MACHEREY-NAGEL
Laboratory-wisdoms

Get highest recoveries with the NucleoBond® Finalizer

Protocol from the manual:

Concentration of NucleoBond® Xtra eluates with the NucleoBond® Finalizers

*Note: Use of the **NucleoBond® Finalizers** is only recommended for vector sizes smaller than 50 kbp.*

Midi - NucleoBond® Finalizer	Maxi - NucleoBond® Finalizer Large

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1 Precipitate DNA

*Note: The **NucleoBond® Finalizer** only holds up to **500 µg** and the **NucleoBond® Finalizer Large** is limited to **2000 µg** of plasmid DNA. Loading more DNA might lead to clogging and complete loss of your sample. Thus, it is highly recommended to determine plasmid yield by measuring A_{260} before precipitating the DNA (see section 4.12). Furthermore, this helps to choose the best buffer volume in step 5 and allows calculation of the recovery after concentration.*

Add **0.7 volumes** of **room-temperature isopropanol** (not supplied with the kit). Vortex well and let the mixture sit for **2 minutes**.

(e.g. for 5 mL NucleoBond® Xtra Midi eluate add **3.5 mL** isopropanol, for 15 mL NucleoBond® Xtra Maxi eluate add **10.5 mL** isopropanol)

3.5 mL for 5 mL eluate	10.5 mL for 15 mL eluate

Laboratory-wisdom:

Don't forget proper mixing! Avoid two-layer formation, otherwise binding efficiency will be low!

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2 Load precipitate

Remove the plunger from a **30 mL Syringe** and attach a NucleoBond® Finalizer to the outlet. Fill the precipitation mixture into the syringe, insert the plunger, hold the syringe in a vertical position, and press the mixture **slowly** through the NucleoBond® Finalizer using **constant force**. Discard the flow-through.

DNA needs some time to bind efficiently! Load really slow, you should still be able to see individual drops coming out of the Finalizer, hold the syringe vertical!

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3 Wash precipitate

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer to the syringe outlet.

Fill **70 % ethanol** (not supplied with the kit) into the syringe, insert the plunger, hold the syringe in a vertical position, and press the ethanol **slowly** through the NucleoBond® Finalizer. Discard the ethanol.

2 mL	5 mL

Don't forget to always remove the Finalizer before you pull out the plunger!

BIOKÉ sharing knowledge

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4 Dry filter membrane

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer. Press air through the NucleoBond® Finalizer **as quickly as possible** while touching a tissue with the tip of the NucleoBond® Finalizer to soak up ethanol.

Repeat this step at least as often as indicated below until **no more ethanol** leaks from the NucleoBond® Finalizer.

Note: A new dry syringe can be used to speed up the procedure (not provided).

≥ 3 times	≥ 6 times

Optional: You can incubate the NucleoBond® Finalizer for 10 minutes at 80 °C to minimize ethanol carry-over. However, the final recovery may be reduced by over-drying the DNA.

Laboratory-wisdom:

Remaining ethanol will inhibit efficient elution. Press the air as fast as possible through the filter. Dry the tip on a tissue.

5 Elute DNA (Buffer TRIS)

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger of a **1 mL Syringe** and attach the NucleoBond® Finalizer to the syringe outlet.

Note: Refer to section 4.11, Table 4 (Midi) or 5 (Maxi) to choose the appropriate volume of elution buffer.

Pipette an appropriate volume of **Redissolving Buffer TRIS** (5 mM Tris/HCl, pH 8.5) or TE buffer into the syringe (see section 4.11). Place the NucleoBond® Finalizer outlet in a vertical position over a fresh collection tube and **elute plasmid DNA** dropwise by inserting the plunger.

200 - 800 µL	400 - 1000 µL

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger and reattach the NucleoBond® Finalizer to the syringe outlet.

! Transfer the first eluate back into the syringe and elute into the same collection tube a second time.

load first eluate completely	load first eluate completely

Remove the NucleoBond® Finalizer from the syringe, pull out the plunger to aspirate air, reattach the NucleoBond® Finalizer and press the air out again to force out as much eluate as possible.

Determine plasmid yield by UV spectroscopy and confirm plasmid integrity by agarose gel electrophoresis (see section 4.12).

For further details, don't forget to have a look into section 4.11 of the manual ("Elution and concentration of plasmid DNA")

Do not lose any precious drops! Place the Finalizer on top of the collection tube before adding elution buffer. Elute really slow, drops instead of flush!

Reload the eluate (second elution) → DNA will be completely redissolved: high recovery & high concentration!

After elution: press air through the filter 2–3 times to recover every highly conentrated drop of DNA.

Also check DNA yield prior to precipitation to get an idea about the recovery rates!

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