

## PuroSPIN™ Plasmid MAXIprep Kit

#NK113-10, #NK113-30

### Product Manual

Version 2.2

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### Kit Contents

Component	10 Preps (#NK113-10)	30 Preps (#NK113-30)
Lysis Buffer LB-PM1	110 mL	250 mL
Lysis Buffer LB-PM2	110 mL	250 mL
Binding Buffer BB-PM	150 mL	200 mL x 2
Wash Buffer WB-PM1	180 mL	250 mL x 2
Wash Buffer WB-PM2 ( <i>after Ethanol addition</i> )	250 mL	250 mL x 2
Elution Buffer EB	50 mL	100 mL
Water, Nuclease-free	50 mL	100 mL
RNase A (20 mg/mL), DNase-free	1 mL	2 mL
PuroSPIN™ MAXI Spin Columns	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 LP-PM1**, the buffer should be stored at **4°C**.

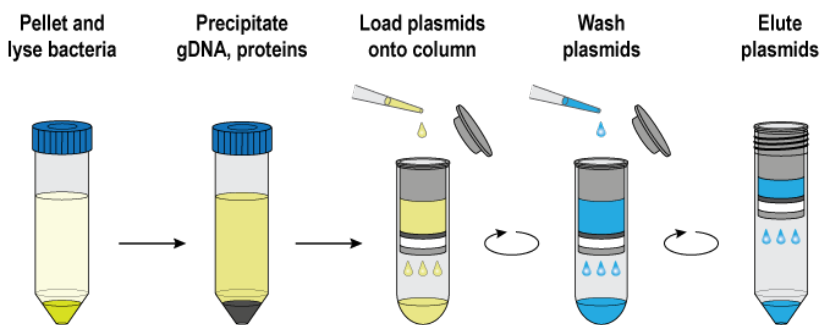
### Additional materials and equipment required

- 95-100% Ethanol
- 50 mL centrifuge tubes with screw caps
- Centrifuge (with 5,000 g capability)
- Appropriate personal protective equipment (gloves, lab coat)

## Kit Description

**PuroSPIN™ Plasmid MAXIprep Kit** is designed to provide simple and easy-to-use method for large scale extraction and purification of plasmid DNA. The purification technology is based on plasmid binding to silica spin-columns, which allows efficient removal of genomic DNA, protein, salt and other contaminants. Silica spin columns offer faster, simpler and safer alternative to traditional nucleic acid purification techniques such as phenol-chloroform extraction. PuroSPIN™ buffer formulations produce highly purified plasmid DNA product for downstream applications, such as transfection, enzymatic digestion, or PCR. **PuroSPIN™ Plasmid MAXIprep Kit** can be used to purify up to **300 mL** of overnight bacterial culture.

## Experimental Workflow



## Important Notes

### BEFORE USE:

- Add RNase A (20 mg/mL) to **Lysis Buffer LB-PM1**.  
*After addition, store the buffer at 4°C.*
  - **#NK113-10** (10 preps): add 1 mL of RNase A
  - **#NK113-30** (30 preps): add 2 mL of RNase A
- Add 96-100% Ethanol to each **Wash Buffer WB-PM2**:
  - **#NK113-10** (10 preps): add 185 mL of Ethanol
  - **#NK113-30** (30 preps): add 185 mL of Ethanol



- **Binding Buffer BB-PM** and **Wash Buffer WB-PM1** contain **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.


## 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 <b>up to 300 mL</b> of the overnight bacterial culture at 5,000g for 10 min. Carefully remove the supernatant without disrupting the bacterial pellet.
3	Add 7 mL of <b>Lysis Buffer LB-PM1</b> to lyse the cells. Resuspend cells by vortexing. <i>NOTE: Do not allow the lysis reaction to proceed longer than 5 min.</i>
4	Add 7 mL of <b>Lysis Buffer LB-PM2</b> and mix thoroughly by inverting tube 4 – 5 times until the solution becomes viscous and slightly clear. <i>NOTE: Do not vortex or vigorously pipet 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 10 mL of <b>Binding Buffer BB-PM</b> and mix by inverting the tube 4 – 5 times. Centrifuge at 4°C for 40 min at 5,000g.
6	Transfer the supernatant into a clean 50 mL centrifuge tube.
7	Transfer 14 mL of the solution into a new <b>PuroSPIN™ MAXI Spin Column</b> . Centrifuge for 2-3 min at 4,000g to bind the sample onto the column. Discard the flow-through. Repeat this step until all of the solution has been passed through the same column.
8	Add 14 mL of <b>Wash Buffer WB-PM1</b> and centrifuge for 3 min at 4,000g. Discard the flow-through.
9	Add 14 mL of <b>Wash Buffer WB-PM2</b> and centrifuge for 3 min at 4,000g. Discard the flow-through.
10	Centrifuge the column for 3 min at 4,000g to remove residual buffer. Transfer the column into a clean 50 mL centrifuge tube.
11	Add 3 mL of <b>Elution Buffer EB</b> or <b>nuclease-free water</b> . Incubate for 2 min at room temperature. Centrifuge the column for 3 min at 4,000g to collect the purified product.

## Troubleshooting

Problem	Possible causes and solutions
Low or no product yield	<p><b>Ethanol was not added to the Wash Buffer WB-PM2.</b> Make sure the indicated amount of 96-100% Ethanol is added to the Wash Buffer WB-PM2 before use.</p> <p><b>Incorrect elution buffer.</b> 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.</p>
Genomic DNA contamination	<p><b>Genomic DNA was sheered into smaller components.</b> 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.</p>
RNA contamination	<p><b>RNase A was not added to Lysis Buffer LB-PM1.</b> Make sure the indicated amount of RNase A is added to the Lysis Buffer LB-PM1 before use.</p>

## Safety

Reagent	Risk Symbols	Risk and Safety Phrases
Binding Buffer BB-PM, Wash Buffer WB-PM1		<p>Contains <b><i>guanidine hydrochloride</i></b></p> <ul style="list-style-type: none"> <li>• Harmful if swallowed.</li> <li>• Irritating to eyes and skin.</li> <li>• Do not breathe vapors and fumes.</li> <li>• Wear suitable protective clothing and gloves.</li> <li>• In case of contact with eyes immediately wash with plenty of water and seek medical advice.</li> <li>• If swallowed, seek medical advice and contact poison control center.</li> </ul>

This product is intended solely for research use only. Full material safety data sheet document is available at [www.lunano.com](http://www.lunano.com).

For further information about this and other products from Luna Nanotech please refer to our website at [www.lunano.com](http://www.lunano.com).