Technical Bulletin #159: Working with RNA

Living with RNase

Most researchers are acutely aware of the risk of RNase contamination, and we do not want to belabor this point or cause undue worry. We do not routinely fin microcentrifuge tubes used with RNA if they are from unopened bags or from bags in which care was taken to avoid contaminating the tubes. Yet we do consis (even those marketed as being RNase-free), the use of which results in RNA degradation. We do recommend that gloves be worn when handling any reagents which have touched refrigerator handles, door knobs, or pipettors are not RNase-free.) When performing procedures that use RNases (eg. ribonuclease protec care should be taken that pipettors are not contaminated by accident. One potential source of contamination is the metal tip ejector mechanism on the side of t bar when it is necessary to insert the pipettor into a larger vessel where the ejector could come into contact with the walls or contents of the vessel will eliminat

A. Detecting RNase

While contaminating RNase can result in a failed experiment, it is often difficult an time-consuming to determine which solution or piece of equipment is respon No. 1964) allows researchers to identify contaminated reagents and equipment quickly, and nonisotopically. In the RNaseAlert Kit procedure, an optimized RN/ both fluorescent and quenching moieties, is introduced as a target for any contaminating RNase. In the presence of RNase, the substrate is cleaved, releasing fluorescence signal can be detected by eye or with a fluorometer.

B. Getting rid of RNase

If RNase contamination of reagents or equipment is suspected to be a problem, extra precautions may be necessary. Autoclaving tips, tubes and solutions is n Glassware can be baked at 300°C for four hours and plasticware, tubes and most solutions can be DEPC-treated (see below). However, both procedures are t expensive and possibly carcinogenic. As an alternative, Ambion's **RNase*Zap***™ (Cat. No. 9780) can be used to eliminate RNase from glassware, plastic surfac *Zap*™ has been shown to effectively inactivate 5 µg of RNase dried onto the bottom of eppendorf tubes without inhibiting subsequent enzymatic reactions perf contains three ingredients known to be active against RNase. RNase *Zap*™ can be poured onto or wiped over surfaces and works immediately upon contact. T with distilled water and is ready for use.

Treating Solutions with DEPC to Remove RNase

To ensure that solutions are free of RNase contamination, they can be treated with diethylpyrocarbonate (DEPC) [WARNING: DEPC is a suspected carcinoger handling; e.g., always wear gloves and handle under an approved fume hood]. DEPC reacts with histidine residues of proteins and will inactivate RNases. How needs to be removed by heat treatment before the solution is used (DEPC breaks down to CO2 and ethanol). Add DEPC to solutions at a concentration of 0.05 liter); stir or shake into solution, incubate for several hours; autoclave at least 45 minutes, or until DEPC scent is gone. Please be aware that compounds conta (2-Amino-2-hydroxymethyl-1,3-propanediol), will also react with DEPC, and thus should be added only after DEPC treatment is complete. Note: We have obse DEPC and thoroughly autoclaved, caused a 20% inhibition of translation in a reticulocyte lysate. We find that distilled water is generally already RNase-free, an

How to Store RNA

RNA may be stored in a number of ways. For short-term storage, RNase-free H2O (**with 0.1 mM EDTA**) or **TE buffer** (10 mM Tris, 1mM EDTA) may be used. to a year without degradation. Magnesium and other metals catalyze non-specific cleavages in RNA, and so should be chelated by the addition of EDTA if RNA important to use an EDTA solution known to be RNase-free for this purpose (older EDTA solutions may have microbial growth which could contaminate the RN suggested that RNA solubilized in formamide may be stored at -20°C without degradation for at least one year (Chomczynski, 1992).

For long term storage, RNA samples may also be stored at -20°C as ethanol precipitates. Accessing these samples on a routine basis can be a nuisance, how pelleted and dissolved in an aqueous buffer before pipetting, if accurate quantitation is important. An alternative is to pipet directly out of an ethanol precipitate

even suspension. We have found, however, that while this method is suitable for qualitative work, it is too imprecise for use in quantitative experiments. RNA d
probably because it forms aggregates; non-uniform suspension, in turn, leads to inconsistency in the amount of RNA removed when equal volumes are pipette

How to Precipitate RNA

A. Precipitating with alcohol

Precipitating RNA with alcohol (ethanol or isopropanol) requires a minimum concentration of monovalent cations (for example: 0.2 M Na+, K+; 0.5 M NH_4+) (W
concentration has been adjusted, the RNA may be precipitated by adding 2.5 volumes of ethanol or 1 volume of isopropanol and mixing thoroughly, followed by
C. While isopropanol is somewhat less efficient at precipitating RNA, isopropanol in the presence of NH_4+ is better than ethanol at keeping free nucleotides in
precipitated RNA. RNA precipitation is faster and more complete at higher RNA concentrations. A general rule of thumb is that RNA concentrations of 10 µg/m
hours to overnight with no difficulty, but at lower concentrations a carrier nucleic acid or glycogen should be added to facilitate precipitation and maximize recov

B. Precipitating with lithium chloride

Lithium Chloride may also be used to precipitate RNA, and has the advantage of not precipitating carbohydrate, protein or DNA. LiCl is frequently used to rem
copurify with RNA prepared by other methods. A final LiCl concentration of 2-3 M is needed to precipitate RNA (adding an equal volume of 4 M LiCl, 20 mM Tri
well). Note that no alcohol is needed for LiCl precipitation. RNA should be allowed to precipitate at -20°C; precipitation time depends on RNA concentration. It i
precipitate for several hours to overnight. After centrifugation to collect the RNA, pellets can be rinsed with 70% ethanol to remove traces of LiCl. LiCl efficientl
length. While LiCl can effectively precipitate RNA from more dilute solutions, for best results, the RNA concentration should exceed 200 µg/ml.

Incorporation and Yield

"Percent incorporation" is calculated by comparing the amount of radioactivity incorporated into synthesized RNA with the total amount of radioactivity in the re
precipitation (see below) but can also be done by simply counting an aliquot of the transcription reaction before and after removal of unincorporated nucleotides
comparison must be adjusted to represent equivalent aliquots. Unincorporated nucleotides may be removed by precipitation using LiCl or NH4OAc and EtOH (
reaction over an RNase-free Sephadex column (e.g., Ambion's **NucAway™ column**), or by gel purification.

The amount of radioactivity incorporated into RNA may also be determined by precipitation with trichloroacetic acid (TCA), filtration, and counting in a liquid sci
RNA labeling reaction to 98 µl of water containing 10 µg of carrier DNA or RNA. To this add 2 ml of cold 10% TCA, vortex and incubate on ice 5 minutes. Colle
vacuum through GF/C glass fiber filters. Wash the sample tube twice with 2 ml 10% TCA and once with 2 ml of 95% ethanol, passing the washes through the f
placed in vials with liquid scintillation cocktail and counted. Note: Both RNA and DNA may be precipitated using this method.

Since percent incorporation of a radiolabeled nucleotide is directly proportional to yield, the actual yield of a transcription reaction is equivalent to that proportio
example, Ambion's **MAXIscript™ Kit** reactions have a theoretical 100% yield of 77 ng when the transcription reaction contains a limiting nucleotide concentrat
reaction the percent incorporation was 80%, then 0.80 X 77 ng or 62 ng of labeled RNA were synthesized.

Some ribosomal subunit size relationships within the eukaryotes are illustrated in Table 1. Both 18S and 28S rRNA contain modified nucleotides, including met
and 37 for 18S; 71 and 60 for 28S, respectively) .

	Avg. # of b
Organism	18S
Drosophila	1976
Rat	1874
Human	1868

Table 1. Ribosomal Subunit Sizes in Representative Eukaryotes.

RNA Size Markers

Ambion offers several different ranges of RNA size markers that can be obtained unlabeled for staining with EtBr or biotinylated for subsequent secondary dete (Cat. No. 7140 - unlabeled, #7175 - biotinylated) contains 5 transcripts evenly spaced between 100 -500 nt, which are ideal for ribonuclease protection assays RNA Century Markers can also be obtained as DNA templates (Cat. No. 7780 and 7782) for the synthesis of radiolabled RNA markers in an in vitro transcriptio **Marker Set** (Cat. No.7150 - unlabeled, #7170 - biotinylated) contains 10 transcripts ranging from 0.5-9.0 kb for use with Northern analysis.

RNA transcripts and double-stranded DNA markers (e.g. pUC 19/Hpa II, Cat. No. **7760** and **7770**) can also be end-labeled with polynucleotide kinase (5' end-l filling reaction) and denatured, for use as labeled size markers.

Other guides to RNA size and migration position are the xylene cyanol and bromophenol blue dyes present in most loading buffers, and rRNA species present Northern analysis. The migration position of the dyes included in loading buffers is affected both by gel percentage and composition (denaturing vs. nondenatu total RNA samples. Both the 18S and 28S species are strongly visible in Northern gels stained with EtBr or UV-shadowed. The table above gives their sizes in

References

- Chomczynski, P. (1992) Solubilization in formamide protects RNA from degradation. Nuc. Acids Res. 20:3791-3792.
- Wallace, D.M. (1987) Precipitation of Nucleic Acids. Methods of Enzymology 152:41-46.

Ordering Information

製品番号（カタログ番号）	**AM1964**
製品名	**RNaseAlert™ Lab Test Kit**
大小	25 reactions
価格 (JPY)	40,100 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM1966**
製品名	**RNaseAlert™ QC System**
大小	480 assays
価格 (JPY)	171,900 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM7000**
製品名	**THE RNA Storage Solution**
大小	10 x 1 mL
価格 (JPY)	10,000 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM7001**
製品名	**THE RNA Storage Solution**
大小	50 mL
価格 (JPY)	10,000 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM7145**
製品名	**Century™-Plus RNA Markers**
大小	1 tube
価格 (JPY)	32,900 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM7150**
製品名	**Millennium™ RNA Markers**
大小	50 µL
価格 (JPY)	39,700 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM7760**
製品名	**pUC19 DNA (Sau3A I digested)**
大小	50 µg
価格 (JPY)	41,700 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM7780**
製品名	**RNA Century™ Marker Templates**
大小	5 µg
価格 (JPY)	41,700 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM7782**
製品名	**RNA Century™-Plus Marker Templates**
大小	5 µg
価格 (JPY)	41,700 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM9480**
製品名	**LiCl Precipitation Solution (7.5 M)**
大小	100 mL
価格 (JPY)	19,500 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM9780**
製品名	**RNase*Zap*™ RNase Decontamination Solution**
大小	250 mL
価格 (JPY)	14,500 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM9782**
製品名	**RNase*Zap*™ RNase Decontamination Solution**
大小	6 x 250 mL
価格 (JPY)	64,500 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM9784**
製品名	**RNase*Zap*™ RNase Decontamination Solution**
大小	4 L
価格 (JPY)	127,900 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM9786**
製品名	**RNase*Zap*™ RNase Decontamination Wipes**
大小	100 sheets
価格 (JPY)	14,500 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM9788**
製品名	**RNase*Zap*™ RNase Decontamination Wipes Refill**
大小	300 sheets
価格 (JPY)	38,700 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM9860**
製品名	**TE, pH 7.0, RNase-free**
大小	10 x 1 mL
価格 (JPY)	10,000 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM9861**
製品名	**TE, pH 7.0, RNase-free**
大小	50 mL
価格 (JPY)	10,000 (**お問い合わせください ›**)
量	-

製品番号（カタログ番号）	**AM9912**
製品名	**EDTA (0.1 mM), pH 8.0, RNase-free**
大小	50 mL
価格 (JPY)	9,800 (**お問い合わせください ›**)
量	-

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