper. Store in small aliquots at –70°C.

5X MOPS Buffer

0.2M MOPS (pH 7.0)
0.05M sodium acetate
0.005M EDTA (pH 8.0)

For 2 liters of buffer, add 83.72g of MOPS (free acid) and 8.23g sodium acetate to 1.6L of DEPC-treated water and stir until completely dissolved. Add 20ml of DEPC-treated 0.5M EDTA and adjust the pH to 7.0 with 10N NaOH. Bring the final volume to 2L with DEPC-treated water. Dispense into 200ml aliquots and autoclave. The solution will turn yellow, but this will not affect the quality of the buffer.

RNA Sample Buffer

10.0ml deionized formamide
3.5ml 37% formaldehyde
2.0ml 5X MOPS Buffer

Dispense into single use aliquots and store at –20°C in tightly sealed, screw-cap tubes. These can be stored for up to 6 months.

RNA Loading Buffer

50% glycerol
1mM EDTA
0.4% bromophenol blue

Prepare in DEPC-treated or Nuclease-Free Water. Use high-grade glycerol to avoid ribonuclease activity. Dispense into single-use aliquots and store at –20°C.

RNA-Qualified Materials	Size	Cat.#
Recombinant RNasin® Ribonuclease Inhibitor[a,b]	2,500 units	N2511
	10,000 units	N2515
RNasin® Plus RNase Inhibitor[c]	2,500 units	N2611
	10,000 units	N2615

For Laboratory Use.

Aerosol Resistant Pipette Tips, pre-sterilized and certified DNase- and RNase-free	Size	Cat.#
Promega 10 Barrier Tips	10 boxes, 96/box	A1491
Promega 10E Barrier Tips	10 boxes, 96/box	A1501
Promega 10F Barrier Tips	10 boxes, 96/box	A1511
Promega 20 Barrier Tips	10 boxes, 96/box	A1521
Promega 100 Barrier Tips	10 boxes, 96/box	A1541
Promega 200 Barrier Tips	10 boxes, 96/box	A1551
Promega 1000 Barrier Tips	10 boxes, 48/box	A1561

Tip and Pipette Compatibility Guide

	Size	Pipetman® Pipette	Eppendorf® Pipette	Oxford Benchmate® Pipette	Finnpipette® Pipette
Promega 10	0.5–10µl	P-2; P-10	—	0.5–10µl	0.5–10µl Digital
Promega 10E	0.5–10µl	P-2; P-10	0.5–10µl	0.5–10µl	—
Promega 10F	0.5–10µl	—	—	—	0.5–10µl
Promega 20	2–20µl	P-20	2–20µl	—	—
Promega 100	10–100µl	P-100	10–100µl	10–50µl	5–40µl
Promega 200	50–200µl	P-200	EDP–250µl	40–200µl	40–200µl
Promega 1000	100–1,000µl	P-1000	—	200–1,000µl	200–1,000µl

Reagents and Chemicals to Study RNA		Size	Cat.#
Agarose, LE Analytical Grade*	For electrophoresis of nucleic acids	100g	V3121
		500g	V3125
Bovine Serum Albumin, Acetylated*	Commonly used in many molecular biology applications	10mg	R3961
		400µl (1µg/µl)	R9461
Dextran Sulphate	Used in hybridization solutions	25g	H5401
EDTA, 0.5M (pH 8.0)*	A chelator of divalent cations suitable for many molecular biology applications	100ml	V4231
EDTA, Disodium Salt*	A chelator of divalent cations suitable many molecular biology applications	100g	H5031
Ethidium Bromide Solution, 10mg/ml	Suitable for staining nucleic acids	10ml	H5041
Formamide*	Used for the denaturation of RNA prior to electrophoresis	100ml	H5051
		500ml	H5052
HEPES (free acid)*	Commonly used in many molecular biology applications	100g	H5302
		500g	H5303
5M Sodium Chloride*	Commonly used in many molecular biology applications	1L	V4221
Sodium Chloride*	Commonly used in many molecular biology applications	500g	H5271
SSC, 20X*	Used in hybridization solutions	1,000ml	V4261
TE Buffer, 1X*	Commonly used in many molecular biology applications	100ml	V6231
		500ml	V6232
Tris Base*	Commonly used in many molecular biology applications	100g	H5133
		500g	H5131
		2,500g	H5135
Tris-HCl*	Commonly used in many molecular biology applications	100g	H5121
		500g	H5123
		2,500g	H5125
Nuclease-Free Water*	Commonly used in many molecular biology applications	50ml	P1193
		150ml	P1195

*For Laboratory Use.

References

1. Blackburn, P., Wilson, G. and Moore, S. (1977) Ribonuclease inhibitor from human placenta. Purification and properties. *J. Biol. Chem.* **252**, 5904–5910.
2. Shultz, J., Hurst, R. and Betz, N. (2001) RNasin® Ribonuclease Inhibitor Part I: Characterization of the protein. *Promega Notes* **77**, 8–11.
3. Shultz, J. *et al.* (2001) RNasin® Ribonuclease Inhibitor Part II: A tale of two proteins. *Promega Notes* **77**, 12–15.
4. Schink, M., Mei, B. and Lepinske, M. (1997) A comparison of ribonuclease inhibitors. *Promega Notes* **61**, 30–32.
5. Ogretmen, B. *et al.* (1993) Effects of staining of RNA with ethidium bromide before electrophoresis on performance of northern blots. *BioTechniques* **14**, 932–935.