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PuroSPIN™ Plasmid MIDIprep Kit

NK112-20, # NK112-50

Product Manual Version 2.2

Kit Contents

Component	20 Preps (#NK112-20)	50 Preps (#NK112-50)
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
Wash Buffer WB-PM2 (*after Ethanol addition*)	*120 mL*	*250 mL*
Elution Buffer EB	50 mL	100 mL
Water, Nuclease-free	50 mL	100 mL
RNase A (20 mg/mL), DNase-free	1 mL	1 mL x 2
PuroSPIN™ MIDI Spin Columns	20	50

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**.

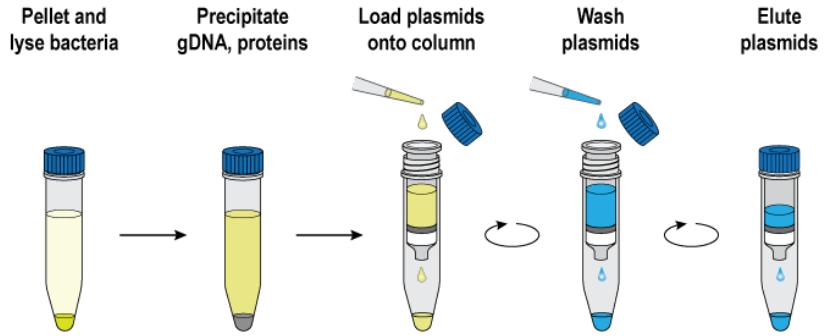
Additional materials and equipment required

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

Kit Description

PuroSPIN™ Plasmid MIDIprep 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 MIDIprep Kit** can be used to purify up to **100 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**.*
  - **#NK112-20** (20 preps): add **1 mL** of RNase A
  - **#NK112-50** (50 preps): add **2** mL of RNase A
- Add 96-100% Ethanol to the **Wash Buffer WB-PM2**:
  - **#NK112-20** (20 preps): add **90 mL** of Ethanol
  - **#NK112-50** (50 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 **up to 100 mL** of the overnight bacterial culture at 5,000g 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. Resuspend cells by vortexing. *NOTE: Do not allow the lysis reaction to proceed longer than 5 min.*
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. ***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.*
5	Add 6 mL of **Binding Buffer BB-PM** 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 15 mL centrifuge tube.
7	Transfer 4 mL of the solution into a new **PuroSPIN™ MIDI Spin Column**. Centrifuge for 4 – 5 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 4 mL of **Wash Buffer WB-PM1** and centrifuge for 3 min at 4,000g. Discard the flow-through.
9	Add 4 mL of **Wash Buffer WB-PM2** 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 15 mL centrifuge tube.
11	Add 1 mL of **Elution Buffer EB** or **nuclease-free water**. 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	**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.
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.

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 from Luna Nanotech please refer to our website at www.lunanano.com.