NucleoBond Xtra Midi – Andrew+ - 3 samples

Protocol details

Application	Transfection-grade plasmid DNA isolation	
Kit	NucleoBond® Xtra Midi	
REF	740410 (.10 / .50 / .100)	
Protocol name	NucleoBond Xtra Midi 3 sample	Rev02

Basic principle

The automated protocol is based on the established anion exchange technology of the NucleoBond® Xtra Midi kit. Preparation of plasmid DNA is achieved by an interlaced and coordinated process of cell lysis, column equilibration and subsequent neutralization of the sample. Purity and integrity of the extracted plasmid DNA are ensured by gentle mixing via pipetting with wide-bore pipette tips and large volumes during the neutralization step. The transfer onto the NucleoBond® column filters is done step-wise to ensure a fast and efficient clearing of the lysate. Plasmid DNA is bound to the NucleoBond® Xtra Silica Resin. After an efficient washing step the plasmid DNA is eluted from the column under high-salt conditions. The following precipitation procedure is based on reversible adsorption of nucleic acids to magnetic beads under appropriate buffer conditions. Plasmid DNA is precipitated from the elution buffer by the addition of isopropanol and bound to the NucleoMag® B-Beads. Beads are washed with 70% EtOH to remove salts and residual contaminants. Finally, highly purified DNA is eluted with low salt elution buffer (TE buffer) and can directly be used for downstream applications.

Five easy steps

Procedure	
1	Cultivate bacteria and harvest cell pellets according to the user manual NucleoBond® Xtra Midi. Samples must be provided in 50 mL conical tubes. Each tube should contain 0.5 - 1 g of pellet wet weight.
2	Select the protocol from the OneLab software and follow the instructions.
3	Fill all reagents in 50 mL conical tubes in the respective dominos according to the table below. Add all the required empty plastic ware according to their positions in the table below. Double-check the correct positions in the OneLab software prior to starting the run.
4	Arrange the deck layout according to the displayed positions in the OneLab software. Remove the filter from the 10 mL pipette and start the run. Insert the filter back into the 10 mL pipette when prompted by the OneLab software (appr. 2 min after start).
5	Remove the NucleoBond® Xtra Midi Column Filters when prompted approx. 40 min after start.

Decklayout

Decklayout	Position	Domino/Device	Labware type
wide	1	Tip Rack Holder 10 mL	0.5-10 mL Sartorius Optifit Tips
	2	Tip Rack Holder 5 mL	100-5000 µL Sartorius SafetySpace™ Filter Tips
	3	Microtube domino	Eppendorf 1.5 mL clear Safe-Lock tubes
	4	50 mL conical centrifuge tube domino	Falcon® 50 mL conical centrifuge tubes
	5	Magnet+	Falcon® 50 mL conical centrifuge tubes
	6	50 mL conical centrifuge tube domino	Falcon® 50 mL conical centrifuge tubes
	7	NucleoBond® Xtra Midi column domino	NucleoBond® Xtra Midi columns with plastic washers

Note: Please make sure to double-check the exact positions of dominos as indicated in the OneLab software prior to start.

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11
52355 Düren · Germany
www.mn-net.com

DE Tel.: +49 24 21 969-0 info@mn-net.com
CH Tel.: +41 62 388 55 00 sales-ch@mn-net.com
FR Tel.: +33 388 68 22 68 sales-fr@mn-net.com
US Tel.: +1 888 321 62 24 sales-us@mn-net.com

Figure 2: Exemplary deck layout (wide)

Loading table

Domino*	Position	Reagent	Approximate volume
50 mL conical centrifuge tube domino (4)	A1	70% EtOH	13 mL
	A2	H_2O	7 mL
	A3	Buffer ELU	16 mL
	A4	Buffer EQU 1	37 mL
	B1	Buffer EQU 2	16 mL
	B2	Isopropanol	12 mL
	B3	Buffer LYS	25 mL
	B4	Buffer NEU	25 mL
50 mL conical centrifuge tube domino (6)	A1	Buffer RES	25 mL
	A2	Sample #1	-
	A3	Sample #2	-
	A4	Sample #3	-
	B1	TE Buffer	4 mL
	B2	Buffer WASH	25 mL
	B3	Waste	-
	B4	Empty	-
Microtube domino (3)	A1	Elution tube #1	-
	A2	Elution tube #2	-
	A3	Elution tube #3	-
	A4	NucleoMag® B-Beads	330 µL
Magnet+ (5)	1	Elution 50 mL conical tube #1	-
	2	Elution 50 mL conical tube #2	-
	3	Elution 50 mL conical tube #3	-
NucleoBond® Xtra Midi column domino (7)	1	NucleoBond® Xtra Midi column #1	-
	2	NucleoBond® Xtra Midi column #2	-
	3	NucleoBond® Xtra Midi column #3	-

Note: Positions and volumes may change and are indicated in the OneLab software prior to start. Please make sure to double-check all positions and volumes prior to starting the run. 50 mL conical tubes should not contain less than the required volumes. For an optimal performance, do not fill in more than additional 20% of the indicated volume. The correct position of the NucleoBond® Xtra Midi column in the domino is essential for an reliable performance. Please check figure 1 for assembling.

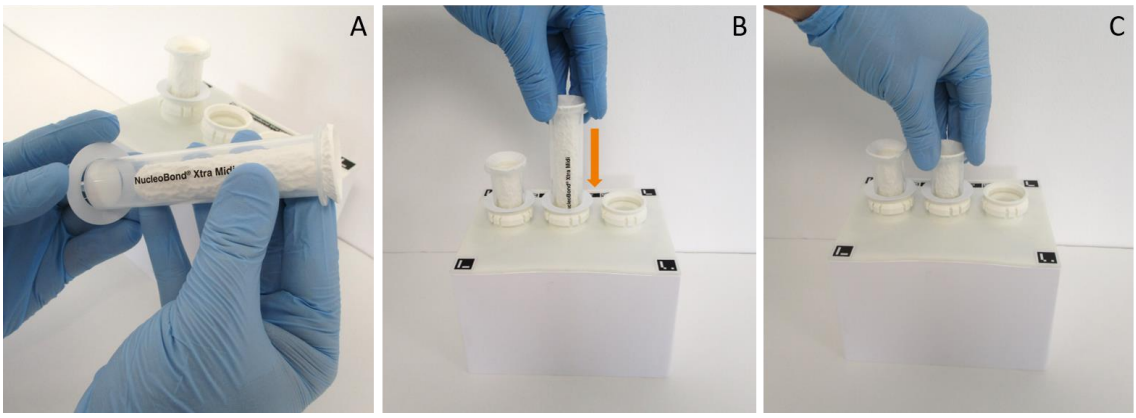

Figure 1: Positioning of the plastic washer on the NucleoBond® Xtra Midi column. A: Insert the NucleoBond® Xtra Midi column into the plastic washer. B: Gently push the column into the NuceoBond Xtra Midi column domino. Push the column down until you feel resistance. C: The column is now in the correct position to start the protocol.

Additional script information

Information	
Number of samples	3 samples per run
Time	~ 2 h 50 min
Hands-on-steps	• Insertion of the 10 mL pipette filter (approx. 2 min after start) • Removal of NucleoBond® Xtra Midi Column Filters after flowthrough of the lysate (approx. 55 min after start)
Tip consumption	18 x 10 mL Filtertips and 28 x 5 mL Filtertips

Additional consumables

Product	Content	REF
NucleoBond® Xtra Midi	Pack of 10 preps 740410 (.10 / .50 / .100)	740410.10
	Pack of 50 preps	740410.50
	Pack of 100 preps	740410.100
NucleoMag® B-Beads	12 mL (sufficient for 100 preps)	744503.12

Note: Eppendorf 1.5 mL clear Safe-Lock tubes and 50 mL conical centrifuge tubes are not provided.

Protocol specifications

Specification	
Pellet wet weight	0.5 – 1.0 g/Pellet or ODV = 250-550
Typical DNA yield	comparable to NucleoBond® Xtra Midi (400 µg/column)

Note: Please refer to the user manual of the NucleoBond® Xtra Midi for a full list of specifications.

Troubleshooting

Problem	Possible cause and suggestions
No or low plasmid DNA yield	*Incomplete lysis of the sample* • Reduce the pellet wet weight of the sample. The maximum wet weight should not exceed 1 g. Alternatively, ODV should be < 550. • Make sure the sample pellet is completely thawed before starting the run. *NucleoMag® B-Beads not homogeneously mixed prior to start* • Mix NucleoMag B-Beads® by vigorous vortexing prior to start
Low purity	*Incomplete lysis/neutralisation of the sample* • Reduce the pellet wet weight of the sample. The maximum wet weight should not exceed 1 g. • Make sure the sample pellet is completely thawed before starting the run.
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnets before aspirating any liquid • Perform a manual separation step with the final elution tubes after the protocol has finished
High ethanol concentration in eluate	*Incomplete drying of the bead pellet* • Increase drying time • Alternatively, the sample can be rinsed once with TE buffer after removal of the wash supernatant. Note that an implementation of the rinse step will shorten the protocol by approx. 25 min and may lead to a decrease in plasmid DNA yield.
Dripping of the pipettes	*Low adhesion of the tip to the pipette shaft* • Use a paper tissue to apply a thin layer of oil to the pipette shaft prior to running the protocol.

Note: Further troubleshooting advice can be found in the NucleoBond® Xtra Midi user manual.

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