Luna Nanotech

PuroAE-KD™ Plasmid MAXIprep Plus Kit

Product Manual

#NKAEKD213-2	2 preps
#NKAEKD213-10	10 preps
#NKAEKD213-30	30 preps

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Luna Nanotech Inc.
85 Citizen Court, Unit 13
Markham, ON, Canada
L6G 1A8

Email: support@lunanano.com
Phone: 1-800-474-4055
Web: www.lunanano.com

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Kit Description

The **PuroAE-KD™ Plasmid MAXIprep Plus Kit** offers a straightforward, easy-to-use workflow for the large-scale extraction and purification of plasmid DNA. Purification relies on the binding of plasmid DNA to an anion-exchange membrane, which efficiently separates it from genomic DNA, protein, salt, and other contaminants. Compared with traditional nucleic acid purification methods such as phenol-chloroform extraction, anion-exchange columns provide a simpler and safer alternative.

Together with the optimized PuroAE-KD™ buffer formulations, this workflow yields highly purified plasmid DNA suitable for demanding downstream applications, including transfection, enzymatic digestion, and PCR.

Kit Contents

Component	2 Preps (#NKAEKD213-2)	10 Preps (#NKAEKD213-10)	30 Preps (#NKAEKD213-30)
Lysis Buffer LB-AE1	40mL	225 mL	450 mL
Lysis Buffer LB-AE2	40 mL	225 mL	450 mL
Neutralization Buffer NB-AE	40 mL	225 mL	450 mL
Equilibration Buffer EB-AE	40 mL	160 mL	470 mL
Wash Buffer WB-AE	80 mL	2 x 400 mL	4 x 500 mL
Elution Buffer EB-AE	40 mL	180 mL	480 mL
Water, Nuclease-free	15 mL	50 mL	150 mL
RNase A (20 mg/mL), DNase-free	1.6 mL	9.0 mL	18.0 mL
PuroAE™ Anion-Exchange Columns	2	10	30
PuroSPIN™ Filter Cartridge for Plasmid Maxiprep Kit	2	10	30
SpinKD Centrifugal Filters	2	10	30

Storage

RNase A should be stored at -20 °C. All components can be stored at room temperature for at least 1 year. Once **RNase A** is added to **Lysis Buffer LB-AE1**, the buffer should be stored at 4 °C.

Additional Materials and Equipment Required

- 50 mL RNase-free centrifuge tubes
- Buffer for reconstitution of DNA: TE buffer or sterile H2O
- Pipettes and sterile nuclease-free pipette tips
- Refrigerated centrifuge (with ≥ 16,000 x g or ≥ 5,000 x g capability)
- Vortex (if not available, can be replaced with vigorous pipetting)
- Appropriate personal protective equipment (gloves, lab coat)

Important Notes

BEFORE USE:

- Add **RNase A** (20 mg/mL) to **Lysis Buffer LB-AE1**. *After addition, store the buffer at 4 °C.*
  - **#NKAEKD113-2** (2 preps): add **1.6 mL** of RNase A
  - **#NKAEKD113-10** (10 preps): add **9.0 mL** of RNase A
  - **#NKAEKD113-30** (30 preps): add **18.0 mL** of RNase A

- If precipitate is observed in the **Lysis Buffer LB-AE2**, re-dissolve it by warming the solution to 37 °C. Cool the solution back to room temperature before use.
- Pre-chill **Neutralization Buffer NB-AE** at 4 °C.

Growth of Bacterial Cultures

DNA yield and quality depend on a lot of variables: the growth medium and antibiotic used, the host strain, the plasmid's type/size/copy number, and the growth conditions themselves.

Medium and conditions: For standard high-copy plasmids, LB medium is the go-to choice, incubated at 37°C with vigorous shaking (200–250 rpm), ideally overnight for 12–16 h. Use flasks 3–4x larger than the culture volume so the medium stays well-oxygenated. Richer media like 2xYT, TB, are alternatives — bacteria grow faster and hit stationary phase much sooner (within 2 h) with these, reaching higher cell densities. But more cells doesn't automatically mean more plasmid DNA: overgrown cultures can accumulate dead or starving cells, leading to degraded plasmid DNA or contamination with genomic DNA. The ideal medium and incubation time really needs to be worked out for each specific strain/plasmid combination. Antibiotic selection: Keep cultures under antibiotic selection throughout growth to maintain the plasmid. Without that pressure, cells readily lose the plasmid during division — and since plasmid-free cells grow faster (they're not burdened with replicating it), they quickly dominate the culture, tanking plasmid yield even if total cell mass looks fine.

Host strain: This mainly affects DNA quality. Strains like DH5α or XL1-Blue give clean, supercoiled plasmid DNA, while strains with high endonuclease activity (like HB101) can degrade DNA quality, causing problems in downstream steps like restriction digests or sequencing.

Plasmid type: Size and origin of replication (ori) strongly affect yield. Bigger plasmids or inserts generally mean lower yield due to reduced copy number — even a normally high-copy ColE1-based construct can act like a low-copy plasmid if it carries a large or problematic insert. The ori itself can shift yield by 10–100x: plasmids/cosmids/BACs built on origins like pBR322 or pACYC sit at under 20 copies per cell (sometimes just 1), while pUC-, pBluescript-, or pGEM-based vectors can reach several hundred copies per cell.

Bottom line: all these factors need to be weighed together when targeting a specific DNA yield, and culture volume and lysis steps should be adjusted accordingly.

Culture Volume for High-Copy Plasmids

The amount of bacterial cells in a culture depends on many factors — the growth medium used (TB, CircleGrow, 2xYT), shaking speed, temperature, host strain, and even the plasmid insert itself. As a general guideline, a 1-liter E. coli LB culture at OD_{600} = 1 contains roughly 1 trillion cells and produces about 1.5–1.8 g of wet cell mass. Shaken overnight LB cultures typically reach OD_{600} values of 3–6, while fermenter-grown cultures can exceed OD_{600} of 10. High-copy plasmids typically yield around 1 mg of DNA per gram of wet cell mass, which is why it's better to base prep on cell mass rather than culture volume. Since weighing cell mass directly is inconvenient, the manual instead uses a calculated value called ODV (optical density x volume):

ODV = OD_{600} × Volume (mL)

If culture's OD_{600} goes above 0.5, it require to dilute it before measuring (a 1:10 dilution with fresh medium works well), then multiply the reading by the dilution factor to get the true OD_{600}. This value is then used with a reference table (**Table #1**) to figure out how much culture volume to use. For example: if the OD_{600} of *E.coli* is 2, calls for 66 mL in a Midi prep or 200 mL in a Maxi prep.

Table #1: Recommended culture volumes for high copy plasmid

Puro-AE™	Pellet Wet Weight	Recommended ODV	OD_{600} = 2	OD_{600} = 4	OD_{600} = 6	OD_{600} = 8	OD_{600} = 10
MAXI	2.25 g	1200	600 mL	300 mL	200 mL	150 mL	120 mL

Culture Volume for Low-Copy Plasmids

PuroAE-KD™ Plasmid MAXIprep Plus Kit can be used to purify both high-copy plasmids (up to several hundred copies per cell) and low-copy plasmids (fewer than 20 copies per cell). For low-copy plasmids, however, both the amount of cells and the volume of lysis buffer must be scaled up by a minimum of two-fold, so that enough DNA is available to make full use of the column's binding capacity.

Table #2 lists the recommended ODV values along with matching combinations of OD_{600} and culture volume for low-copy plasmid cultures. (See section "**Culture Volume for High-Copy Plasmids**" for details on how ODV is calculated, where ODV = OD_{600} × volume.) For example, an *E. coli* culture with an OD_{600} of 6 would require 400 mL for a Maxi prep.

Table #2: Recommended culture volumes for low copy plasmid

Puro-AE™	Pellet Wet Weight	Recommended ODV	OD_{600} = 2	OD_{600} = 4	OD_{600} = 6	OD_{600} = 8	OD_{600} = 10
MAXI	4.5 g	2400	1200 mL	600 mL	400 mL	300 mL	240 mL

Scaling the culture and lysis buffer volumes up even further — by a factor of 3–5, for instance — will give still better yields. Extra lysis buffer is available as a separate purchase.

Cell Lysis

The bacterial pellet is first resuspended in Lysis Buffer LB-AE1, then lysed with Lysis Buffer LB-AE2 via a sodium hydroxide/SDS treatment. These conditions denature proteins along with both chromosomal and plasmid DNA, while RNase A breaks down RNA. Adding Neutralization Buffer NB-AE — which contains potassium acetate — causes the SDS to come out of solution as potassium dodecyl sulfate (KDS), a precipitate that carries proteins, chromosomal DNA, and other cell debris down with it. At the same time, the potassium acetate neutralizes the lysate, allowing plasmid DNA to return to its native supercoiled conformation and stay dissolved.

In the standard protocol, buffer volumes are calibrated for the culture volumes listed for high-copy plasmids in section "Culture Volume for High-Copy Plasmids", Table #1. Overloading the system with cell material compromises both lysis and precipitation efficiency, which can lower plasmid yield and purity. Lysis buffer volumes therefore need to be scaled up whenever larger cultures are processed — as is the case for low-copy vector purification section "Culture Volume for Low-Copy Plasmids", Table #2.

As a rough guide, the required volumes of Lysis Buffer LB-AE1, Lysis Buffer LB-AE2, and Neutralization Buffer NB-AE can be estimated with:

Volume [mL] = Culture volume [mL] × OD_{600} ÷ 50

For example, 400 mL of a low-copy culture at OD_{600} = 2, you would use 16 mL of each of the buffers. Should the kit not contain enough buffer for your preparation, a supplementary set comprising Lysis Buffer LB-AE1, Lysis Buffer LB-AE2, Neutralization Buffer NB-AE, and RNase A can be purchased separately.
Using an adequate buffer volume keeps lysis short — 3–4 minutes is sufficient, and 5 minutes should be treated as the upper limit. Leaving the sample in alkaline conditions for longer risks irreversible denaturation and degradation of the plasmid DNA, and can release contaminating chromosomal DNA into the lysate.

Lysate Clarification Using the PuroSPINTM Filter Cartridge for Plasmid MAXIprep Kit

Following alkaline lysis and neutralization, the lysate contains a bulky white flocculate of potassium dodecyl sulfate (KDS) in which precipitated proteins, chromosomal DNA, and cell debris are trapped. This precipitate must be removed completely before the cleared lysate is applied to the PuroAE™ Anion-Exchange Column, since residual particulate matter will block the column bed and compromise both flow rate and yield.

The PuroSPIN™ Filter Cartridge for Plasmid Maxiprep Kit supplied with the kit is designed for this purpose. It can be loaded with the crude neutralized lysate directly — supernatant together with the suspended SDS–protein complex — without a prior centrifugation step. The filter retains the flocculate while the clarified solution passes through.

Maximum loading volume for PuroSPIN™ Filter Cartridge for Plasmid Maxiprep Kit: 60 mL. Do not exceed this volume.

The barrel must retain sufficient headspace for the plunger to be seated without displacing liquid over the rim, and the frit has a finite capacity for retained precipitate. Overloading leads to premature clogging, and forcing lysate through a saturated frit compacts the precipitate cake and can drive contaminating chromosomal DNA into the filtrate.

The total volume to be clarified is the sum of the three buffer volumes: **Total lysate volume = Lysis Buffer 1 LB-AE1 + Lysis Buffer 2 LB-AE2 + Neutralization Buffer NB-AE**. The precipitated SDS–protein complex forms within this volume and does not add appreciably to it. Depending on the total lysate volume there are two way to proceed.

Total lysate volume	Procedure
≤ 60 mL	Load the crude neutralized lysate directly onto the filter unit (see **Direct filtration**, below).
≤ 60 mL	Clarify by centrifugation first, then filter the supernatant (see **Clarification by centrifugation**, below). Alternatively, divide the lysate between two or more filter units.

Note that scaled-up preparations — for example low-copy plasmid purifications, where cell mass and lysis buffer volumes are increased by a factor of 2 or more — will routinely exceed 60 mL, the same is true for high-copy plasmids with low OD_{600}. Check the calculated buffer volumes before beginning lysis so that the appropriate clarification route can be prepared in advance, see sections "Culture Volume for High-Copy Plasmids", Table #1 and Culture Volume for Low-Copy Plasmids", Table #2.

Direct filtration (total volume ≤ 60 mL):

1. Invert the neutralized lysate 8-10 times immediately beforehand to obtain a homogeneous suspension of the flocculate.

2. Pour the lysate, including the white precipitate, into the barrel of the Puro-SPIN™ Filter Cartridge for Plasmid Maxiprep Kit placed onto a suitable bottle or flask (Make sure to remove the cap attached to the tip of the filter!). Do not exceed 60 mL.

3. When all sample is loaded, insert the plunger and press gently to filter the sample. Retain the collected filtrate for loading onto the column.

Note: Apply only moderate pressure. Excessive force compacts the cake and may force fine particulate and chromosomal DNA through the frit.
Optional: Incubating the neutralized lysate for 5 min on ice before filtration produces a coarser flocculate, which filters considerably faster.

Clarification by centrifugation (total volume > 60 mL):

Transfer the neutralized lysate to appropriate centrifuge tubes or bottles and spin according to the equipment available.

High-speed centrifugation: Centrifuge at 20,000 × g for 30 min.

Low-speed centrifugation: Centrifuge at 5,000 × g for 45 min.

In both cases, proceed as follows:
1. Carefully decant or pipette the cleared supernatant, leaving the pellet and any floating precipitate layer undisturbed.

2. Apply 60 mL of the cleared supernatant to the PuroSPIN™ Filter Cartridge for Plasmid Maxiprep Kit and allow it to drip through by gravity flow, collecting the filtrate in suitable bottle or flask. Keep adding the supernatant. The filter serves here as a polishing step, removing any residual suspended matter carried over from the centrifugation.

3. For the final 60 mL fraction, insert the plunger and push the remaining solution through.

Note: If the supernatant remains visibly turbid after centrifugation, transfer it to a fresh tube and repeat the spin before filtration.

Note: Precipitate can re-form in cleared lysates that are left standing. If the filtrate is not to be loaded immediately, inspect it before use and re-filter if any precipitate has appeared.

Protocol for High-Copy Plasmid Purification

Step	Procedure
1	Prepare overnight bacterial culture in LB media by inoculating a single bacterial colony into 2 mL of LB media and incubating overnight. Dilute the starter culture up to 1:10,000 in fresh LB media. Incubate the culture overnight or until OD600 reaches 2 – 3.
2	Determine the OD_{600} of your culture and use it to work out the appropriate culture volume: **V [mL] = 1200 ÷ OD_{600}** Collect the cells by centrifuging at 5,000 × g for at least 10 min at 4 °C, then pour off all of the supernatant. *NOTE: Efficient lysis depends on maintaining a specific ratio between the cell mass and the volumes of Buffers LB-AE1, LB-AE2, and NB-AE. Recommendations for the optimal pelleted culture volume are given in section, "Culture Volume for High-Copy Plasmids" for cells carrying high-copy plasmids and in section, "Culture Volume for Low-Copy Plasmids for those with low-copy plasmids on page 6 of the manual* . **The buffers volumes suggested in steps below assume a final OD_{600} of the culture = 4 and culture volume of 200 mL.**
3	Add 16 mL of **Lysis Buffer LB-AE1** (*with RNase A added*) to collected pellet. Resuspend cells by vortexing. No clumps should be left behind, as these prevent efficient lysis. *NOTE: If started with more than the recommended cell mass, scale the LB-AE1 volume up accordingly. (See section "Cell Lysis" on page 7 of the manual).*
4	Add 16 mL of **Lysis Buffer LB-AE2** to the cell suspension and mix gently by inverting the tube five times. Never vortex — shearing releases contaminating chromosomal DNA from the cell debris. Incubate 5 min at room temperature. *NOTE 1: Inspect Lysis Buffer LB-AE2 for precipitated SDS before use; if white precipitate is visible, warm the buffer at 37 °C for a few minutes until it redissolves completely, then let it return to room temperature.* *NOTE 2: Scale the Lysis Buffer LB-AE2 volume proportionally for larger cell inputs (See section "Cell Lysis" on page 7 of the manual).* *NOTE 3: Extended exposure to alkali can irreversibly denature and degrade the plasmid DNA and free chromosomal DNA into the lysate. Do not exceed 5 minutes of Lysis.*

5	*Equilibrate the* **PuroAE™ Anion-Exchange Column.** *Pour 15 mL of* **Equilibration Buffer EB-AE** *onto the rim of the column, (angle the pipette tip against the inner wall of the column,* **AVOID** *dispensing the buffer directly onto the membrane surface) let the column drain by gravity. The column will not run dry.*
6	Add 16 mL of pre-chilled **Neutralization Buffer NB-AE** and mix the lysate gently by inverting the tube for 8 - 10 times, Do not vortex. *NOTE 1: Chill Buffer Neutralization Buffer NB-AE on ice or refrigerate it before use.* *NOTE 2: Complete neutralization is essential for precipitating all protein and chromosomal DNA: the lysate should change from slimy and viscous to a thin, uniform suspension containing an off-white flocculate.* *NOTE 3: Scale the Neutralization Buffer NB-AE volume proportionally for larger cell inputs (See section "Cell Lysis" on page 7 of the manual).*
7	Determine the total lysate volume (Lysis Buffer LB-AE1 + Lysis Buffer LB-AE2 + Neutralization Buffer NB-AE); if it does not exceed 60 mL, invert the tube 3 times and pour the neutralized lysate — supernatant together with the white SDS–protein precipitate — directly into the filter unit, then allow it to drip through by gravity into a collection bottle (*not provided*). Once flow has essentially stopped, insert the plunger and push the final fraction through using only gentle pressure. If the total volume exceeds 60 mL, first centrifuge the lysate at 20,000 × g for 30 min (or 5,000 × g for 45 min if only a low-speed centrifuge is available), then load the cleared supernatant onto the filter, let it drip through by gravity, and recover the last fraction with the plunger as above. *NOTE : Do not load more than 60 mL — the barrel requires headspace for the plunger and the frit has a limited capacity for precipitate. Do not force the plunger, as this compacts the cake and can drive fine particulate and chromosomal DNA into the filtrate. Incubating the neutralized lysate 5 min on ice before filtration yields a coarser flocculate that filters considerably faster, and the collected filtrate should be loaded onto the column promptly since precipitate can re-form on standing*. Please read senction, "Lysate Clarification Using the PuroSPIN™ Filter Cartridge for Plasmid MAXIprep Kit" on page 9 of the manual.
8	Carefully apply cleared supernatant containing plasmid DNA onto the rim of the equilibrated **PuroAE™ Anion-Exchange Column**, (angle the pipette tip against the inner wall of the column, AVOID

	dispensing the supernatant directly onto the membrane surface) let the column drain by gravity.
9	Wash the column by applying 30 mL of **Wash Buffer WB-AE**. Angle the pipette tip against the inner wall of the column, AVOID dispensing the buffer directly onto the membrane surface let the column drain by gravity.
10	Repeat *STEP 9* with another 30 mL of **Wash Buffer WB-AE**.
11	Add 15 mL of **Elution Buffer EB-AE** to the column. Collect the eluate in a 50 mL centrifuge tube (*not provided)*. NOTE: Warming **Elution Buffer EB-AE** to 50 °C beforehand can improve yields for large constructs such as BACs.
12	Continue with, **"Desalting and Concentration of Anion-Exchange Eluate Using SpinKD Centrifugal Filter"** Protocol on Page 18 of the manual.

Protocol for Low-Copy Plasmid Purification

The lysis buffer volumes provided in the kit are adjusted for high copy plasmid purification. Therefore, additional buffer has to be ordered separately for routine purification of low copy plasmids.

Step	Procedure
1	Prepare overnight bacterial culture in LB media by inoculating a single bacterial colony into 2 mL of LB media and incubating overnight. Dilute the starter culture up to 1:10,000 in fresh LB media. Incubate the culture overnight or until OD600 reaches 2 – 3.
2	Determine the OD_{600} of your culture and use it to work out the appropriate culture volume: **V [mL] = 2400 ÷ OD_{600}** Collect the cells by centrifuging at 5,000 × g for at least 10 min at 4 °C, then pour off all of the supernatant. *NOTE: Efficient lysis depends on maintaining a specific ratio between the cell mass and the volumes of Buffers LB-AE1, LB-AE2, and NB-AE. Recommendations for the optimal pelleted culture volume are given in section, "Culture Volume for High-Copy Plasmids" for cells carrying high-copy plasmids and in section, "Culture Volume for Low-Copy Plasmids for those with low-copy plasmids on page 6 of the manual* . **The buffers volumes suggested in steps below assume a final OD_{600} of the culture = 6 and culture volume of 400 mL.**
3	Add 48 mL of **Lysis Buffer LB-AE1** (*with RNase A added*) to collected pellet. Resuspend cells by vortexing. No clumps should be left behind, as these prevent efficient lysis. *NOTE: If started with more than the recommended cell mass, scale the LB-AE1 volume up accordingly. (See section "Cell Lysis" on page 7 of the manual).*
4	Add 48 mL of **Lysis Buffer LB-AE2** to the cell suspension and mix gently by inverting the tube five times. Never vortex — shearing releases contaminating chromosomal DNA from the cell debris. Incubate 5 min at room temperature. *NOTE 1: Inspect Lysis Buffer LB-AE2 for precipitated SDS before use; if white precipitate is visible, warm the buffer at 37 °C for a few minutes until it redissolves completely, then let it return to room temperature.*

	NOTE 2: Scale the Lysis Buffer LB-AE2 volume proportionally for larger cell inputs (See section "Cell Lysis" on page 7 of the manual). *NOTE 3: Extended exposure to alkali can irreversibly denature and degrade the plasmid DNA and free chromosomal DNA into the lysate. Do not exceed 5 minutes of Lysis.*
5	*Equilibrate the* **PuroAE™ Anion-Exchange Column.** *Pour 15 mL of* **Equilibration Buffer EB-AE** *onto the rim of the column, (angle the pipette tip against the inner wall of the column,* **AVOID** *dispensing the buffer directly onto the membrane surface) let the column drain by gravity. The column will not run dry.*
6	Add 48 mL of pre-chilled **Neutralization Buffer NB-AE** and mix the lysate gently by inverting the tube for 8 - 10 times, Do not vortex. *NOTE 1: Chill Buffer Neutralization Buffer NB-AE on ice or refrigerate it before use.* *NOTE 2: Complete neutralization is essential for precipitating all protein and chromosomal DNA: the lysate should change from slimy and viscous to a thin, uniform suspension containing an off-white flocculate.* *NOTE 3: Scale the Neutralization Buffer NB-AE volume proportionally for larger cell inputs (See section "Cell Lysis" on page 7 of the manual).*
7	Determine the total lysate volume (Lysis Buffer LB-AE1 + Lysis Buffer LB-AE2 + Neutralization Buffer NB-AE); if it does not exceed 60 mL, invert the tube 3 times and pour the neutralized lysate — supernatant together with the white SDS–protein precipitate — directly into the filter unit, then allow it to drip through by gravity into a collection bottle (*not provided*). Once flow has essentially stopped, insert the plunger and push the final fraction through using only gentle pressure. If the total volume exceeds 60 mL, first centrifuge the lysate at 20,000 × g for 30 min (or 5,000 × g for 45 min if only a low-speed centrifuge is available), then load the cleared supernatant onto the filter, let it drip through by gravity, and recover the last fraction with the plunger as above. *NOTE : Do not load more than 60 mL — the barrel requires headspace for the plunger and the frit has a limited capacity for precipitate. Do not force the plunger, as this compacts the cake and can drive fine particulate and chromosomal DNA into the filtrate. Incubating the neutralized lysate 5 min on ice before filtration yields a coarser flocculate that filters considerably faster, and the collected filtrate should be loaded onto the column promptly since precipitate can re-form on standing*. Please read

	senction, "Lysate Clarification Using the PuroSPIN™ Filter Cartridge for Plasmid MAXIprep Kit" on page 9 of the manual.
8	Carefully remove supernatant containing plasmid DNA and apply onto the rim of the equilibrated **PuroAE™ Anion-Exchange Column**, (angle the pipette tip against the inner wall of the column, AVOID dispensing the supernatant directly onto the membrane surface) let the column drain by gravity.
9	Wash the column by applying 30 mL of **Wash Buffer WB-AE**. Angle the pipette tip against the inner wall of the column, AVOID dispensing the buffer directly onto the membrane surface let the column drain by gravity.
10	Repeat *STEP 9* with another 30 mL of **Wash Buffer WB-AE6**
11	Add 15 mL of **Elution Buffer EB-AE** to the column. Collect the eluate in a 50 mL centrifuge tube (*not provided)*. NOTE: Warming **Elution Buffer EB-AE** to 50 °C beforehand can improve yields for large constructs such as BACs.
12	Continue with, "**Desalting and Concentration of Anion-Exchange Eluate Using SpinKD Centrifugal Filter**" Protocol on Page 18 of the manual.

Desalting and Concentration of Anion-Exchange Eluate Using SpinKD Centrifugal Filter

Important notes before proceeding:

1. Use a swinging-bucket rotor if available. Fixed-angle rotors concentrate the retentate against one side of the membrane, increasing localized fouling and making residual volume harder to judge.
2. Mix between every cycle. Concentration polarization forms a viscous gel layer of DNA at the membrane surface. If undisturbed it collapses flux and traps plasmid that never resuspends. Pipette-mixing (gently — avoid shearing) restores both flux and recovery.
3. Don't over-speed. Higher g-force does not proportionally increase flux once the gel layer forms, but it does increase shear on supercoiled DNA and the risk of pushing past the residual-volume mark.
4. Never spin to dryness. A dry membrane binds plasmid irreversibly and is the most common cause of catastrophic yield loss with UF devices. Monitor and stop early rather than adding time.

1	Pre-rinse (*optional but recommended*). Add 10 mL TE to the sample reservoir, spin 5 min at 4,000 × g, discard filtrate. This removes membrane glycerol/preservative and wets the membrane evenly.
2	Transfer the full anion-exchange eluate to the sample reservoir. Do not exceed the rated fill line. Cap the device.
3	Spin at 4,000 × g, until the retentate reaches **0.5 mL** (typically 15–20 min; time varies with plasmid load and lysate cleanliness). Check volume against the graduations rather than relying on a fixed time. Discard the filtrate.
4	Add TE to bring the retentate back to the original fill volume (~15 mL). Pipette-mix the retentate 3–4 times against the membrane face to disperse the concentrated DNA layer. Spin to 0.5 mL as in step 3. Discard filtrate. *NOTE: If the plasmid is destined for enzymatic downstream work or transfection, exchange into 10 mM Tris-HCl pH 8.0 or nuclease-free water instead of TE — EDTA carryover chelates Mg^{2+} and can inhibit restriction digests, ligations, and some transfection reagents.*
5	Repeat step 4 twice more.
6	After the third wash, spin to the target final volume (typically 0.5–1.0 mL). Do not spin below ~200 µL. Carefully pipette solution up and down on each side of the membrane to reconstitute all DNA. Transfer the solution into a new 1.5 or 2 mL microcentrifuge tube.

Troubleshooting

Problem	Possible causes and solutions
No or low plasmid DNA yield	**The plasmid failed to propagate** Before committing to a large-scale purification, gauge how much plasmid is present by running a quick PuroSPIN ™ Plasmid Miniprep Purification Kit. **Alkaline lysis was incomplete** The cell input was too high. See sections “Culture volume for High-Copy Plasmids” on Page 6 of the manual and “Culture Volume for Low-Copy Plasmids” on Page 7 of the manual for the culture and lysis buffer volumes that are recommended. Look for SDS precipitation in Lysis Buffer LB-AE2, before use — this is especially likely after storage below 20 °C. If needed, warm the bottle at 37 °C for several minutes and mix well until the SDS has fully redissolved. **SDS or other precipitates remain in the sample** After centrifugation when transferring the lysate to the column make sure no SDS precipitates are added with supernatant. Whenever precipitate is visible re-centrifuge the lysate again as indicated before loading it. **The sample or lysate is too viscous** Too much cell mass was used — consult sections “Culture volume for High-Copy Plasmids” on Page 6 of the manual and “Culture Volume for Low-Copy Plasmids” on Page 7 of the manual for appropriate culture and lysis buffer volumes. Mix thoroughly after neutralization so that SDS and chromosomal DNA precipitate fully. Otherwise, residual SDS blocks DNA from binding to the column.

PuroSPIN™ Filter Cartridge Filter clogs; flow stops early and little or no filtrate is recovered	**Loading volume was exceeded** Do not load more than 60 mL of lysate per filter unit. For larger volumes, clarify by centrifugation first or divide the lysate between two units. **Too much cell mass was used** Excess cell input produces disproportionately more precipitate and a more viscous lysate. Refer to the recommended culture volumes and, for scaled-up preparations, increase lysis buffer volumes proportionally. **The flocculate is too fine** Incubate the neutralized lysate for 5 min on ice before pouring; a coarser precipitate filters far more readily.
Excessive force is needed on the plunger	**The precipitate cake is saturated or compacted** Do not force the plunger — this extrudes fine particulate and chromosomal DNA into the filtrate. Stop, transfer the remaining lysate to a fresh filter unit, or clarify the remainder by centrifugation (20,000 × g for 30 min, or 5,000 × g for 45 min) and filter the supernatant. **The plunger was inserted too early** Allow the bulk of the lysate to pass by gravity flow first and use the plunger only to recover the final fraction.
Liquid overflows the barrel when the plunger is inserted	**The barrel was filled too close to the rim** The plunger tip displaces volume as it seats. Keep the fill volume at or below 60 mL so that adequate headspace remains.
PuroAE™ Anion-Exchange Column is blocked or running very slowly	**The sample is too viscous** Never try to purify a lysate made from a culture larger than the recommendation for that column size while using standard lysis buffer volumes. Beyond blocking the column, incomplete lysis can cut yields substantially. Refer ee sections “Culture volume for High-Copy Plasmids” on Page 6 of the manual. manual and “Culture Volume for Low-Copy Plasmids” on Page 7 of the manual. Mix well after neutralization to precipitate all SDS and chromosomal DNA.

	The lysate was not fully cleared Filtration failed due to excess of culture or force used on a filter. Centrifuge the lysate. Precipitate can also form during storage; re-clear the lysate before loading the column.
Genomic DNA contamination of the plasmid	**Lysis was too harsh** Do not exceed 5 min of lysis in Lysis Buffer LB-AE2. **The lysate was mixed too vigorously, or vortexed after lysis** Invert the tube exactly five times as indicated in the protocol; never vortex once Lysis Buffer LB-AE2 has been added. Switch to larger tubes, or scale the culture down, to make gentle mixing easier.
RNA contamination of the plasmid	**RNase digestion was inefficient** Either no RNase was added to Lysis Buffer LB-AE1, or it was stored incorrectly. Add fresh RNase to Lysis Buffer LB-AE1 and store at 4 °C when is not used. **Wash buffer pH or salt concentration is too low** Keep all buffers tightly closed as exposure to oxygen can effect the pH of buffers. **Washing with Wash Buffer WB-AE was insufficient** Make sure to wash the column with Wash Buffer WB-AE two times as indicated in the protocol.
Low purity (A260/A280 < 1.8)	**Only a small amount of DNA was loaded** With a lot of binding capacity left unoccupied, more thorough washing is needed — perform the wash twice as indicated. Shorten lysis to under 5 min.
The purified plasmid performs poorly in downstream reactions	**Chromosomal DNA or RNA is contaminating the plasmid** See the detailed troubleshooting sections above. **DNA is degraded** Confirm that all equipment — pipettes, centrifuge tubes, and so on — is clean and free of nucleases. Keep lysis in Lysis Buffer LB-AE2 to no more than 5 min. **The DNA is irreversibly denatured** On a gel, denatured plasmid migrates faster than the supercoiled form. Do not leave the sample in Lysis Buffer LB-AE2 for longer than 5 min after adding it.

SpinKD Centrifugal Filter Desalting Issues	**Low overall recovery (< 70 %)** The membrane may have run dry. Ultrafiltration membranes bind plasmid irreversibly once dewetted, and this is the most common cause of severe loss. Monitor the retentate against the reservoir graduations and stop the run early rather than adding time; never concentrate below 200 µL. **Yield declines progressively across wash cycles** Concentration polarisation is trapping plasmid in a viscous gel layer at the membrane that does not resuspend on refill. Pipette-mix the retentate 3–4 times against the membrane face before adding TE at each cycle. **Retentate volume overshoots the target** Fixed-angle rotor. These concentrate the retentate against one side of the membrane, which increases localised fouling and makes the residual volume hard to read. Use a swinging-bucket rotor where available. **Low A_{260}/A_{23_0}** Residual salt or buffer components. Almost always caused by refilling from 2–3 mL instead of 0.5 mL: a 30-fold volume ratio gives ~30× salt reduction per cycle, but a 5-fold ratio gives only ~5×, and three such cycles leave several millimolar behind. Run one or two additional wash cycles at the correct ratio. **Restriction digest, ligation or other enzymatic step inhibited** EDTA carryover from TE chelates Mg^{2+}. For enzymology or transfection, perform the final exchange into 10 mM Tris-HCl pH 8.0 or nuclease-free H_2O rather than TE.
	Increased nicked or linear species on agarose Excessive shear. Pipette-mix gently with a wide-bore tip; avoid vortexing the retentate, and do not exceed the rated g-force for the device.

User Notes

Safety

Reagent	Risk Symbols	Risk and Safety Phrases
Lysis Buffer LB-AE2, Netralization Buffer NB-AE, Wash Buffer WB-AE, Elution Buffer EB-AE		Contain *Sodium Dodecyl Sulfate* and *Glacial Acetic Acid* • Harmful if swallowed. • Irritating to eyes and skin. • Do not breathe vapors and fumes • Wear suitable protective clothing and gloves. • In case of contact with eyes immediately wash with plenty of water and seek medical advice. • If swallowed, seek medical advice and contact poison control center.

This product is intended solely for research use only. Full material safety data sheet document is available at www.lunanano.com.

For further information about this and other products please visit our website at www.lunanano.com.

Learn more at lunanano.com/dna-rna-purification-products

Luna Nanotech Inc.
85 Citizen Court, Unit 13
Markham, ON, Canada
L6G 1A8

Email: support@lunanano.com
Phone: 1-800-474-4055
Web: www.lunanano.com

Luna Nanotech
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