

November 2007

**** NEW: improved buffers! ****



mi-Gel Extraction Kit

**Cat. No mi-GE100
[100 Preparations]**

**This kit is for research purposes only.
Not for use in diagnostic procedures.
For *in vitro* use only.**

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Introduction

The mi-Gel Extraction Kit is a high performance simple to use product to recover pure DNA from all kind of agarose gels. The isolation procedure can be performed in about 10 to 20 minutes. It is not necessary to use low melt agarose to get high yields. The resulting DNA can be used for any downstream application.

Specifications

DNA size range: 80 bp - 50 kb

Types of agarose: All

Types of gel buffers: All

Maximum gel band weight: 200 mg per spin column

Recovery rates: 70 - 95%

Final volume of DNA: 30 μ l

Kit Contents

Preparation

Gel Extraction Buffer

Column Wash Buffer

Spin column

Collection tube

mi-Gel Extraction Kit

100rxn /kit

110 ml (room temperature)

25 ml (room temperature)

Add 100 ml of pure ethanol (99.9%) to the "Column Wash Buffer" up to a final volume of 125 ml prior to first use

100

100

Required Equipment

Microcentrifuge (13.000 rpm or 12.000 x g)

Vortexer

Microcentrifuge tubes

Water bath or heating block (65°C)

Distilled water (pH 7-8) or 10 mM Tris-HCL (pH 8.0)

Kit Storage

At room temperature for 6 months.

Precautions

See MSDS on our homepage.

Wear gloves and goggles. Avoid contact with all reagents. If eye or skin contact occurs, wash thoroughly with water.

Protocol

Note: Before starting, please make sure....

- Add 100 ml of pure ethanol (99.9%) to the "Column Wash Buffer" up to a final volume of 125 ml prior to first use
- If the Gel Extraction Buffer is precipitated, heat to dissolve at 50°C for 10 min.

1. Cut the desired DNA band with a clean, sharp scalpel from a TAE or TBE agarose gel.
2. Place the gel piece (maximum 200 mg) in a 1.5 ml microcentrifuge tube.
3. Add 3 volumes of Gel Extraction Buffer to the 1.5 ml microcentrifuge tube. For example: 100 mg of agarose require 300 µl of Gel Extraction Buffer. Be sure the gel is submerged in the Gel Extraction Buffer.
4. Incubate for 5 - 10 min at 65°C.
Mix thoroughly for 10 sec. *Do not vortex!!!*
Invert the tube every 2-3 min. Incubate until the gel is completely melted. (> 2% gels require up to 10 min for melting)
5. If the colour of the mixture becomes brown after melting the gel slice, add 10µl of 3M sodium acetate (pH 5.0). The colour will turn to yellow.
If the loading dye is included in the gel piece, the colour will change to purple.
6. Insert the spin column into the collection tube and add the solution into the spin column.
7. Centrifuge the spin column at 13,000 rpm (12.000 x g) for 1 min at room temperature.
8. Discard the flow through liquid and replace spin column in collection tube.
9. Add 500 µl of Column Wash Buffer to the spin column.
(Make sure to have completed the "Column Wash buffer" by adding 100 ml of pure ethanol (99,9%) before first use.)
10. Centrifuge at 13,000 rpm (12.000 x g) for 1 min at room temperature.
11. Discard the flow through liquid. Repeat steps 9 and 10 once more.
12. To eliminate any possibility of Column Wash Buffer carryover, spin at 13,000 rpm (12,000 x g) for 1 min at room temperature. Replace the spin column into a clean microcentrifuge tube (not included).

13. Elute the DNA by adding 10 - 30 µl of nuclease-free water or 10 mM Tris-HCl (pH 8.0) directly onto the centre of the white spin column membrane.
14. Spin at 13,000 rpm (12,000 x g). The DNA is now ready to use.

Hints and Troubleshooting

Concentrating the DNA

If your final elution volume is too diluted for your purposes, add 1.5 µl of 5 M NaCl and mix. Then add 70 µl of 100% cold ethanol. Mix and centrifuge at 13,000 rpm (12,000 x g) for 5 min at 4°C. Decant all liquids. You can dry the residual ethanol in a speed vac, desiccator or with ambient air. Resuspend the precipitated DNA in desired volume.

Melting the gel slice

Always make sure you have melted the gel completely at 55-65°C before proceeding to step 6. If the tube is floating too high out of the water / heating block, the gel will take longer to melt. Repeat the steps 4+5 to ensure complete melting.

Low Recovery (recovery below 50% is considered poor)

- The gel was not completely melted before step 6.
- The melting temperature was too high.
- The centrifuge did not spin with sufficient force.
- Distilled water or 10mM Tris-HCl (pH 8.0) were not loaded directly onto the centre of the spin column.

Incubating the spin column loaded with TE buffer or distilled water for 1-5 min at room temperature before the centrifugation generally increases the DNA yield. The elution efficiency will decrease when using the elution buffer with a pH <7.0

If you have any questions or comments, please contact us at:

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