



Isopropanol precipitation of DNA

Alcohol precipitation is commonly used for concentrating, desalting, and recovering nucleic acids. Precipitation is mediated by high concentrations of salt and the addition of either isopropanol or ethanol. Precipitation of DNA with isopropanol is typically carried out using 0.6–0.7 volumes of alcohol, while ethanol precipitation of DNA requires 2–3 volumes of alcohol. Since less alcohol is required for isopropanol precipitation, this is the preferred method for precipitating DNA from large volumes. In addition, isopropanol precipitation can be performed at room temperature which minimizes co-precipitation of salt and minimizes the risk that co-precipitated salt will interfere with downstream applications.

In QIAGEN® anion-exchange purification procedures, such as plasmid or lambda purification, or high-molecular-weight genomic DNA isolation, purified nucleic acids are eluted from the columns in high-salt buffer and then concentrated and de-salted by isopropanol precipitation.

This article provides hints on how to perform an effective isopropanol precipitation to help ensure maximum recovery of DNA. The range of values given for some specifications reflects the variation between protocols depending on the scale and type of DNA preparation. For exact values please refer to the specific protocols.

Isopropanol precipitation procedure

1. Adjust the salt concentration if necessary, for example, with sodium acetate (0.3 M, pH 5.2, final concentration) or ammonium acetate (2.0–2.5 M, final concentration).



Eluates obtained from QIAGEN anion-exchange columns have a suitable salt concentration for efficient isopropanol precipitation, so there is no need to adjust the salt concentration.

2. Add 0.6–0.7 volumes of room-temperature isopropanol to the DNA solution and mix well.



Use of all solutions at room temperature minimizes co-precipitation of salt.



Do not use polycarbonate tubes for precipitation as polycarbonate is not resistant to isopropanol.

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3. Centrifuge the sample immediately at 10,000–15,000 x g for 15–30 minutes at 4°C.

Tip Centrifugation should be carried out at 4°C to prevent overheating of the sample. (When precipitating from small volumes, centrifugation may be carried out at room temperature.)

Tip Genomic DNA can alternatively be precipitated by spooling the DNA using a glass rod following addition of isopropanol. The spooled DNA should be transferred immediately to a microfuge tube containing an appropriate buffer and redissolved (see step 8).

4. Carefully decant the supernatant without disturbing the pellet.

Tip Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Pellets from isopropanol precipitation have a glassy appearance and may be more difficult to see than the fluffy salt-containing pellets resulting from ethanol precipitation.

Tip Care should be taken when removing the supernatant as pellets from isopropanol precipitation are more loosely attached to the side of the tube.

Tip To decant by pouring, carefully tip the tube with the pellet on the upper side to avoid dislodging the pellet (Figure 1).

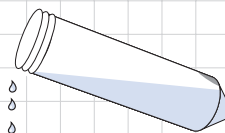


Figure 1 Decanting the supernatant

Tip For valuable samples, the supernatant can be retained until recovery of the precipitated DNA has been verified.

5. Wash the DNA pellet by adding 1–10 ml (depending on the size of the preparation) of room-temperature 70% ethanol. This removes co-precipitated salt and replaces the isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

6. Centrifuge at 10,000–15,000 x g for 5–15 minutes at 4°C.

Tip Centrifuge the tube in the same orientation as previously to recover the DNA into a compact pellet.

7. Carefully decant the supernatant without disturbing the pellet.

8. Air-dry the pellet for 5–20 minutes (depending on the size of the pellet).

Tip Do not overdry the pellet (e.g., by using a vacuum evaporator) as this will make DNA, especially high-molecular-weight DNA, difficult to redissolve.

9. Redissolve the DNA in a suitable buffer.

Tip

Choose an appropriate volume of buffer according to the expected DNA yield and the desired final DNA concentration.

Tip

Use a buffer with a pH ≥ 8.0 for redissolving, as DNA does not dissolve easily in acidic buffers.

Tip

Redissolve by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting up and down to promote resuspension may cause shearing and should be avoided.

Tip

High-molecular-weight DNA, such as genomic DNA, should be redissolved very gently to avoid shearing, e.g., at room temperature overnight or at 55°C for 1–2 hours with gentle agitation.

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