HiYield Plasmid Mini Kit 3.0

(SYG011-001/FYG011-100/FYG011-300) Store at RT/4℃ Ver. S0521

Sample：1-4 ml of bacterial cultures Yield：Up to 50 µg

Contents

	SYG011-001 (4 preps)	FYG011-100 (100 preps)	FYG011-300 (300 preps)
PN1 Buffer	1 ml	25 ml	65 ml
PN2 Buffer	1 ml	25 ml	65 ml
PN3 Buffer	1.5 ml	35 ml	95 ml
W1 Buffer	2 ml	45 ml	125 ml
W2 Buffer	300 µl x2	15 ml	25 ml x2
Elution Buffer	1 ml	10 ml	30 ml
RNase A (50 mg/ml)	Added	50 µl	150 µl
PN Column	4 pcs	100 pcs	300 pcs
Collection Tube	4 pcs	100 pcs	300 pcs
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***Please add provided RNase A to PN1 Buffer and store at 4°C.**

Buffer Preparation

- **Add ethanol (96-100%) to the W2 Buffer prior to initial use**

	SYG011-001 (4 preps)	FYG011-100 (100 preps)	FYG011-300 (300 preps)
PN1 Buffer **RNase A**	Added	25 ml 50 µl	60 ml 150 µl
W2 Buffer **Ethanol (96-100%)**	300 µl x2 1.2 ml x2	15 ml 60 ml	25 ml 100 ml

Important Notes

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. If any precipitation formed in PN2 Buffer, please warm the buffer in a 37°C water bath for dissolving.
3. To maintain the pH value of PN2 Buffer, please close the bottle immediately after use.
4. Please centrifuge at 14,000 x g in all the centrifuge steps.

Growth of Bacteria for plasmid isolation

1. Pick a single colony from a fresh selective plate to inoculate 1-4 ml of LB medium in culture tube with the appropriate selection antibiotic.
2. Incubate the bacteria at 37°C while shaking at 200-250 rpm for 16-18 hours.
3. Harvest the bacterial culture by centrifugation at 8000 rpm (6,800 x g) in a microcentrifuge for 2 minutes at room temperature.
4. Discard the supernatant and remove the remaining medium carefully.

Purification Protocols

Step 1. Sample harvesting

a. Transfer **1-4 ml of bacterial culture** to a 1.5 ml microcentrifuge tube (not provided).
b. Centrifuge the bacterial culture at 14,000 x g for 1 minute and discard the supernatant.

- **Repeat Step 1a and 1b until total bacterial culture was harvested.**

Step 2. Resuspension

a. **Add 200 µl PN1 Buffer (RNase A added)** to resuspend the pellet completely by pipetting or vortexing.

- Make sure that RNase A was added prior to the initial use.
- No cell pellet should be observed after the resuspension.

Step 3. Cell Lysis

a. **Add 200 µl PN2 Buffer** and mix gently by inverting the tube 10 times to lyse the cells and incubate at room temperature for 2 minutes.

- Do not vortex, vortex may shear genomic DNA.
- Do not proceed this step over 5 minutes.

Step 4. Neutralization

a. **Add 300 µl PN3 Buffer** and gently invert the tube 10 times immediately.

- Do not vortex, vortex may shear genomic DNA.
- Invert immediately after adding PN3 Buffer will avoid asymmetric precipitation.

b. Centrifuge at 14,000 x g for 3 minutes. During centrifuging, place the **PN Column** into **Collection Tube.**

Step 5. DNA Binding

a. Transfer the supernatant to **PN Column** carefully.
b. Centrifuge at 14,000 x g for 30 seconds.
c. Discard the flow-through and place **PN Column** back in the **Collection Tube**.

- Do not transfer any white pellet into the column to avoid blockage.

Step 6. Wash

a. **Add 400 µl W1 Buffer** to **PN Column**.
b. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place **PN Column** back into the **Collection Tube**.
c. Add **600 µl W2 Buffer** (ethanol added) to **PN Column**.
d. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through then place **PN Column** back into the **Collection Tube**.

Step 7. Dry

a. Centrifuge at 14,000 x g for 2 minutes to dry the column.

- This Step will remove the residual liquid, which will affect the quality and the yield of the isolation product.

Step 8. Elution

a. Place **PN Column** to a clean 1.5 ml microcentrifuge tube (not provided).
b. Add **50-100 µl of Elution Buffer** or ddH_2O into the center of the column matrix.
c. Stand for 2 minutes.
d. Centrifuge at 14,000 x g for 2 minutes to elute the purified DNA.

Step 9. Pure DNA

a. Store the DNA fragment at 4°C or -20°C.

- To elute effectively, make sure that the elution solution is absorbed completely by the membrane.
- If the plasmid DNA is larger than 10 kb, use preheated 70 °C Elution Buffer to improve the elution efficiency.

Product Use Limitation & Warranty

This product is intended to be used for life science research only.
It has not been approved for drug or diagnostic purpose.
YEASTERN's products should not be resold, modified for resale, or used to manufacture commercial products without written approval by YEASTERN. YEASTERN guarantees the performance of all products in the manner described in our protocol. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, YEASTERN will replace it free of charge.