Ethanol precipitation of nucleic acids (Eppendorf tubes)

OpenWetWare

Abstract

Nucleic acid precipitation is used to concentrate and/or purify nucleic acids. The below protocol is based on the fact that nucleic acids are less soluble in alcohol than in more polar water. Addition of salt further decreases solubility by competing for water dipoles; as does low temperature. Please see the OpenWetWare website for more details.

Citation: OpenWetWare contributors, 'Ethanol precipitation of nucleic acids', *OpenWetWare, ,* 1 July 2012, 10:48 UTC, <http://openwetware.org/index.php?title=Ethanol_precipitation_of_nucleic_acids&oldid=611420 > [accessed 21 January 2015]

Citation: OpenWetWare Ethanol precipitation of nucleic acids (Eppendorf tubes). **protocols.io** dx.doi.org/10.17504/protocols.io.cdrs55
Published: 21 Jan 2015

Protocol

Step 1.
Add 1/10 volume of 3M sodium acetate, pH 5.2 or 1/2 volume of 5M ammonium acetate.

REAGENTS
ammonium acetate View by P212121

Step 2.
Add 2-3 volumes of 100% Ethanol.

Step 3.
Mix and freeze overnight in -20.

NOTES
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In general, the time you need to incubate in the freezer depends on how much nucleic acid you have, how big it is and the volume it is in. My general protocol is to freeze for 20 min to 1 hr at -80 ˚C. This seems to work well for most things, but you may want to freeze longer if you have only a small concentration of nucleic acid or if it is small in size(<15 nucleotides). (Kathleen)
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If you are in a hurry, you can also dip you epi shortly into liquid nitrogen. If you added enough ethanol, the mix won't freeze. Careful with isopropanol - it freezes more quickly. This works well for me and saves me a lengthy incubation in the fridge. (Jasu)

Step 4.
Spin at full speed in a standard microcentrifuge at 4 degrees for 30 minutes.

DURATION
00:30:00

Step 5.

Decant (or carefully pipet off) the supernatant.

Step 6.
Dry the pellet.

NOTES

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For this you can air dry (tubes open, ~15 min) or dry in a speedvac. DNA and RNA (if you don't have RNases in your sample) are typically hearty enough for you to air dry at 37 ˚C, if desired.

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Overdrying can make DNA hard to re-dissolve. Especially for longer DNA, I avoid vacuum drying and airdry only briefly before re-dissolving. (Jasu)

Step 7.
Add your desired quantity of water. Vortex and spin down to resuspend.

NOTES

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Beware of using water unless you are sure of what you are getting in to. The "pH" of water can vary widely (I've seen from pH 5 to pH 8.5), and depurination of DNA at low pH or degradation of RNA at high pH are possibilities. Water also typically contains trace metals, which can accelerate these reactions. I typically recommend resuspension in TE (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). This makes sure your nucleic acid is at a neutral pH and the EDTA will chelate any trace metals. Since they are in such small amounts, neither the buffer nor the EDTA will affect most downstream reactions. (Kathleen)