ISOSPIN Plasmid

Manual (Ver. 05)

Code No. 318-07991

NIPPON GENE CO., LTD.

I Description

The ISOSPIN Plasmid is an easy and rapid method for the purification of highly pure plasmid DNA from 1-5 mL overnight culture of *E. coli*.

II Kit components

Component	(100 preps)	Note
IS1 Buffer	30 ml x 1	(see next page)
IS2 Buffer	30 ml x 1	
IS3 Buffer	40 ml x 1	
ISPW Buffer	60 ml x 1	
ISW Buffer	100 ml x 1	(includes ethanol)*
ISE Buffer	10 ml x 1	10 mM Tris-HCl (pH 8.5)
RNase A (100 mg/ml)	60 µl x 1	(see next page)
Spin Column (a Spin Column and Collection Tube set)	50 sets x 2	

* Keep the buffer bottle tightly closed after use.

III Storage conditions

All the kit components can be stored at room temperature (15-25°C). RNase A is stable for longer periods when stored at 4°C or -20°C. After adding the RNase A to the IS1 Buffer, store the IS1 Buffer [+ RNase A] at 2-10°C.

IV Precautions

- The kit is intended research use only.

V Protocol

Material not supplied

- Micropipette
- Pipette tips
- 1.5 mL microcentrifuge tubes
- Microcentrifuge

Before You Begin

Buffer Preparation

☐ Add all the RNase A (100 mg/ml) to the IS1 Buffer, and mix well.
Store the IS1 Buffer [+ RNase A] at 2-10°C.
Please mark the buffer bottle to indicate whether RNase A has been added or not.

Prepare the Starting Materials

Pick a fresh single colony from an LB agar plate with appropriate antibiotic, and inoculate in liquid LB medium containing the same selective antibiotic. Incubate bacterial cultures at 37°C for 12-16 hours. Do not incubate the cultures for longer than 16 hours because the cells will begin to lyse.

You can now isolate your plasmid DNA from the bacterial culture:

- Prepare 1-5 ml *E.coli* culture for High-copy plasmids, such as pUC.
- Prepare 1-10 ml *E.coli* culture for Low-copy plasmids, such as pBR.

Checklist

☐ Please check whether the RNase A had been added to the IS1 Buffer bottle.
☐ If precipitates appear in the IS2 Buffer, please incubate at 37°C, inverting periodically to dissolve.

Plasmid DNA Extraction Protocol

1. Prepare **1-5 ml overnight (12-16 hours) culture of *E.coli***, as described in the previous page. Add the culture to a 1.5 mL microcentrifuge tube. Centrifuge at 10,000 x g for 5 min at room temperature. Discard the supernatant and repeat Step 1 to collect more cells.

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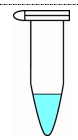

2. Add 250 µl of **IS1 Buffer [+ RNase A]** to the pellet of bacterial cells, and mix by vortex or pipet. Resuspend completely the pellet until there are no visible clumps.

3. Add 250 µl of **IS2 Buffer** and gently invert the tube 4-6 times to mix until the solution is clear.
   Note) Do not allow this step for more than 5 min. Do not Vortex to avoid shearing of genomic DNA.

4. Add 350 µl of **IS3 Buffer** and gently invert the tube 4-6 times to mix well.
   Note) Do not vortex or vigorous shake.

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

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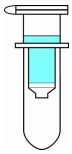

6. Prepare a **Spin Column** (a Spin Column and Collection Tube set). Apply all the **supernatant** to the Spin Column.
   Note) Carefully transfer ~800 µl supernatant to Spin Column without disturbing a white pellet form.

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

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

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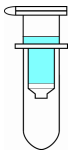

9. Add 500 µl of **ISPW Buffer** to the Spin Column.

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

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

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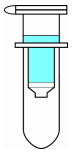

12. Apply 750 µl of **ISW Buffer** to the Spin Column.

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

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

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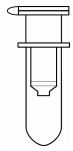

15. Centrifuge at 12,000 x g, for 1min at room temperature to remove residual liquid.

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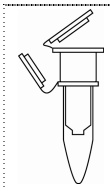


16. Transfer the Spin Column to a new 1.5 mL microcentrifuge tube.

17. Add 50 µl of **ISE Buffer** to the center of the Spin Column. Incubate 3 min at room temperature.

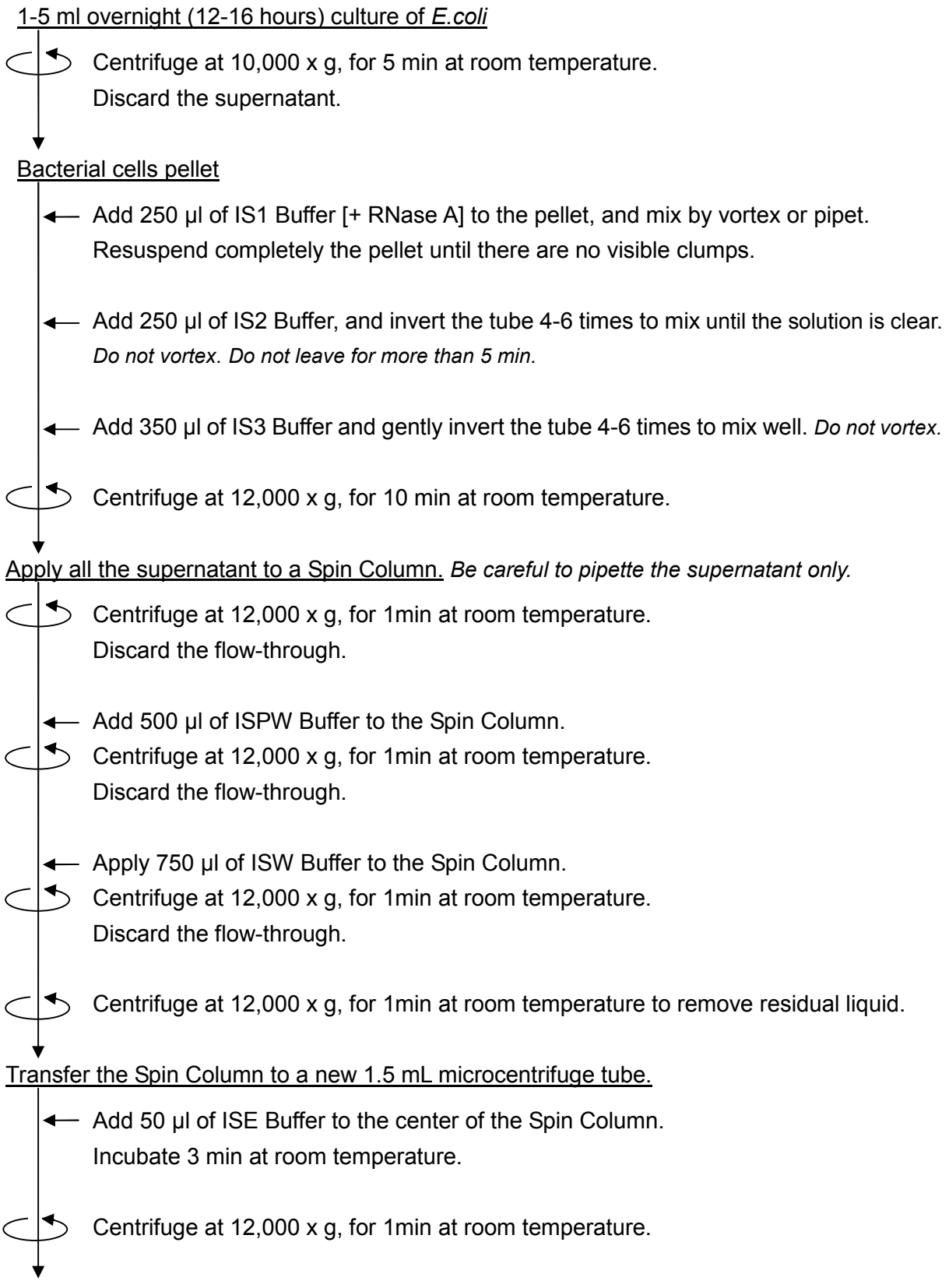
    Note) Nuclease-free water or TE (pH8.0) can also be used to elute the DNA in place of ISE Buffer (10 mM Tris-HCl, pH 8.5). We recommend using TE (pH8.0) containing EDTA if you store the eluted DNA for long term storage.

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

19. Recover your purified plasmid DNA in the microcentrifuge tube.

    Note) The purified plasmid DNA 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.
For Low-copy plasmid, use 1-10 ml of an overnight *E.coli* culture as starting material.

Contaminated with genomic DNA

Do not vortex or leave after adding the IS2 Buffer. Please read instructions carefully.

Low DNA Performance

If ethanol has been carried-over, spin for 5 min, instead of 1 min in Step 15.

VII Data

Technical Information

Binding Capacity:	up to 20 μg
Column Volume:	900 μl
Elution Volume:	50 μl
Plasmid Size:	up to 20 kbp

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

NIPPON GENE CO., LTD.

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

http://www.nippongene.com/

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