EchoCLEAN Organic Solvent DNA CleanUp Kit – Protocols

for 1-step removal of inhibitors and a wide range of impurities from DNA solutions.

This protocol has been developed to remove impurities like inhibitors, salts, nucleotides, TRIzol™, phenol, chloroform and other organic solvents from DNA solutions.

Materials and equipment needed

- 80 to 110 µl of DNA sample. For DNA samples less than 80 µl, dilute with Tris buffer to a minimum of 80 µl
- Microcentrifuge with rotor for 1.5 and 2 ml reaction tubes. If available, switch to *relative centrifugal force* (rcf)*
- Vortexer
- One reaction tube (2 ml) per sample for column preparation
- One reaction tube (1.5 ml) per sample for elution and collection of the purified DNA
- Pipets for 10 µl and 200 µl scale, corresponding pipet tips
- For fastest procedure (PROTOCOL 1): Cap Puncher (BioEcho product no. 050-001-001)

Preparation before starting

- Set the microcentrifuge to 800 x g.*

PROTOCOL 1: Purification using the Cap Puncher

BioEcho cap puncher

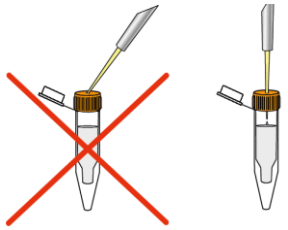
Column preparation

1. Vortex the EchoLUTION Spin Column briefly and place into a 2 ml reaction tube. . Place the 2 ml tube with the spin column into a rack.
2. Use the cap puncher (scan QR code to watch a video): Punch a hole into the column cap and lift the column together with the Cap Puncher out of the 2ml collection tube. Snap off bottom closure of the column and detach the Cap Puncher. Place the punched spin column back into the 2 ml reaction tube.
3. Centrifuge for 1 min at 800 x *g**. Discard the 2 ml reaction tube containing the column buffer.
4. Place the prepared spin column into a new 1.5 ml reaction tube for elution of the sample and place both together in a rack.

   Continue with “Purification” (below).

Purification (1–2 minutes)

5. Transfer 80 – 110 µl sample containing the DNA to the prepared column from step 4 as illustrated below:
   Note: volume should be 80–110 µl



Insert pipet tip vertically through the hole in the column cap and pipet the sample slowly (~5 sec) into the column.

6. Centrifuge 1 min at 800 x *g**. The purified DNA (final volume 80–100 µl; 10 mM Tris·Cl, pH 7.8) flows through the column into the 1.5 ml elution tube. Discard the spin column.

   The eluted DNA can be used immediately or stored at 4°C or for long-term storage at –20°C. For spectrophotometric analysis, use 1x Tris Buffer as blank after 1:10 dilution of the 10x Tris Buffer (T) supplied with the kit.

PROTOCOL 2: Purification without the use of a Cap Puncher

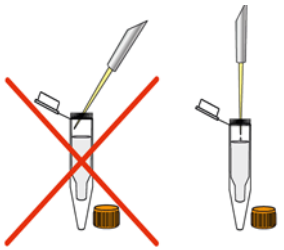
Column preparation

1. Vortex the EchoLUTION Spin Column briefly and place into a 2 ml reaction tube. . Place the 2 ml tube with the spin column into a rack.
2. Loosen the screw cap of the spin column a half turn and snap off the bottom closure. Important: Do not close the screw cap of the spin column. The screw cap must stay loosened a half turn to avoid generation of a vacuum. Place back the column into the 2 ml collection tube.
3. Centrifuge for 1 min at 800 x *g**. Discard the 2 ml reaction tube containing the column buffer.
4. Place the prepared spin column into a new 1.5 ml reaction tube for elution of the sample DNA and place back into the rack.

   Continue with “Purification” (below).

Purification (1–2 minutes)

5. Transfer 80 – 110 µl sample containing the DNA to the prepared column from step 4 as illustrated below:
   Note: volume should be 80–110 µl, see section “Materials and equipment needed” for details



Open cap and pipet the sample slowly (~5 sec) onto the center of the resin bed of the prepared spin column. Close screw cap and loosen again half a turn.
Important: Don´t close the screw cap of the spin column tightly!

6. Centrifuge 1 min at 800 x *g**. The purified DNA (final volume 80–100 µl; 10 mM Tris·Cl, pH 7.8) flows through the column into the 1.5 ml elution tube. Discard the spin column.

   The eluted DNA can be used immediately or stored at 4°C or for long-term storage at –20°C. For spectrophotometric analysis, use 1x Tris Buffer as blank after 1:10 dilution of the 10x Tris Buffer (T) supplied with the kit.

Product use limitation

The EchoCLEAN DNA CleanUp Kits are for research use only. They have not been registered or authorized to be used for diagnosis, prevention or treatment of a disease.

Please note: The depletion of nucleic acids of a specific length is a function of loaded concentration and amount of fragment to be depleted. In certain cases depletion may not be quantitative.

* Most centrifuges offer the choice between rpm and g-force (rcf); if not, calculate the rpm corresponding to 800 x g using the formula: rpm = 1000 x $\sqrt{(\frac{800}{1.12 \, x \, r})}$, where r = radius of rotor in mm. E.g., with a radius of 150 mm, the corresponding rpm to 800 x g is approx. 2200 rpm.

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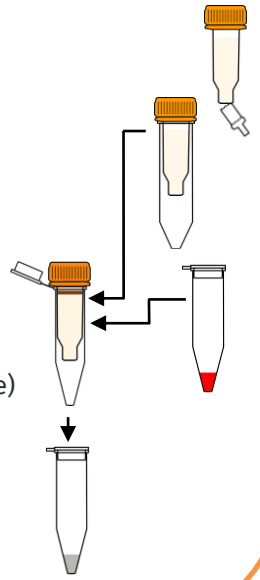
Product no. (rxn’s)	020-002-040-010 (10)	020-002-040-010 (50)	020-002-040-250 (250)
Kit contents	EchoCLEAN Organic Solvent DNA CleanUp Spin Columns		

Quick PROTOCOL 1

Column preparation

- Homogenize column resin by vortexing and place in a 2 ml tube.
- Punch a hole in the cap, and break off bottom closure (scan QR code to watch a video).
- Place spin column back into 2 ml tube.
- Centrifuge 1 min at 800 *x g** to elute column buffer.
- Place column in a 1.5 ml tube for elution.



Purification of DNA

- Transfer 80-110 µl of sample by pipetting slowly through cap hole – see PROTOCOL 1 or watch video (scan QR code)
- Centrifuge 1 min at 800 x g*.

Purified DNA is ready to use.

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