Safe RNA concentration without loss using RNAstable™

Summary:
RNAstable is designed to prevent degradation of RNA samples. Based on its unique stabilization properties it can also be used to concentrate RNA without loss during recovery. Currently, traditional ethanol precipitation is the most commonly used method for concentrating RNA. Precipitation is associated with large sample loss of more than 50% and also increased salt concentrations. RNAstable based concentration is rapid and efficient with close to 100% recovery and is particularly useful for samples containing limited quantities of RNA.

Introduction:
RNAstable is a novel RNA stabilization product developed to protect RNA samples from degradation during dry storage at room temperature. RNAstable is based on the natural principles of anhydrobiosis (meaning "life without water"), a biological mechanism employed by some multicellular organisms that enables their survival while dry for over 100 years. Anhydrobiotic organisms such as tardigrades and brine shrimp protect their DNA, RNA, proteins, membranes and cellular systems for survival and can be revived by simple re-hydration. By exploiting these unique characteristics, RNAstable was designed to stabilize RNA *dry* at ambient temperatures. Complete sample recovery is achieved by a simple one-step rehydration method, thus eliminating losses associated with traditional ethanol precipitations. RNAstable can also be used to conveniently concentrate RNA samples with significantly improved yields; this application is particularly advantageous for concentrating dilute samples containing limited amounts of RNA. Purified RNA in up to 100 µl total volume can be applied directly into RNAstable and dried. Sample concentration is accomplished by simply rehydrating in the desired volume. Since there is no sample loss resulting from extraneous precipitation and wash steps, recovery yields are greatly improved using RNAstable's one-step rehydration sample recovery and concentration protocol. Rehydrated samples can be used directly in downstream applications with no further purification, thus eliminating lengthy procedures and consequent sample loss. Extensive studies indicate no inhibition or loss due to degradation following storage of precious labile RNA samples in RNAstable.

Materials and Methods:
Experiments were designed to directly compare recovering and concentrating RNA samples with RNAstable to traditional ethanol precipitation methods. An aliquot of 50 µl of 293T total RNA (20 ng/µl) was applied to RNAstable in the 1.5 ml standard microfuge tube format (Biomatrica catalog #93221-001) and dried in a vacuum concentrator for 30 min at room temperature (no heat). The dried sample was then kept at room temperature until ready for use.* An identical amount (1000 ng) of the same RNA was concentrated using traditional ethanol precipitation. A 1/10th volume of sodium acetate at pH 5.2 and 2.5 volumes of 100% ethanol were added to the RNA sample and stored at -80°C for 1 hour. The frozen sample was centrifuged for 15 min at 12,000x*g* to pellet the sample and then washed once with 70% ethanol to remove any residual salt. The pellet was dried for 10 min at room temperature in a laminar flow hood. Both the RNAstable sample and ethanol precipitated sample were rehydrated in 10 µl of DEPC water. The yield of each recovered RNA sample was quantified by A_{260} absorbance readings on a UV spectrophotometer (Beckman). Results are shown as shown in Figure 1. The final concentration of the RNA recovered from RNAstable was 91.2 ng/µl, while the concentration of the ethanol precipitated sample was 42.9 ng/µl.

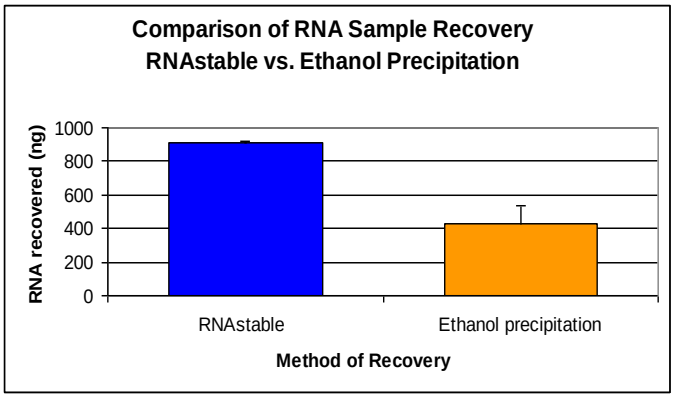

Figure: 1 µg of total RNA was concentrated either using RNAstable technology or ethanol precipitation.

Results and Discussion:

A comparison of sample recovery methods indicates significantly improved yields using RNAstable as compared to traditional ethanol precipitation for recovering and concentrating nucleic acids. Recovery using RNAstable was >90% compared to 42% using conventional ethanol precipitation. Samples dried into RNAstable can be recovered and concentrated by a simple one-step rehydration protocol. Since the rehydrated samples can be used directly without further purification, there is no sample loss typically associated with ethanol precipitations and subsequent wash steps that are necessary to remove residual salt. Rehydrated RNA samples containing RNAstable can be used directly for cDNA synthesis, RT-PCR, quantitative PCR, agarose gel electrophoresis, bioanalyzer analysis and microarray analysis without any inhibition or interference. RNAstable thus eliminates the need for laborious protocols and resultant sample loss associated with traditional ethanol precipitation methods, while simultaneously protecting samples from degradation, even after extended periods of storage at room temperature.

*For best results, it is recommended that samples stored over 1 month be kept either in a desiccating chamber or heat-sealed, moisture barrier bag with a desiccant packet and then stored at room temperature (15-25˚C) with ≤50% relative humidity.