EchoCLEAN Organic Solvent DNA CleanUp Kit-Protocol

for simgle-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
  Important: Switch centrifuge to *relative centrifugal force, rcf* (x *g**); if this is not possible please use formula below* to calculate the conversion of round per minute (rpm) into 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 **1000 x *g*.***
- Important: ***Switch to relative centrifugal force, rcf* (x *g**, not rpm)

Product use limitation

The EchoCLEAN Organic Solvent 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.

* Most centrifuges offer the choice between rpm and g-force (rcf); if not, calculate the rpm matching the g-force using the formula: rpm = 1,000 x $\sqrt{(\frac{g}{1.12 \times r})}$, where r = radius of rotor in mm. and g the required g-force.
E. g., with a radius of 150 mm, the corresponding rpm to 1,000 x g is approx. 2,400 rpm.

PROTOCOL 1: Purification **using** the Cap Puncher

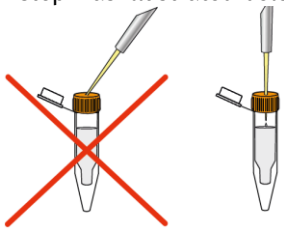
BioEcho Cap Puncher

Column preparation

1. Vortex the **EchoCLEAN Organic Solvent Spin Column** briefly and place into a **2 ml** reaction tube.
   Let stand for about 5 min.
2. Use of 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 2 ml collection tube. Snap off bottom closure of the column and detach the Cap Puncher by twisting while pulling out.
   Place the punched spin column back into the 2 ml reaction tube
3. Centrifuge for **1 min at 1000 x *g**.** Discard the flow-through volume (“void volume”) collected in the 2 ml reaction tube.
4. Place the prepared spin column into a new **1.5 ml** reaction tube for elution of the purified DNA and place back into the rack.
   Continue with “Clean up of DNA”.

Clean up of DNA

5. Transfer **80 – 110 µl of the DNA sample** to the prepared **EchoCLEAN Spin Column** from step 4 as illustrated below:



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

Note:

- Do not punch pipette tip into the resin bed during loading of lysate!

6. Centrifuge **1 min at 1000 *x g**.** The purified genomic DNA elutes into the 1.5 ml elution tube and can be immediately applied in downstream applications.

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 (T) supplied with the kit as blank.

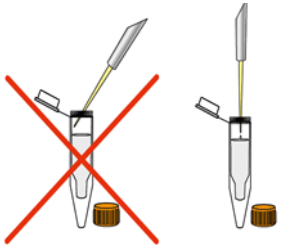
PROTOCOL 2: Purification **without** a Cap Puncher

Column preparation

1. Vortex the **EchoCLEAN Organic Solvent Spin Spin Column** briefly and place into a **2 ml** reaction tube. Let stand for 5 min.
2. **Loosen** the screw cap of the spin column **half a turn** and **snap off the bottom closure.**
   Important: Do not close the screw cap of the spin column. The screw cap must stay loosened **half a turn** to avoid generation of a vacuum. Place the column back into the 2 ml collection tube and both into the centrifuge.
3. Centrifuge for **1 min at 1000 x *g**.** Discard the flow-through volume ("void volume") collected in the 2 ml reaction tube.
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 "Clean up of DNA.

Clean up of DNA

5. Transfer **80 – 110 µl of the DNA sample** to the prepared **EchoCLEAN Spin Column** from step 4 as illustrated below



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: Do not close the screw cap of the spin column tightly!**

Note:

- Do not punch pipette tip into the resin bed during loading of lysate!

6. Centrifuge **1 min at 1000 x *g****. The purified genomic DNA elutes into the 1.5 ml elution tube and can be immediately applied in downstream applications

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 (T) supplied with the kit as blank.

EchoCLEAN Organic Solvent DNA CleanUp Kit

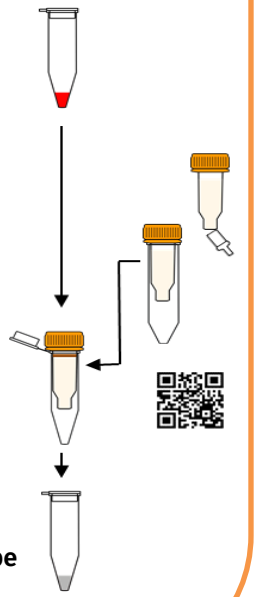
for 1-step removal of inhibitors, TRIzol™, organic solvents and a wide range of impurities from DNA solutions

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, 1x Tris Buffer		

Quick PROTOCOL (please read protocol first)

Column preparation

- Vortex **EchoCLEAN spin column** and place in a **2 ml** tube
  Let stand for 10 min
- Punch a hole in the cap with the Cap Puncher, and
  break off bottom closure (scan QR code to watch a video)
- Place spin column back into 2 ml tube
- Centrifuge **1 min at 1000 *x g**** to elute column buffer
- Place column in a **1.5 ml** tube



Clean up of DNA

- Transfer **DNA sample (max. 110 µl)** by pipetting
  slowly through cap hole (scan QR code to watch a video)
- Centrifuge **1 min at 1,000 *x g**** to elute DNA into **Elution tube**
- Eluted DNA is ready to use

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