Sodium Acetate Precipitation of Small Nucleic Acids

This precipitation can be used to concentrate small nucleic acids from dilute solutions. When work with extremely low concentration of nucleic acids, we recommend overnight sodium acetate/ethanol precipitation with a carrier such as linear acrylamide or glycogen for maximum nucleic acid recovery from the Buffer. We do not recommend using glycogen as a carrier for samples that will be used for microarray analysis.

DNA and RNA precipitation using alcohol is based on the principle of salting out in the presence of salts that renders nucleic acids preferentially to become insoluble and the precipitate is collected by centrifugation. The process also purifies the DNA & RNA leaving alcohol soluble salts, organic solvents and detergents.

The backbone of DNA & RNA is negatively charged due to the phosphate groups of the phosphodiester linkages and is thus hydrated and readily soluble in neutral water. Addition of cationic salts and ethanol or isopropanol disrupts the hydrate shell and promotes ionic bond formation between the negatively charged phosphate groups and positively charged ions effectively neutralization of the DNA or RNA molecule leading to precipitation.

Ethanol versus Isopropanol

Ethanol and isopropanol are both used for precipitation of nucleic acids. We recommend, Ethanol is used at 3 - 5 volumes for RNA and isopropanol at 2 - 4 volume of DNA and RNA solutions, for maximal efficiency.

Isopropanol has the advantage of requiring less volume and is typically used when precipitating large volumes of nucleic acid solutions. If necessary, less volume, such as, 1 volume of isopropanol is added to 1 volume of DNA solution. In contrast, 2 volumes of ethanol is standard for DNA and 2.5 volumes for RNA solutions. However, isopropanol has the disadvantage of co precipitating more salts and is less volatile compared to ethanol and is slow to air dry increasing the risk of alcohol carry-over into the final sample. Nevertheless, the higher amount alcohol is useful for smaller RNA fragments.

Carriers /Coprecipitants

DNA and RNA are quite effectively precipitated at a concentration of 100-200 ng/mL by standard ethanol precipitation and usually a pellet is also visible at this concentration. Quantitative precipitation is usually not achieved below 100 ng/mL in addition a pellet is not visible thus making it difficult to accomplish ethanol precipitation.

Traditionally tRNA and glycogen were used as carrier to aid precipitation and visibility of the pellet. Both of these are from biological sources and thus contain traces of nucleic acids and their use requires extensive removal of nucleic acids and nucleases. Linear poly acrylamide (LPA) is a synthetic inert carrier and thus free of nucleic acids and nucleases.

Glycogen and linear acrylamide (LPA) both have minimal inhibitory effect on most molecular biology applications and thus can be used quite confidently.

Salts

Most common salts used in ethanol precipitations are sodium acetate, ammonium acetate, sodium chloride and lithium chloride. 3M sodium acetate pH of 5.2-5.5 solution is the standard reagent for nucleic acid precipitation in most laboratories.

Frequently used salts are listed below with some attributes.

Salt	Stock	Final Molarity	Attributes
Sodium acetate	3M, pH 5.5	0.3M	Precipitates proteins so should be avoided if solution contains high amount of protein.
Sodium Chloride	3M or 5M	0.2-0.3M	Preferred salt in high detergent content solutions. SDS remains soluble in 70% ethanol and ensures detergent free DNA precipitation.
Ammonium Acetate	7.5M	2.5M	Preferred salt in high dNTP's and oligosaccharides content solutions as these remain in solution. Avoid using if kinasing as ammonium ions inhibit polynucleotide kinase.
Potassium Acetate	3M, pH 5.5	0.3M	1. Potassium acetate is particularly useful in the precipitation of RNA for cell-free translation as it avoids the addition of sodium ions. 2. Precipitates proteins so should be avoided if solution contains high amount of protein. 3. Avoid using with DNA and RNA solutions containing SDS. Potassium salt of SDS is very insoluble.
Lithium Chloride	7.5M	2.5M	Useful in precipitation of RNA 300 nucleotides and above without ethanol precipitation. Avoid if downstream application involves translation. Ethanol precipitation will precipitate DNA, RNA, oligos and proteins.

Common Contaminant and Recommended Salt Usage		
Contaminant	**Salt**	**Recommended Protocol**
High Protein	Ammonium acetate 2.5M precipitates proteins in the absence of ethanol while DNA remain in solution	Use ammonium acetate 2.5M final concentration and without adding ethanol centrifuge at 12 K rpm for 10 minutes. Proteins should be precipitated. Decant solution to fresh tube and proceed with ethanol precipitation.
High Carbohydrate and high dNTP	Ammonium acetate 2.5M with ethanol precipitates DNA while carbohydrates and dNTP's remain in solution	Follow standard ethanol precipitation procedure with 70% ethanol wash after precipitation.
High Detergent	Sodium chloride 0.2M. Detergents including SDS remain in solution in 0.2M sodium chloride- 65-70% ethanol solution.	Follow standard ethanol precipitation procedure with 70% ethanol wash after precipitation.

Chilling Temperature & Time

Classic chilling temperature is -20°C for 10-15 minutes, this may be preferred when the DNA concentration is lower than 100 ng but usually not necessary as precipitation occurs very rapidly when DNA concentration is not limiting.

Precipitation chilling time varies amongst different laboratories from –70°C, -20°C, 0°C, or room temperature for periods of 5 min to overnight. For low DNA concentrations longer centrifugation times of up to 20 minutes may be required for efficient recovery.

At the lower temperatures, the viscosity of the alcohol is greatly increased and centrifugation for longer times may be required to effectively pellet the precipitated DNA. The efficiency of precipitation for small concentrations or amounts of DNA may be increased by incubation at –70°C, but these solutions should be brought to 0°C before centrifugation.

Centrifugation Speed & Time

Generally centrifugation at 12K rpm for 5-10 minutes is sufficient for DNA and RNA precipitation. Longer spin time is effective in recovery of low concentration of nucleic acids but increasing beyond 20 minutes is not usually necessary.

Drying

Care should be observed in drying the precipitated DNA and RNA to completely evaporate the ethanol or isopropanol before reconstitution. Nucleic acids should not be over dried and speedvac with heat should be avoided or performed for an observed short duration sufficient to evaporate the alcohol. Usually leaving the tubes open on the bench for a few minutes is sufficient.

Sodium Acetate Protocol

Sodium Acetate precipitates proteins so should be avoided if solution contains high amount of protein. 0.3 M Sodium Acetate final concentration and 3 to 5 volume ethanol. Routine precipitation of DNA or RNA at Concentrations ≥20 ng/mL

Carrier Usage: 1 µL (10 µg) of 10mg/mL glycogen solution is adequate for 500 µL DNA or RNA solution. If coprecipitate with LPA, 1 -2µL (5-10 µg) of 5mg/mL Linear polyacrylamide (LPA) solution is adequate for 500 µL DNA or RNA solution. Linear polyacrylamide pellet does not stick tightly on the bottom of microfuge tube. Be careful not to discard pellet when you remove supernatant. 大多数情况不需要加 carriers，会引入 glycogen 杂质，只有很稀样品才需要。

1. Add 2 µl carrier (e.g. linear acrylamide or glycogen) to the nucleic acid solution and mix well. This step is optional.
2. Add 1:10 volume of 3 M sodium acetate and mix thoroughly; for 230 µl of sample buffer, this will be 23 µl of 3 M sodium acetate. (sodium acetate 可以多加，到 2:10 体积都没有问题，太多也没必要)
3. Add 4 volumes of 100% ethanol or 3 volumes of isopropanol; i.e. 1 ml 100% ethanol for 230 µl sample Buffer plus 23 µl 3 M sodium acetate.
4. Mix thoroughly and incubate at –20°C overnight (16 hr). We found that overnight incubation is important for maximizing recovery in this precipitation. 大部分情况-80°C 冷却 10-20 分钟就够。
5. Microcentrifuge at top speed for 10 min at 4° C.
6. Carefully remove and discard the supernatant.
7. Resolve the pellet in PBS or nuclease-free water. Repeat the precipitation step 2-6 once if this is for purification RNA from dye labeling reaction. The free chemical dye can be completely removed after the second precipitation.
8. Wash the pellet by adding 500 µl 80% cold ethanol. Microcentrifuge at top speed for 10 min at 4°C or room temp, and carefully remove and discard the 80% ethanol. This step is optional.
9. To remove the last traces of alcohol, quickly re-spin the tube, and aspirate any residual fluid with a very fine tipped pipette or syringe needle. Air dry the pellet.