ISOSPIN Agarose Gel

Manual (Ver. 04)

Code No. 311-07981

NIPPON GENE CO., LTD.

I Description

The ISOSPIN Agarose Gel enables an easy, rapid and effective isolation of DNA fragments from agarose gels for 30 min. The kit is applicable for DNA isolation from up to 5% standard or low-melt agarose gels in TAE or TBE buffer.

II Kit components

Component	(100 preps)	Note
ISAE Buffer	75 ml x 2	Yellow *
ISW Buffer	100 ml x 1	(includes ethanol) **
ISE Buffer	10 ml x 1	10 mM Tris-HCl (pH 8.5)
Spin Column (a Spin Column and Collection Tube set)	50 sets x 2	

* If precipitates appear in the ISAE Buffer, please incubate at 37-60°C, inverting periodically to dissolve.

** Keep the buffer bottle tightly closed after use.

III Storage conditions

All the kit components can be stored at room temperature (15-25°C).

IV Precautions

- The kit is intended research use only.

V Protocol

Material not supplied

- Micropipette
- Pipette tips
- 1.5 mL microcentrifuge tubes
- Microcentrifuge
- Heater (at 50°C)
- Isopropanol

Material, as necessary

- 2.0 mL microcentrifuge tubes
- 3M Sodium Acetate (pH 5.2)

Protocol

1. Excise DNA fragment out of the gel with a clean scalpel and transfer it to a clean 1.5-2 mL microcentrifuge tube. Weigh **the gel slice**.

   Note)

   - The gel slice should be ≤250 mg in a 1.5 mL microcentrifuge tube.
   - The gel slice should be ≤340 mg in a 2.0 mL microcentrifuge tube.
   - UV light Exposure time should be kept as short as possible.

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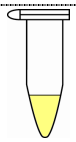

2. Add 3 volumes of **ISAE Buffer** (Yellow) to 1 volume of gel slice.

   Example) Add 300 µl of ISAE Buffer to 100 mg of gel.

3. Incubate at 50°C for 10 min, and mix by inverting the tube every 2 min during the incubation until the gel slice has completely dissolved.

4. After the gel slice has completely dissolved, check that the color of the mixture is yellow.

   Note) If the color of the mixture becomes orange or purple, add 1/60 volumes of 3M Sodium Acetate (pH 5.2) to 1 volume of the mixture. The color will return yellow.

   e.g., Add 6.7 µl of 3M Sodium Acetate (pH 5.2) to about 400 µl of the mixture (100 mg of the gel and 300 µl of the ISAE Buffer).

5. Add 1 volume of **isopropanol** to 1 gel volume (*i.e.,* add 1/4 volumes of isopropanol to 1 volume of the mixture), and mix well by inverting. Spin down lightly.

   Example) Add 100 µl of isopropanol to about 400 µl of the mixture (100 mg of the gel and 300 µl of the ISAE Buffer).

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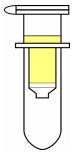

6. Prepare a **Spin Column** (a Spin Column and Collection Tube set). Apply the **Mixture** to the Spin Column.

7. Centrifuge for 1 min at 12,000 x g at room temperature.

8. Discard the flow-through. Place the Spin Column back into the same Collection Tube.

   Note) If there are the Mixture left over, apply the remaining Mixture to the Spin Column, and repeat 7 and 8.

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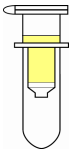

9. Apply 500 µl of **ISAE Buffer** (Yellow) to the Spin Column.

10. Centrifuge for 1 min at 12,000 x g at room temperature.

11. Discard the flow-through. Place the Spin Column back into the same Collection Tube.

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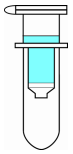

12. Apply 750 µl of **ISW Buffer** to the Spin Column.

13. Centrifuge for 1 min at 12,000 x g at room temperature.

14. Discard the flow-through. Place the Spin Column back into the same Collection Tube.

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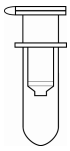

15. Centrifuge for 1 min at 12,000 x g at room temperature to remove residual liquid.

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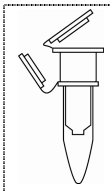


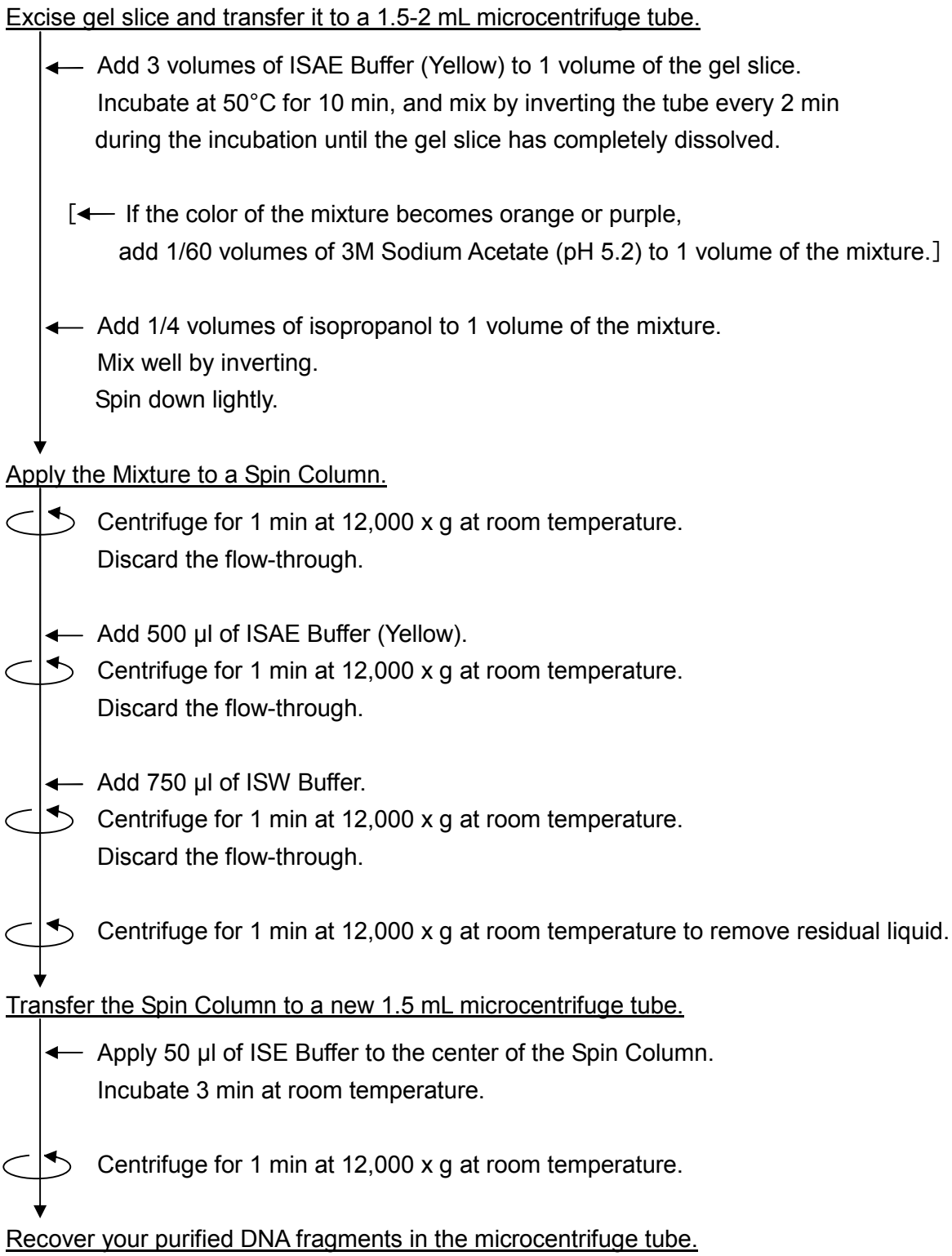
16. Transfer the Spin Column to a new 1.5 mL microcentrifuge tube.

17. Add 50 µl of **ISE Buffer** to the center of the Spin Column. Incubate 3 min at room temperature.
Note) Nuclease-free water or TE (pH8.0) can also be used to elute the DNA in place of ISE Buffer (10 mM Tris-HCl, pH 8.5).

18. Centrifuge for 1 min at 12,000 x g at room temperature.

19. Recover your purified DNA fragments in the microcentrifuge tube.
Note) The DNA fragments can be used directly or stored at -20°C for long term storage.

Simplified Protocol



VI Troubleshooting

Low DNA Yield

Ensure the ISE Buffer is applied to the center of the Spin Column so that elution is efficient.
Larger elution volumes can increase yield of DNA at the cost of dilution of the sample.
Ensure reagents will be added correctly.
Be sure to incubate until the gel slice has completely dissolved.
The ISAE Buffer (Yellow) is acidic. If the pH increases during the gel melting process, the color of the mixture will become Orange or Purple. In such case, add 3M Sodium Acetate (pH 5.0) to adjust the pH of the mixture.

Low DNA Performance

Please repeat wash step (Step 12, 13 and 14). 80% Ethanol can also be used to wash the DNA in place of ISW Buffer.
If ethanol has been carried-over, spin for 5 min, instead of 1 min in Step 15.

Sheared DNA

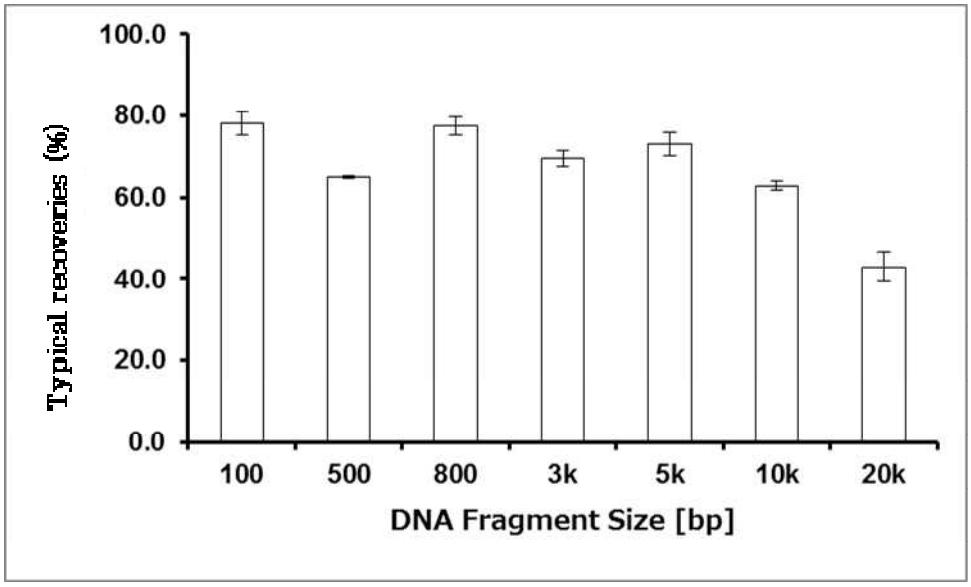
Mix well by inverting or flicking the tube. Do not mix by pipetting.

VII Data

Technical Information

DNA Binding Volume	20 µg
Column Volume	900 µl
Minimum Elution Volume:	10 µl
Typical DNA Recovery	40 - 80% (100 bp - 20 kbp)

Typical recoveries with smaller and larger fragment sizes



The information in the descriptions of the products may be changed without prior notification.

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If you have any questions, please contact us by web form.

http://www.nippongene.com/