Ambion
THE RNA COMPANY

Technical Bulletin #160

The Use of LiCl Precipitation for RNA Purification

LiCl has been frequently used to precipitate RNA, although precipitation with alcohol and a monovalent cation such as sodium or ammonium ion is much more widely used. LiCl precipitation offers major advantages over other RNA precipitation methods in that it does not efficiently precipitate DNA, protein or carbohydrate (Barlow et al., 1963). It is the method of choice for removing inhibitors of translation or cDNA synthesis from RNA preparations (Cathala et al., 1983). It also provides a simple rapid method for recovering RNA from in vitro transcription reactions.

Ambion provides LiCl as an RNA recovery agent in its MEGAscript® and mMESSAGE mMACHINE® large scale in vitro transcription kits. However, while providing telephone technical service, we have noticed that many users are reluctant to use LiCl, presumably because there is not good data in the literature describing its properties. We have conducted a systematic study of the use of LiCl and find that it is a very effective method for precipitating RNA, especially from in vitro transcription reactions.

The three key variables we studied were: (a) the temperature at which the precipitate is allowed to form, (b) the concentration of the RNA and the lithium chloride used and, (c) the time and speed of centrifugation used to collect the precipitated RNA. All of these variables have been explored and are discussed below. We find that LiCl precipitated RNA samples prepared in this way require no further purification for use in hybridization and in vitro translation reactions. It has been reported that lithium chloride is unsuitable for cell free translations due to the inhibition of chloride ions (Maniatis, et al., 1989); However, we have not been able to document any deleterious effect in either translation or microinjection experiments. Another advantage is that lithium precipitation efficiently removes unincorporated NTPs, which allows for more accurate quantitation by UV spectrophotometry.

Experimental Procedures

Unlabeled RNA transcripts with the lengths of 100, 300, and 500 bases were synthesized in large amounts using the MEGAscript in vitro Transcription Kit. Lithium chloride was used to precipitate the RNA followed by resuspension in water. The concentration of each RNA was determined by spectrophotometry. An additional set of labeled transcripts were synthesized in the presence of 50 µCi of alpha-[^{32}P] UTP (800 Ci/mmol) to produce the three RNA transcripts with a specific activity of 3.3 x 10^6 cpm/µg.

Comparison of Lithium Chloride and Ammonium Acetate/Ethanol

In preliminary experiments, we compared the precipitation efficiency of 2.5 M lithium chloride with 0.5 M ammonium acetate and 2.5 volumes of ethanol with RNA transcripts of 100 and 300 bases in length. The average recovery with the lithium chloride was 74% compared to 85% with the ethanol. Gel analysis of the precipitated products suggested that the lithium chloride may not precipitate the smallest RNA fragments as efficiently as the ethanol. This can be an advantage when preparing labeled probe for ribonuclease protection assays in that the lithium chloride precipitated product will give a cleaner band on gel analysis, especially with non-gel purified probe.

Precipitation Parameters of Lithium Chloride RNA Concentration

Decreasing amounts of each size of RNA were precipitated using a constant concentration of 2.5 M LiCl to determine if there is a threshold of precipitation for a given size and concentration of RNA. The three stocks of non-radioactive RNA mixed with tracer labeled RNA (5 x 10^4 cpm) were aliquotted in tubes. Water and then lithium chloride were added to a final volume of 50 µl, with a constant concentration of 2.5 M lithium chloride. Each size transcript was tested separately to observe possible size effects on precipitation efficiency. All samples were chilled 30 minutes at -20°C then centrifuged for 15 minutes at 16,000 x g at 4°C. The supernatant was removed by aspiration and dried for 10 minutes. The pellets were resuspended in 10 µl of gel loading buffer (80% formamide, 0.1% bromophenol blue, 0.1% xylene cyanol, and 1mM EDTA) and heated for 5 minutes at 95°C. A portion of each sample was run on a 4% PAGE-urea gel. The gel was dried and exposed directly to film for 30 minutes. **Figure 1** shows the effect of RNA concentration on lithium chloride precipitation of the 100 base transcript. It appears that RNA as small as 100 nucleotides and as dilute as 5 µg/ml can be efficiently precipitated by lithium chloride. This was a surprising result since it is generally thought that RNA must be at relatively high concentrations in order to be efficiently precipitated with lithium chloride.

Lithium Chloride Concentration

The effect of lithium chloride concentration on precipitation efficiency was tested on three different sized transcripts. Each size transcript was kept at a constant concentration of 1 µg/ml while the lithium chloride was tested at 2.5, 1.0, and 0.5 molar concentrations. Labeled RNA (5 x 10^4 cpm) was also added as a tracer. The samples were centrifuged 10 minutes at 4°C, aspirated and dried. The pellets were resuspended in 10 µl of gel loading buffer, heated for 10 minutes at 95°C, and a portion of each was run on a 4% PAGE-urea gel. The gel was dried and exposed for 30 minutes without intensifying screens. **Figure 2** shows the effect of lithium chloride on precipitation of the 300 base transcript. It appears that lithium chloride is effectively precipitating RNA at a 0.5 molar concentration and recovery was similar at all concentrations of lithium chloride. Lane 5 is a zero lithium chloride control to analyze the effect of centrifugation.

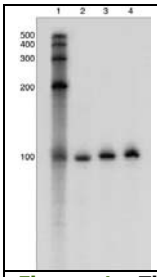

Figure 1. The Effect of RNA Concentration on Lithium Chloride Precipitation. Lane 1, RNA size standards. Lane 2, 5µg/ml RNA, Lane 3, 50 µg/ml RNA, and Lane 4, 500 µg/ml of RNA.

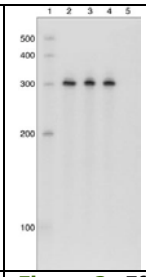

Figure 2. Effect of Lithium Chloride Concentration on Precipitating RNA. Lane 1, RNA size standards. Lane 2, 2.5 M LiCl. Lane 3, 1.0 M LiCl. Lane 4, 0.5 M LiCl, and lane 5, no LiCl.

Chilling Time

The RNA was kept at a constant concentration of 1 µg/ml, with 1.0 M lithium chloride. The length of time for precipitation was tested at 0, 0.5, and 1.0 hour. The 0.5 and 1.0 hour time points were incubated at -20°C and 25°C to test precipitation time and temperature independently. Samples were prepared as before, and visualized on a 4% PAGE-urea gel. In **Figure 3**, it appears that allowing precipitation to occur for a 30 minute period is more efficient than immediate centrifugation; compare Lane 2 to Lane 3. Although it appears there is no difference in precipitating 30 or 60 minutes at -20°C and 25°C, as seen in Lanes 3-6, it is advisable to precipitate at -20°C for 30 minutes to lower the activity of any possible RNases that might be present.

Centrifugation Time

Using a constant concentration of 1 µg/ml RNA, in a volume of 50 µl with 1.0 M lithium chloride, samples were centrifuged for 0.5, 1, 2, 5, 10, and 20 minutes at 4°C at 16,000 x g. The different sized transcripts, with radioactive RNA, were tested independently. **Figure 4** shows that centrifugation time is a major factor in recovery of RNA. As little as 50 ng of RNA can be

quantitatively recovered by centrifugation at 16,000 x g for 20 minutes at 4°C. Lanes 2-7 show decreasing recovery as spin time is lowered.

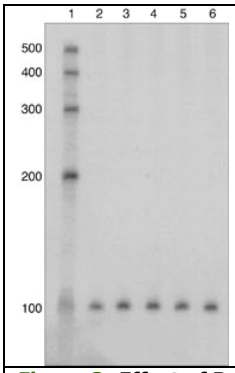

Figure 3. Effect of Precipitation Temperature Using Lithium Chloride. Lane 1, RNA size standards. Lane 2, RNA centrifutged immediately without chilling. Lane 3, RNA chilled at -20°C for 30 minutes before centrifugation. Lane 4, RNA incubated at 25°C for 30 minutes to test precipitation time independently of chilling. Lane 5, RNA chilled at -20°C for 1 hour. Lane 6, RNA incubated at 25°C for 1 hour.

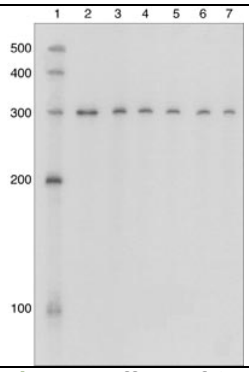

Figure 4. Effects of Centrifugation Time in Precipitating RNA. Lane 1, RNA size standards. Lane 2, RNA centrifuged for 20 minutes, Lane 3, 10 minutes, Lane 4, 5 minutes, Lane 5, 2 minutes, Lane 6, 1 minutes, and Lane 7, 30 seconds.

Discussion

The use of lithium chloride in RNA precipitation is a fast, convenient method of isolating transcripts from in vitro transcription reactions with very low carry over of unincorporated nucleotides. A major advantage of lithium chloride is that it does not efficiently precipitate either protein or DNA. For some applications, gel purification may be necessary, as in a ribonuclease protection assay. For in vitro or in vivo translation, the lithium chloride method may be preferable to ethanol precipitation since full-length transcripts are often preferentially recovered. Moreover, RNAs precipitated by this method give more accurate values when quantitated by UV spectroscopy since lithium chloride is so effective at removing free nucleotides. This strategy is similar to the use of isopropanol rather than ethanol to precipitate nucleic acids. Isopropanol is less efficient than ethanol at precipitating nucleotides and thus, gives more accurate values when RNA concentration is quantitated by UV spectrophotometry. Contrary to previously published reports, we find that lithium chloride does not appear to preferentially precipitate higher molecular weight RNA rather than smaller RNA. Lithium chloride precipitations using mixtures of equal amounts of RNA of lengths 100, 200, 300, 400, and 500 bases (RNA Century™ Size Standards) showed that all sizes were precipitated equally well (data not shown). Since it was thought that the larger sizes might aid in the precipitation of smaller size transcripts, the experiments in this paper were performed using each size of transcript separately. No differences in precipitating a single size of RNA (e.g. 100 bases) as compared to a mix of all sizes of the RNA markers was seen. It should be noted, however, that some small RNAs such as tRNAs are not efficiently precipitated by lithium chloride. This is likely due to the high degree of secondary structure in tRNA. While we recommend the routine use of lithium chloride for precipitating RNA from solutions containing at least 400 µg/ml RNA, we are cautious about recommending its use with lower concentrations of RNA until we have tested its use with a wider range of RNAs.

References

- Barlow, J.J., Mathias, A.P., Williamson, R., and Gammack, D.B., (1963). A Simple Method for the Quantitative Isolation of Undegraded High Molecular Weight Ribonucleic Acid. *Biochem. Biophys. Res. Commun.* **13**:61-66.
- Cathala, G., Savouret, J., Mendez, B., West, B.L., Karin, M., Martial, J.A., and Baxter, J.D., (1983). A Method for Isolation of Intact, Translationally Active Ribonucleic Acid. *DNA* **2**:329-335.
- Maniatis, Sambrook, Fritsch, (1989). *Molecular Cloning: A Laboratory Manual 2nd ed.*, Vol. 3, Appendix E.12.

Cat#	Product Name	Size
9480	7.5 M LiCl Precipitation Solution	100 ml