



Luna Nanotech

PuroSPIN™ Plasmid MIDIprep Kit

Product Manual

#NK112-2

2 Preps

#NK112-20

20 preps

#NK112-50

50 preps

Manual Version 2.7
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Kit Description

The **PuroSPIN™ Plasmid MIDIprep 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 extraction. The PuroSPIN™ buffer formulations produce highly purified plasmid DNA for downstream applications, such as transfection, enzymatic digestion, or PCR. The **PuroSPIN™ Plasmid MIDIprep Kit** can be used to purify plasmid DNA from **up to 50 mL** of overnight bacterial culture.

Kit Contents

Component	2 Preps (#NK112-2)	20 Preps (#NK112-20)	50 Preps (#NK112-50)
Lysis Buffer LB-PM1	10 mL	100 mL	225 mL
Lysis Buffer LB-PM2	10 mL	100 mL	225 mL
Binding Buffer BB-PM	18 mL	180 mL	200 mL (x2)
Wash Buffer WB-PM1 *	12 mL	120 mL *	225 mL *
Wash Buffer WB-PM2 **	12 mL	120 mL **	225 mL **
Elution Buffer EB	5 mL	30 mL	55 mL
Water, Nuclease-free	5 mL	30 mL	55 mL
RNase A (20 mg/mL), DNase-free	400 µL	4 mL	9 mL
PuroSPIN™ MIDI Spin Columns	2	20	50

* 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

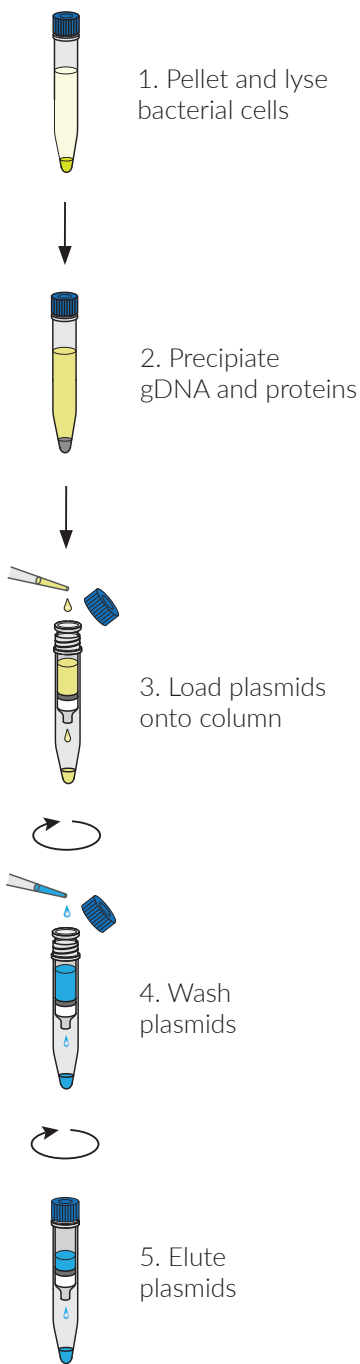
- 96 – 100% Ethanol
- 100 % Isopropanol
- 3M NaOH
- Pipettes and sterile nuclease-free pipette tips
- 15 mL RNase-free centrifuge tubes with screw caps
- Centrifuge (with 12,000 x g capability)
- Vortex (if not available, can be replaced with vigorous pipetting)
- Appropriate personal protective equipment (gloves, lab coat)

Important Notes

BEFORE USE:

- Add **RNase A** (20 mg/mL) to **Lysis Buffer LB-PM1**. *After addition, store the buffer at 4 °C.*
 - **#NK112-2** (2 preps): add **400 µL** of RNase A
 - **#NK112-20** (20 preps): add **4 mL** of RNase A
 - **#NK112-50** (50 preps): add **9 mL** of RNase A
- Add 100 % Isopropanol to the **Wash Buffer WB-PM1**:
 - **#NK112-2** (2 preps): add **3.6 mL** of Isopropanol
 - **#NK112-20** (20 preps): add **36 mL** of Isopropanol
 - **#NK112-50** (50 preps): add **75 mL** of Isopropanol
- Add 96 – 100% Ethanol to the **Wash Buffer WB-PM2**:
 - **#NK112-2** (2 preps): add **9.6 mL** of Ethanol
 - **#NK112-20** (20 preps): add **96 mL** of Ethanol
 - **#NK112-50** (50 preps): add **180 mL** of Ethanol
- **Binding Buffer BB-PM** 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-PM** 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



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 50 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 4 mL of Lysis Buffer LB-PM1 to lyse the cells. Re-suspend cells by vortexing. <i>NOTE: Do not allow the lysis reaction to proceed longer than 5 min.</i>
4	Add 4 mL of Lysis Buffer LB-PM2 and mix thoroughly by inverting tube 4 – 5 times until the solution becomes viscous and slightly clear. <i>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.</i>
5	Add 6 mL of Binding Buffer BB-PM and mix by inverting the tube 10 times. Centrifuge at 4 °C for 30 min at 12,000 x g.
6	Transfer 4 mL of the solution into a new PuroSPIN™ MIDI 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.
7	Add 4 mL of Wash Buffer WB-PM1 and centrifuge for 3 min at 4,000 x g. Discard the flow-through.
8	Add 4 mL of Wash Buffer WB-PM2 and centrifuge for 3 min at 4,000 x g. Discard the flow-through.
9	Centrifuge the column for 3 min at 4,000 x g to remove residual buffer. Transfer the column into a clean 15 mL centrifuge tube.
10	Add 1 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 3 min at 5,000 x g to collect the purified product.

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. Insufficient incubation time. Make sure after adding Elution Buffer or Nuclease-free water to the column incubate the sample for 2 minutes at room temperature.
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 <u>Step 4</u>. 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.

Safety

Reagent	Risk Symbols	Risk and Safety Phrases
Binding Buffer BB-PM, Wash Buffer WB-PM1		Contains <i>guanidine hydrochloride</i> • 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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