

Concentration of DNA in Solution

DNA is most commonly precipitated using ethanol. Isopropanol can be used in the place of ethanol if the total volume of solution must be kept at a minimum, since one volume of isopropanol can be used in the place of two volumes of ethanol. However, isopropanol is less volatile than ethanol and solvents such as sucrose and NaCl coprecipitate more easily with the DNA, making ethanol precipitation preferable unless the volume must be kept at a minimum. Buffers containing >1mM phosphate or >10mM EDTA should not be used for ethanol precipitation as they coprecipitate with nucleic acids. These agents should be removed by conventional or spun-column chromatography before ethanol precipitation is performed. The efficiency of recovery of very short (<100 nucleotides) can be improved by the addition of MgCl₂ to a final concentration of 0.01M.

There are three major variables to consider:

1. **Temperature:** Until a few years ago, precipitations were routinely carried out at low temperatures (e.g. dry ice/EtOH baths). It is now known that this is unnecessary. At 0°C DNA concentrations as low as 20 ng/ml can be quantitatively recovered by centrifugation in the absence of carrier DNA.

2. **Type of monovalent cations used to precipitate mixture:** The most commonly used:

- **Ammonium Acetate:** Reduces coprecipitation of dNTPs. However, ammonium ions inhibit T4 polynucleotide kinase, and therefore its use should be avoided with DNA that is to be phosphoralated. Stock concentration: 10 M. Final concentration: 2-2.5 M.

- **Lithium Chloride (LiCl):** Useful when using high concentrations of ethanol (e.g. RNA precipitations). Its use should be avoided, however, when RNA is to be used in a cell free translation system. Stock concentration: 8 M. Final concentration: 0.8 M.

- **Sodium Chloride (NaCl):** Useful if SDS has been used to isolate DNA from cells, as SDS will go into solution in 70% ethanol. Stock concentration: 5M. Final concentration: 0.2 M.

- **Sodium Acetate pH 5.2(NaOAc):** Used most routinely in procedures. Stock concentration: 3 M. Final concentration: 0.3 M

3. **Time and Speed of Centrifugation:** As little as 20 ng of DNA in a 1 ml volume can be quantitatively recovered in the absence of carrier DNA by centrifugation at 12,000 g/ 15 minutes/ 0-4°C in a microfuge. At lower concentrations or with very small fragments (< 100 nucleotides) more extensive centrifugation is required. The pellet can then be washed with 70% ethanol and recovered by centrifugation at 12,000 g/ 10 minutes/ 0-4°C. This procedure allows the recovery of picogram amounts of nucleic acid without the use of carrier DNA. [NOTE: Ken adds with low concentrations of DNA the longer you spin, the better.]

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1. Estimate the volume of DNA solution.

2. Adjust the monovalent cation concentration appropriately by dilution with TE or by the addition of salt solution.

3. Add exactly 2 volumes of ice cold ethanol (or one volume of isopropanol). Store on ice and allow DNA to precipitate for 15 - 30 minutes. For small (<100 nucleotides) or dilute (<0.1µg/ml) solutions of DNA keep solution on ice for at least an hour and add MgCl₂ to a final concentration of 0.01M. NOTE: Ken adds that some protocols allow up to 4 volumes of EtOH. Volume of EtOH addition will vary depending upon the experimental situation, so keep this in mind.
4. Recover DNA by centrifugation at 0°C (12,000 g x 10 minutes is sufficient for most DNA unless the solution contains small fragments or the solution is dilute. In this case longer centrifugation is desirable).
5. Remove the supernatant without disturbing the pellet. (It is best to save the supernatant from important samples until the recovery of DNA from the precipitate is verified).
6. Wash pellet with 70% ethanol and recentrifuge at 12,000 rpm x 2 minutes. Repeat.
7. Allow ethanol to evaporate and dissolve pellet (sometimes invisible) in desired volume of TE 7.6 - 8.0. Rinse the walls of the tube well with buffer.

Concentration of Nucleic Acids by Extraction with Butanol

During extraction of aqueous solutions with solvents such as secondary butyl alcohol (2-butanol) or *n*-butyl alcohol (1-butanol), some of the water molecules are partitioned into the organic phase. By carrying out several cycles of extractions, the volume of a nucleic acid solution can be reduced significantly. This method of concentration is used to reduce the volume of dilute solutions to the point where the nucleic acid can easily be recovered by precipitation with ethanol.

1. Measure the volume of the nucleic acid solution and add an equal volume of 2-butanol. Mix the solution well by vortexing. NOTE: Addition of too much 2-butanol can result in the removal of all the water and precipitation of the nucleic acid. If this happens, add water to the organic phase until an aqueous phase (which should contain the nucleic acids) reappears.
2. Centrifuge the solution at room temperature at 12,000g for 20 seconds in a microfuge or at 1600g for 1 minute in a bench-top centrifuge. Use an automatic micropipetter to remove and discard the upper (2-butanol) phase.
3. Repeat steps (1) and (2) until the desired volume of aqueous phase is achieved.

Because 2-butanol extraction does not remove salt, the salt concentration increases in proportion to the reduction in volume of the solution. The nucleic acid can be transferred to the desired buffer by spun-column chromatography or by precipitation with ethanol.

Taken from: Sambrook et.al Molecular Cloning , A Laboratory Manual; second edition; pp. E10 - E16. Look here for more detailed information.

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