5. **Transfer the sample to Spin column. Centrifuge for 1 min at 6,000 x g. Discard the flow-through and place the column back into the same collection tube.**
   If the sample volume is more than 750 µL, load the sample and centrifuge spin column again. If the sample volume is less than 750 µL, you will just need to centrifuge the sample only once.
6. **Add 500 µL Extraction Buffer to Spin column, centrifuge for 30~60 sec at 12,000 x g. Discard the flow-through. Add 750 µL Wash Buffer to Spin column, centrifuge for 30~60 sec at 12,000 x g. Discard the flow-through.**
   If the DNA will be used for salt sensitive applications, leave the spin column at room temperature for 2~5 mins after adding the Wash Buffer before centrifugation.
7. **Centrifuge for an additional 1 min at 12,000 x g and transfer the Spin column to a sterile 1.5 mL microcentrifuge tube.**
   This helps to prevent residual liquid in the column.
8. **Add 30~100 µL Elution Buffer, H_2O or TE Buffer to the Spin column and incubate it at room temperature for 1 min.**
   The volume of elution buffer can be adjusted according to needs, but should not be less than 20 µL.
9. **Centrifuge the sample tube for 1 min at 12,000 x g. Discard the spin column and store the purified DNA at -20°C.**
   The extracted DNA can be used directly for all kinds of downstream molecular biological experiments.

Troubleshooting

No DNA recovery

If the DNA fragment is not found in elution buffer, check whether ethanol has been added to Wash Buffer according to the volume marked on bottle label.

Low DNA recovery

1. Check the color of the solution after the gel slice is completely dissolved. The extraction buffer has an acidic pH. If the pH increases after gel melts, i.e. binding mixture turns purple, DNA will not bind efficiently. If this happens, add 0.1 volume 3 M sodium acetate (pH 5.0) to the sample and mix. The color of the mixture should become yellow.
2. If electrophoresis buffer has been repeatedly used, DNA recovery will become low as well. Replace the electrolysis buffer to increase DNA recovery.
3. Incubate the Elution Buffer at 30~60°C to increase DNA yields.

Recovery counting

1. It is not recommended to test DNA recovery rate by comparing the absorbance via spectrophotometer because the samples would contain non-targeted DNA fragments, primer, dNTP and other components before extraction.
2. We recommend using electrophoresis and gel imaging system to test recovery rate by grey level contrast analysis.

RESEARCH INSTRUMENTS
Enabling Next-Generation Science

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PRODUCT INFORMATION

PureNA™ Biospin Gel Extraction Kit
Cat# KN02-50, KN02-250

www.ri.com.sg

Kit Components

Cat#	KN02-50	KN02-250
Components	**50 preps**	**250 preps**
Extraction Buffer	85 mL	213 mL x 2
Wash Buffer	10 mL	25 mL x 2
Elution Buffer	10 mL	50 mL
Collection tubes (2 mL)	50	250

Storage and Transportation

- The kit should be stored at room temperature (15~25℃) for up to 18 months if all components are kept well.
- The kit can be transported at room temperature.

Description

PureNA™ Biospin Gel Extraction Kit provides a simple, rapid and effective method for purification of DNA fragments from agarose gel in TAE or TBE buffer. DNA fragments ranging from 60 bp to 23 kb are purified from up to 3% standard or high / low-melt gel using spin column. Purified DNA can be used directly for all kinds of downstream molecular biological experiments such as cloning, sequencing, restriction enzyme digestion, PCR and sequencing.

Technical Information

Method	Working time	Column volume	DNA size range	Elution recovery	Sample volume
Spin column	16 mins for 2 samples	750 µL	60 bp~23 kb	≥99%	Up to 400 mg gel slice

Agarose type	Electrophoresis	Incubate temperature
High / low melt agarose	TAE/TBE buffer	50°C (low-melt agarose) 55°C (standard agarose)

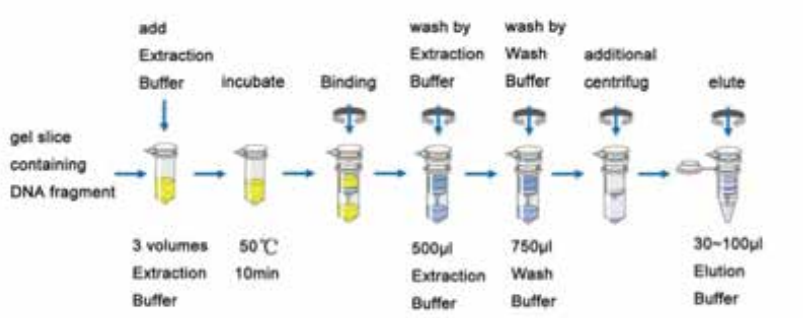
Apparatus and materials to be prepared by the user

- Sterile 1.5 and 2.0 mL microcentrifuge tubes
- Ethanol (>95%)
- Isopropanol
- 3 M sodium acetate (pH 5.0), if necessary
- Microcentrifuge capable of 14,000 x g
- Vortex mixer

IMPORTANT NOTES

1. The yellow color of Extraction Buffer indicates pH ≤7.8.
2. Add 40 mL ethanol (volume is specified on bottle label) to Wash Buffer and mix well.
3. Close the lid after using the Extraction Buffer as soon as possible.
4. The fitting elution volume is 50 µL. However, user can adjust its volume if required.
5. 3 M sodium acetate (pH 5.0) can be used if required.

Centrifugation Protocol



1. **Excise the DNA fragment from the agarose gel with a clean and sharp scalpel.**
   Minimize the size of the gel slice by removing extra agarose.
2. **Weigh the gel slice and add 3 volumes of Extraction Buffer to 1 volume of gel slice (100 mg=100 µL).**
   For example, add 300 µL Extraction Buffer to each 100 mg of gel. The gel slice should not be more than 400 mg per prep.
3. **Incubate the gel mixture at 50°C for 10 mins or until the gel melts in a heating block and vortex the tube every 2-3 mins during the incubation.**
   If the color of the mixture is purple, add 10ul of 3 M sodium acetate (pH5.0) and mix. The color of the mixture will become yellow.
4. **Optional: Add 1 volume of isopropanol to 1 volume of gel and mix.**
   There is no need to add isopropanol if the fragments are > 500 bp or < 4 kb long.