March 13,04

RNA info:

Starting template

The efficiencies of reverse transcription and PCR are highly dependent on the quality and quantity of the starting RNA template.

It is important to have intact RNA as starting template. Even trace amounts of contaminating RNases in the RNA sample can cause RNA cleavage, resulting in shortened cDNA products. Chemical impurities, such as protein, poly-anions (e.g., heparin), salts, EDTA, ethanol, phenol, and other solvents, can affect the activity and processivity of the reverse transcriptases and the *Taq* DNA polymerase. To ensure reproducible and efficient RT-PCR, it is important to determine the quality and quantity of the starting RNA.

For best results, we recommend starting with RNA purified using silica-gel–membrane technology. For example, RNeasy® Kits, QIAamp® Viral RNA Kits, and the QIAamp RNA Blood Mini Kit can be used to isolate RNA from a variety of starting materials and provide high-quality RNA ideal for use in reverse-transcription and RT-PCR applications.

Alternatively, high-quality mRNA can be used, purified, for example, with Oligotex™ mRNA and Oligotex Direct mRNA Kits. For more information about RNeasy, QIAamp, and Oligotex products, please call one of the QIAGEN Technical Service Departments or local distributors listed on the last page.

Storage of RNA

Purified RNA may be stored at **–20°C or –70°C** in RNase-free water. Under these conditions, no degradation of RNA is detectable for at least 1 year.

Determining concentration and purity of RNA

• The concentration of RNA should be determined by measuring the absorbance at 260 nm (*A*260) in a spectrophotometer. Note that absorbance measurements cannot discriminate between DNA and RNA.

• To determine RNA concentration, we recommend dilution of the sample in water since the relationship between absorbance and concentration (*A*260 reading of 1 = 40 µg/ml RNA) is based on an extinction coefficient calculated for RNA in water. To ensure significance, readings should be between 0.1 and 1.0.

• The ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity. To determine RNA purity, we recommend measuring absorbance in10 mM Tris·Cl, pH 7.5. Pure RNA has an *A*260/*A*280 ratio of 1.9–2.1* in 10 mMTris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer using the same solution.

Integrity of RNA

The integrity and size distribution of total RNA can be checked by denaturing agarose-gel electrophoresis and ethidium bromide* staining. The respective ribosomal bands (Table) should appear as sharp bands on the stained gel. The intensity of the 28S ribosomal RNA band should be approximately twice that of the 18S rRNA band. If the ribosomal bands in a given lane are not sharp, but appear as a smear of lower molecular weight species, it is likely that the RNA sample suffered major degradation during preparation.

Table. Sizes of ribosomal RNAs from various sources

Source	rRNA	Size (kb)
Mouse	18S	1.9
	28S	4.7
Human	18S	1.9
	28S	5.0

DEPC (diethyl pyrocarbonate) for RNase inactivation

This info was quoted from Qiagen one step RT PCR manual (cat#: 210212)

RNase and Handling RNA:

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to degrade RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently, and keep tubes closed whenever possible.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% (v/v) to inactivate RNases on glass or plasticware, or to create RNase-free solutions and water.

DEPC inactivates RNases by covalent modification. **Add 0.1 ml DEPC to 100 ml of the solution** to be treated and shake vigorously to bring the DEPC into solution. Let the solution **incubate for 12 hours at 37°C. Autoclave for 15 minutes** to remove any trace of DEPC. **DEPC will react with primary amines and cannot be used directly to treat Tris buffers**. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240°C for four or more hours (overnight, if more convenient) before use. **Autoclaving alone will not fully inactivate many RNases.**

Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases. Nondisposable plasticware should be treated before use to ensure that it is RNase-free. **Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water.** Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.