Ethanol Precipitation of DNA and RNA: How it Works

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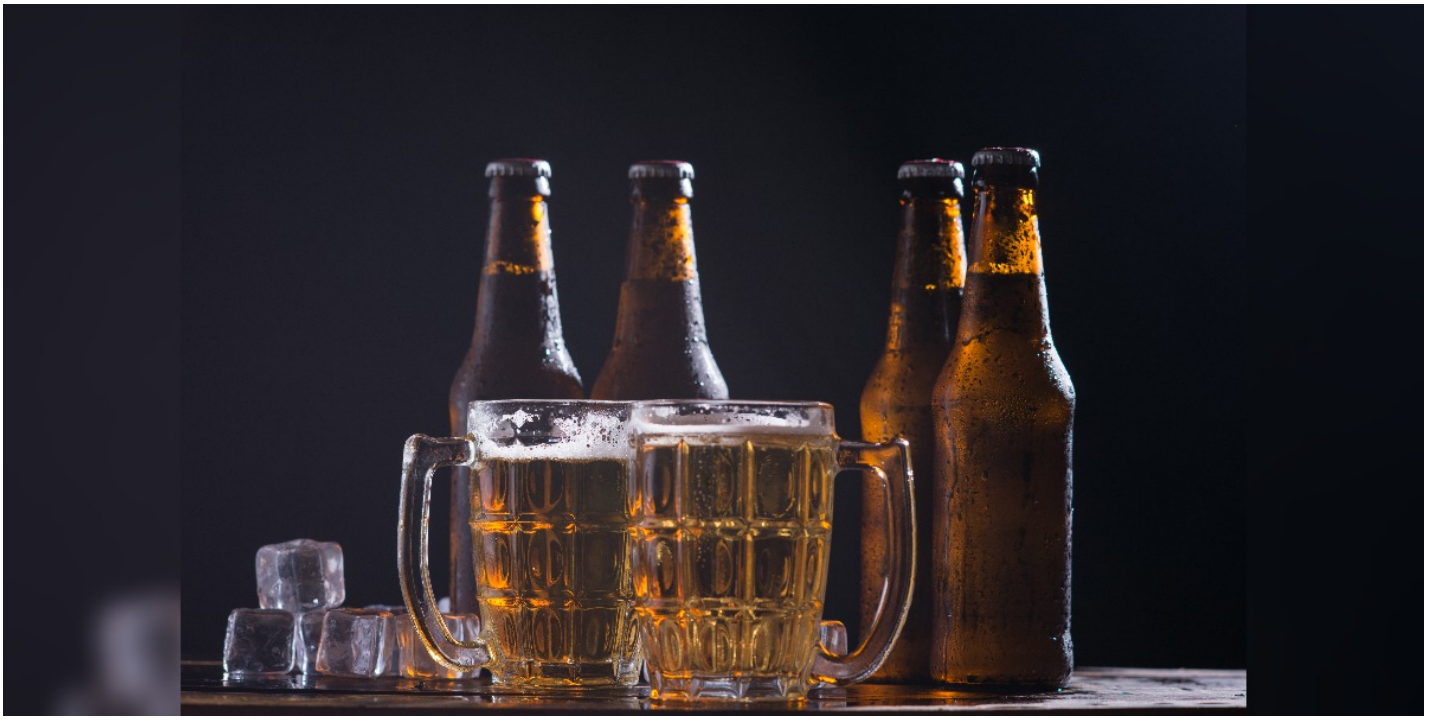

Ethanol precipitation is a commonly used technique for concentrating and de-salting nucleic acid (DNA or RNA) preparations in an aqueous solution.

The basic procedure is that salt and ethanol (https://bitesizebio.com/articles/dna-precipitation-ethanol-vs-isopropanol/) are added to the aqueous solution, which forces the precipitation of nucleic acids out of the solution. After precipitation, the nucleic acids can then be separated from the rest of

the solution by centrifugation. The pellet is then washed in cold 70% ethanol. After a further centrifugation step, the ethanol is removed and the nucleic acid pellet is allowed to dry before resuspending in a clean aqueous buffer.

So how does this work?

It's All About Solubility…

First, we need to know why nucleic acids are soluble in water. Water is a polar molecule – it has a partial negative charge near the oxygen atom due to the unshared pairs of electrons, and partial positive charges near the hydrogen atoms. Because of these charges, polar molecules like DNA or RNA can interact electrostatically with the water molecules, allowing them to easily dissolve in water. Polar molecules can therefore be described as hydrophilic and non-polar molecules, which can't easily interact with water molecules, are hydrophobic. Nucleic acids are hydrophilic due to the negatively charged phosphate (PO_3^-) groups along the sugar-phosphate backbone.

The Role of Salt…

OK, so back to the protocol. The role of salt in the protocol is to neutralize the charges on the sugar-phosphate backbone. A commonly used salt is sodium acetate. In solution, sodium acetate breaks up into Na^+ and $[CH_3COO]^-$. The positively charged sodium ions neutralize the negative charge on the PO_3^- groups on the nucleic acids, making the molecule far less hydrophilic and, therefore, much less soluble in water.

The Role of Ethanol…

The electrostatic attraction between the Na^+ ions in solution and the PO_3^- ions are dictated by Coulomb's Law (http://en.wikipedia.org/wiki/Coulomb%27s_law), which is affected by the dielectric constant of the solution. Water has a high dielectric constant, which makes it fairly difficult for the Na^+ and PO_3^- to come together. Ethanol, on the other hand, has a much lower dielectric constant, making it much easier for Na^+ to interact with the PO_3^-. This shields its charge and makes the nucleic acid less hydrophilic, thus causing it to drop out of the solution.

The Role of Temperature…

Incubation of the nucleic acid/salt/ethanol mixture at low temperatures (e.g. –20° or –80°C) is commonly cited as a necessary step in protocols. However, according to Maniatis *et al.* (Molecular Cloning, A Laboratory Manual 2nd Edition… 2nd edition?? – I need to get a newer version!), this is not required, as nucleic acids at concentrations as low as 20 ng/mL will precipitate at 0–4°C, so incubation for 15–30 minutes on ice is sufficient.

The Wash Step With 70% Ethanol…

This step is to wash any residual salt away from the pelleted DNA.

A Few Tips on Ethanol Precipitation…

- **Choice of salt**
  - Use **sodium acetate** (0.3 M final conc, pH 5.2) for routine DNA precipitation.
  - Use **sodium chloride** (0.2 M final conc) for DNA samples containing SDS, since NaCl keeps SDS soluble in 70% ethanol so that it doesn't precipitate with the DNA.

- Use **lithium chloride** (0.8 M final conc) for RNA. Since 2.5–3 volumes of ethanol are needed for RNA precipitation and LiCl is more soluble in ethanol than sodium acetate, it will not precipitate. But beware – chloride ions will inhibit protein synthesis and DNA polymerase, so LiCl is no good for RNA preps for *in vitro* translation or reverse transcription. In these cases, use sodium acetate.
- Use **ammonium acetate** (2 M final conc) for the removal of dNTPs, but do not use it for the preparation of DNA for T4 polynucleotide kinase reactions as ammonium ions inhibit the enzyme.

- **To increase the yield in precipitations of low concentration or small nucleic acid pieces (less than 100 nucleotides)**
  - Add $MgCl_2$ to a final concentration of 0.01 M.
  - Increase the time of incubation on ice before centrifugation to 1 hour.

This explanation should bring you up to speed on how ethanol precipitation works. If you want to learn more about the ins and outs of ethanol precipitation and other DNA clean-up approaches, you might want to check these out…

More Articles on Ethanol Precipitation

- Ethanol precipitation – DNA vs Isopropanol (https://bitesizebio.com/articles/dna-precipitation-ethanol-vs-isopropanol/)
- How to get the perfect pellet after DNA/RNA ethanol precipitation (https://bitesizebio.com/13513/how-to-get-a-perfect-pellet-after-dnarna-precipitation/)

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Image Credit: ()

154 Comments

Ful Ful on February 16, 2021 at 5:42 pm

How can DNA extraction without ethanol?

Laura Grassie on August 31, 2021 at 10:30 am

You can use isopropanol to precipitate DNA – see our article DNA Precipitation: Ethanol vs. Isopropanol for more information here: https://bitesizebio.com/2839/dna-precipitation-ethanol-vs-isopropanol/ (https://bitesizebio.com/2839/dna-precipitation-ethanol-vs-isopropanol/)

bjorn on October 13, 2020 at 6:13 pm

The link to "How to get the perfect pellet after DNA/RNA ethanol precipitation"
Seems dead. Should be
https://bitesizebio.com/13513/how-to-get-a-perfect-pellet-after-dnarna-precipitation
(https://bitesizebio.com/13513/how-to-get-a-perfect-pellet-after-dnarna-precipitation)
?

Laura Grassie on August 31, 2021 at 10:34 am

Thanks, we've fixed this link now!

rrc on April 26, 2019 at 6:53 am

Let's say that disrupted cells and cell free DNA are placed into 90ml of 95% ethanol at room temperature, how would you go about extracting the DNA directly from the ethanol?

hannnah on January 17, 2018 at 5:35 pm

its cooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooool……but a doubt why dna called dna

Halahath on February 21, 2018 at 10:25 pm

DeoxyriboNucleic Acid… in other words, DNA has the sugar deoxyribose, and obviously, it's a nucleic acid.

Fatemeh on May 7, 2017 at 4:20 pm

Thank you very much for your great article. How can we cite your article? I could not find the date published?

« Older Comments (https://bitesizebio.com/253/the-basics-how-ethanol-precipitation-of-dna-and-rna-works/comment-page-26/#comments)

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