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NucleoSpin® Gel and PCR Clean-up

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1 Works for me dx.doi.org/10.17504/protocols.io.77ahrie

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ABSTRACT

- Buffer NTI
- NucleoSpin® Gel and PCR Clean-up Column
- NucleoSpin® Gel and PCR Clean-up Collection Tube (2ml)
- Buffer NT3
- Buffer NE / nuclease-free water
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Solubilize gel slice

For each 100 mg of agarose gel < 2 % add 200 µL Buffer NTI. For gels containing > 2 % agarose, double the volume of Buffer NTI. Incubate sample for 5–10 min at 50 °C. Vortex the sample briefly every 2–3 min until the gel slice is completely dissolved!

Bind DNA

Place a NucleoSpin® Gel and PCR Clean-up Column into a Collection Tube (2 mL) and load up to 700 µL sample. Centrifuge for 30 s at 11,000 x g. Discard flow-through and place the column back into the collection tube. Load remaining sample if necessary and repeat the centrifugation step.

Wash silica membrane

Add 700 µL Buffer NT3 to the NucleoSpin® Gel and PCR Clean-up Column. Centrifuge for 30 s at 11,000 x g. Discard flow-through and place the column back into the collection tube.

Dry silica membrane

Centrifuge for 1 min at 11,000 x g to remove Buffer NT3 completely. Make sure the spin column does not come in contact with the flow-through while removing it from the centrifuge and the collection tube. Note: Residual ethanol from Buffer NT3 might inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns for 2–5 min at 70 °C prior to elution.

Elute DNA

Place the NucleoSpin® Gel and PCR Clean-up Column into a new 1.5 mL microcentrifuge tube (not provided). Add 15–30 µL Buffer NE and incubate at room temperature (18–25 °C) for 1 min. Centrifuge for 1 min at 11,000 x g.