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PuroSPIN™ Gel Extraction Kit

NK107-50, # NK107-250

Product Manual Version 2.2

Kit Contents

Component	50 Preps (#NK107-50)	250 Preps (#NK107-250)
Binding Buffer BB-GE	100 mL	2 x 220 mL
Wash Buffer WB-GE (*after Ethanol addition*)	120 mL	2 x 225 mL
Elution Buffer EB	15 mL	50 mL
Water, Nuclease-free	15 mL	50 mL
PuroSPIN™ MINI Spin Columns	50	250
Collection Tubes (2 mL)	50	250

Storage

All components can be stored at room temperature for at least 1 year.

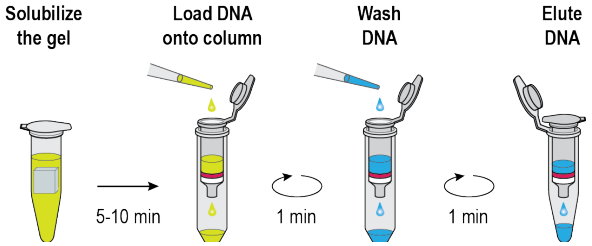
Additional materials and equipment required

- 95-100% Ethanol
- 100% Isopropanol
- 1.5 mL microcentrifuge tubes
- Microcentrifuge (with 12,000g capability)
- Appropriate personal protective equipment (gloves, lab coat)

Kit Description

PuroSPIN™ Gel Extraction Kit is designed to provide simple and easy-to-use method for extraction and purification of DNA samples (100 bp − 10 kbp) from agarose and PAGE gels. The purification technology is based on dissolving the gel embedded DNA in a buffer solution, followed by nucleic acid binding to silica spin-columns, which allows efficient removal of gel residues 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 DNA product for downstream applications, such as PCR, restriction digestion, ligation, labeling, or sequencing. PuroSPIN™ columns can bind up to 200 µg of DNA product.

Experimental Workflow



Important Notes

BEFORE USE:

- Add 96-100% Ethanol to the **Wash Buffer WB-GE**:
  - **#NK107-50** (50 preps): add **90 mL** of Ethanol
  - **#NK107-250** (250 preps): add **170 mL** of Ethanol

- **Binding Buffer BB-GE** contains ***guanidine thiocyanate***. 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-GE** re-dissolve it by warming the solution to 37°C. Cool the solution back to room temperature before use.

Protocol A. DNA Extraction from Agarose Gel

Step	Procedure
1	Excise the DNA fragment from the agarose gel with a clean, sharp razor blade. Weigh the gel slice in a clean 1.5 mL microcentrifuge tube.
2	Add 3 volumes of **Binding Buffer BB-GE** to 1 volume of the agarose gel (≤ 2% agarose). For example, add 150 µL of **Binding Buffer BB-GE** to 50 mg gel slice. *NOTE: For gels with > 2% agarose, add 5 gel volumes of **Binding Buffer BB-GE**. A maximum of 500 mg of agarose gel is recommended to be used with each spin column.*
3	Incubate the tube at 50°C for 5-10 min (or until the gel slice has completely dissolved). Vortex the tube every 5 min during the incubation to help dissolve the gel. *NOTE: If at this stage the color of the solution changes from yellow to purple or violet, increase the solution acidity by adding 5-10 µL of 3 M potassium acetate buffer (pH 5).*
4	Add 1.5 volumes of **100% Isopropanol** to the sample. For example, add 75 µL of 100% isopropanol to 50 mg gel slice. *NOTE: For gels with > 2% agarose, add 2.5 gel volumes of **Isopropanol.***
5	Transfer up to 800 µL of the sample into the **PuroSPIN™ MINI Spin Column.** Centrifuge at 12,000g for 1 min to allow DNA to bind to the column. Discard the flow-through. *NOTE: If the total sample volume exceeds 800 µL, repeat this step until all of the sample has been loaded onto the column.*
6	Add 700 µL of **Wash Buffer WB-GE** and centrifuge at 12,000g for 1 minute. Discard the flow-through.

7	Add another 700 µL of **Wash Buffer WB-GE** and centrifuge at 12,000g for 1 minute. Discard the flow-through. *NOTE: If more than 800 µL of the dissolved gel solution were loaded onto the column in Step 5, it is recommended to wash the solution 1 additional time with **Wash Buffer WB-GE** to better remove all contaminating salts and yield higher purity product.*
8	Centrifuge at 12,000g for 1 min to remove the residual washing buffer.
9	Transfer the column into a clean 1.5 mL microcentrifuge tube.
10	Add 30-100 µL of **Elution Buffer EB** or **nuclease-free water**. Incubate for 2 min at room temperature. *NOTE: Smaller volume will yield higher concentration DNA product, while larger volume will maximize the total amount of eluted DNA. The eluted DNA amount can be further increased by repeating the DNA elution step using another 100 µL of **Elution Buffer EB** or **nuclease-free water**.*
11	Centrifuge the column at 12,000g for 1 minute to collect the purified product.

Protocol B. DNA Extraction from PAGE Gel

Step	Procedure
1	Excise the DNA fragment from the PAGE gel with a clean, sharp razor blade. Weigh the gel slice in a clean 1.5 mL microcentrifuge tube.
2	Mince the gel slice into smaller pieces inside the tube using a clean pipette tip.
3	Add 5 volumes of **Binding Buffer BB-GE** to 1 volume of the PAGE gel. For example, add 250 µL of **Binding Buffer BB-GE** to 50 mg gel slice.
4	Incubate at 50°C for 35 min to allow diffusion of DNA from the gel into the buffer. Vortex the tube every 5 min during the incubation. *NOTE: If at this stage the color of the solution changes to purple or violet, increase the acidity by adding 5-10 µL of 3 M potassium acetate buffer (pH 5).*
5	Add 2.5 volumes of **100% Isopropanol** to the gel sample and centrifuge at 12,000g for 2 min. Transfer the supernatant into a clean 1.5 mL microcentrifuge tube.
6	Transfer up to 800 µL of the sample into the **PuroSPIN™ MINI Spin Column.** Centrifuge at 12,000g for 1 min to allow DNA to bind to the column. Discard the flow-through. *NOTE: If the total sample volume exceeds 800 µL, repeat this step until all of the sample has been loaded onto the column.*
7	Add 700 µL of **Wash Buffer WB-GE** and centrifuge at 12,000g for 1 minute. Discard the flow-through.
8	Add another 700 µL of **Wash Buffer WB-GE** and centrifuge at 12,000g for 1 minute. Discard the flow-through.
9	After the last wash step, place the column back into the collection tube and centrifuge at 12,000g for 1 min to remove the residual washing buffer.

10	Transfer the column into a clean 1.5 mL microcentrifuge tube.
11	Add 30-100 µL of **Elution Buffer EB** or **nuclease-free water**. Incubate for 2 min at room temperature. *NOTE: Smaller volume will yield higher concentration DNA product, while larger volume will maximize the total amount of eluted DNA. The eluted DNA amount can be further increased by repeating the DNA elution step using another 100 µL of* ***Elution Buffer EB*** *or* ***nuclease-free water****.*
12	Centrifuge the column at 12,000g for 1 minute 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-GE.** Make sure the indicated amount of 96-100% Ethanol is added to the Wash Buffer WB-GE before use. **Dissolved gel solution is purple instead of yellow in color.** Add 3M potassium acetate (pH 5) to the dissolved gel solution to increase its acidity before loading it onto the column. **Isopropanol was not added to the sample.** Make sure the indicated amount of isopropanol is added to the sample before loading it onto the column. **Too much starting material was used.** Make sure the starting DNA amount does not exceed 200 μg. **Incorrect elution buffer is used.** Make sure to use low salt and high pH (8 – 8.5) elution buffer. Make sure to pipette the elution buffer directly onto the center of the membrane.

Safety

Reagent	Risk Symbols	Risk and Safety Phrases
Binding Buffer BB-GE		Contains ***guanidine thiocyanate*** • Harmful if swallowed, irritating to eyes / 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.

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