Luna Nanotech

PuroSPIN-KD™ Plasmid MAXIprep Plus Kit

Product Manual

#NKKD213-2	2 preps
#NKKD213-10	10 preps
#NKKD213-30	30 preps

Manual Version 1.0
September 2026

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Kit Description

The **PuroSPIN-KD™ Plasmid MAXIprep Plus Kit** is designed to provide a simple and easy-to-use method for large scale extraction and purification of plasmid DNA. This purification technology is based on plasmid binding to silica spin-columns, which allows for efficient removal of genomic DNA, protein, salt, and other contaminants. Silica spin columns provide a faster, simpler, and safer alternative to traditional nucleic acid purification techniques such as phenol-chloroform extractions. The PuroSPIN™ buffer formulations produce highly purified plasmid DNA for downstream applications, such as transfection, enzymatic digestion, or PCR. The **PuroSPIN-KD™ Plasmid MAXIprep Plus Kit** can be used to purify plasmid DNA from up to **150 mL** of overnight bacterial culture.

Kit Contents

Component	2 Preps (#NKKD213-2)	10 Preps (#NKKD213-10)	30 Preps (#NKKD213-30)
Lysis Buffer LB-PM1	30 mL	160 mL	2 x 220 mL
Lysis Buffer LB-PM2	30 mL	160 mL	2 x 220 mL
Precipitation Buffer PB-PM	30 mL	160 mL	2 x 220 mL
Binding Buffer BB-S	30 mL	160 mL	2 x 220 mL
Wash Buffer WB-PM1 *	40 mL *	225 mL *	480 mL *
Wash Buffer WB-PM2 **	40 mL **	225 mL **	480 mL **
Elution Buffer EB	15 mL	50 mL	100 mL
Water, Nuclease-free	15 mL	50 mL	100 mL
RNase A (20 mg/mL), DNase-free	1.2 mL	6.4 mL	2 x 8.8 mL
PuroSPIN™ MAXI Spin Columns	2	10	30
PuroSPIN™ Filter Cartridges for Plasmid Maxiprep Kit	2	10	30
SpinKD Centrifugal Filters	2	10	30

* After addition of 100% Isopropanol
** After addition of 96 – 100% Ethanol

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 LP-PM1**, the buffer should be stored at 4 °C.

Additional Materials and Equipment Required

- 96 – 100% Ethanol
- 100% Isopropanol
- 3M NaOH
- 50 mL RNase-free centrifuge tubes with screw caps
- Pipettes and sterile nuclease-free pipette tips
- Refrigerated centrifuge (with 16,000 x g capability, OR 5,000 x g) (preferably with swinging-bucket rotor available)
- Vortex (if not available, can be replaced with vigorous pipetting)
- Appropriate personal protective equipment (gloves, lab coat)

Important Notes

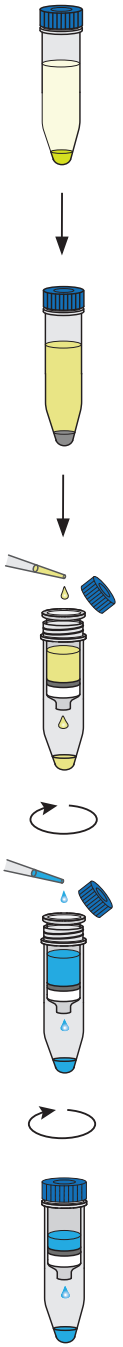
BEFORE USE:

- Add **RNase A** (20 mg/mL) to **Lysis Buffer LB-PM1**. *After addition, store the buffer at 4 °C.*
  - **#NKKD213-2** (2 preps): add **1.2 mL** of RNase A
  - **#NKKD213-10** (10 preps): add **6.4 mL** of RNase A
  - **#NKKD213-30** (30 preps): add **8.8 mL** of RNase A to each bottle
- Add **100% Isopropanol** to **Wash Buffer WB-PM1**:
  - **#NKKD213-2** (2 preps): add **12 mL** of Isopropanol
  - **#NKKD213-10** (10 preps): add **68 mL** of Isopropanol
  - **#NKKD213-30** (30 preps): add **144 mL** of Isoproanol
- Add **96 – 100% Ethanol** to the **Wash Buffer WB-PM2**:
  - **#NKKD213-2** (2 preps): add **32 mL** of Ethanol
  - **#NKKD213-10** (10 preps): add **180 mL** of Ethanol
  - **#NKKD213-30** (30 preps): add **384 mL** of Ethanol

- **Binding Buffer BB-S** and **Wash Buffer WB-PM1** contains ***guanidine hydrochloride***. It is a known irritant that is harmful if inhaled, swallowed, or contacted with skin. Wear appropriate protective equipment when working with this reagent.

- If precipitate is observed in the **Binding Buffer BB-S** and **Wash Buffer WB-PM1**, re-dissolve it by warming the solution to 37°C. Cool the solution back to room temperature before use.

Experimental Workflow



1. Pellet and lyse bacterial cells

2. Precipiate gDNA and proteins

3. Load plasmids onto column

4. Wash plasmids

5. Elute plasmids

Protocol

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	Centrifuge **up to 150 mL** of the overnight bacterial culture at 5,000 x g for 10 min. Carefully remove the supernatant without disrupting the bacterial pellet.
3	Add 14 mL of **Lysis Buffer LB-PM1** to lyse the cells. Resuspend cells by vortexing. *NOTE: Do not allow the lysis reaction to proceed longer than 5 min.*
4	Add 14 mL of **Lysis Buffer LB-PM2** and mix thoroughly by inverting tube 4 – 5 times until the solution becomes viscous and slightly clear. *NOTE: Do not vortex or vigorously pipette the solution during this step. This might shear genomic DNA into smaller pieces, which will be purified together with the plasmids, contaminating the preparation.*
5	Add 14 mL of **Precipitation Buffer PB-PM** and mix by inverting the tube 10 times.
6	Take the new **PuroSPIN™ Filter Cartridges for Plasmid Maxiprep Kit.** Make sure to keep the plug attached to the Luer Lock at the bottom of syringe filter. Place the syringe filter upright in a 50 mL tube rack and load the lysate. Wait for 5 minutes for white precipitate to float to the top.
7	Remove the Luer Lock plug from the bottom of the syringe and place it into a clean 50 ml conical tube. Place a new **PuroSPIN™ Syringe Plunger** in the syringe and push the solution through the **PuroSPIN™ Filter Cartridges for Plasmid Maxiprep Kit** in one continuous motion until approximately 30 ml of cleared lysate is recovered. Save the cleared lysate. Discard the used syringe filter and plunger.
8	Add 14 mL of **Binding Buffer BB-S** to the cleared lysate. Mix by inverting tube 10 times. *NOTE: If a lot of plasmid DNA is present in the lysate it may appear slightly cloudy.*

9	Activate the **PuroSPIN™ MAXI Silica Spin Column** inserted into the provided collection tube by adding 3 mL of **3M NaOH** *(not provided)* solution to the column**.** Incubate for 5 min at room temperature. Centrifuge the column for 4 min at 5,000 x g. Discard the flow-throughth. *NOTE: Column activation step is optional. However, this step can significantly increase the yield of purified plasmid DNA*
10	Transfer 14 mL of the solution into the activated **PuroSPIN™ MAXI Spin Column**. Centrifuge for 3 min at 5,000 x g to bind the sample onto the column. Discard the flow-through. Repeat this step until all the solution has been passed through the column.
11	Add 14 mL of **Wash Buffer WB-PM1** and centrifuge for 3 min at 5,000 x g. Discard the flow-through.
12	Add 14 mL of **Wash Buffer WB-PM2** and centrifuge for 3 min at 5,000 x g. Discard the flow-through.
13	Centrifuge the column for 3 min at 5,000 x g to remove residual buffer. Transfer the column into a clean 50 mL centrifuge tube.
14	Add 5 mL of **Elution Buffer EB** (pre-heated to 55 °C) or **nuclease-free water** (pre-heated to 55 °C). Incubate for 3 min at room temperature. Centrifuge the column for 5 min at 5,000 x g to collect the purified product.
15	Continue with, "**Concentration of Eluate Using SpinKD Centrifugal Filter**" Protocol on Page 8 of the manual.

Concentration of 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
Low or no product yield	**Isopropanol was not added to the Wash Buffer WB-PM1** Make sure to add indicated amount of 100% Isopropanol to the Wash Buffer WB-PM1 before use. **Ethanol was not added to the Wash Buffer WB-PM2.** Make sure the indicated amount of 96 – 100% Ethanol is added to the Wash Buffer WB-PM2 before use. **Incorrect Elution Buffer.** Make sure to use low salt and high pH (8 – 8.5) elution buffer. Make sure to pipette that elution buffer right into the center of the purification column membrane. **Elution Buffer or Nuclease-free water were pre-heated.** Make sure to pre-heat the Elution Buffer or nuclease-free water to 55 °C before adding to the column. **Silica Spin Column was not activated** Make sure to activate the Silica Spin column with 3M NaOH solution prior to loading a sample. This step can significantly increase the yield of plasmid DNA.
Genomic DNA contamination	**Genomic DNA was sheered into smaller components.** Do NOT use vortexing or vigorous pipetting to mix the product after the addition of Lysis Buffer LB-PM2 in Step 4. Only mix the solution by gently inverting the tube 4 – 5 times.
RNA contamination	**RNase A was not added to Lysis Buffer LB-PM1.** Make sure the indicated amount of RNase A is added to the Lysis Buffer LB-PM1 before use.
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.

SpinKD Centrifugal Filter Desalting Issues (*continue*)	**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_{230}** 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.

Safety

Reagent	Risk Symbols	Risk and Safety Phrases
Binding Buffer BB-PM, Wash Buffer WB-PM1		Contains ***guanidine hydrochloride*** • 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

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