GSure® Plasmid MIDI Kit

#G4615 25 preparations

Storage: GSPB1 at 4°C on arrival;

Rest at Room Temperature

Procedure:

1. Pellet down overnight grown cells in a 50ml centrifuge tube by centrifugation at 4000 rpm 40C for 10 minutes. If require please increase the centrifugation time.

2. Resuspend harvested bacterial cells in 2.5 ml Buffer GSPB1 (GSPB1 needed to be shaken very thoroughly before use). Resuspension should be done by vigorous vertexing, for better efficiency, tap vortex to resuspend the cells. No cell clumps should be visible after complete resuspension of the pellet.

3. After completion of resuspension of total cell pellet in GSPB1, incubate the tube in ice for 10 minutes. Incubation at room temperature doesn't make any remarkable difference in the isolated DNA quality.

4. Add 2.5ml Buffer GSPB2 and and mix by inverting the tube 4-6 time. For complete and proper mixing of GSPB2, inversion should be done by rolling the tube.

5. Incubate the tube at room temperature for 15 minutes. GSure plasmid DNA isolation buffer contains a chromogenic indicator (sheerPINK) which turns pink after addition of buffer GSPB2. Properly lysed cells will show a clear pink solution; whereas a turbid pink appearance represents incomplete lysis of cells. **Do not vortex to avoid genomic DNA contamination in the plasmid preparation**. If required, continue inverting the tube until the solution becomes viscous and slightly clear. **Do not allow** the lysis reaction to proceed for more than 15 min.

6. Add 3.5ml Buffer GSPB3 and invert the tube immediately. Addition of GSPB3 buffer will turn the pink solution colorless and a cloudy appearance would be visible. To avoid localized precipitation, invert the tube thoroughly and immediately after addition of buffer. Continue mixing until the solution becomes colorless completely because a trace of pink color represents incomplete neutralization.

7. Incubate the tube in ice for more than 15 minutes. For better yield, mix the sample by inverting the tube after 2 minutes interval. The tube could be incubated at this stage upto 2hr.

8. Centrifuge the tube for 15 minutes at 4,000 rpm in a bench top cold centrifuge at 40C. A compact white pellet will form at the bottom of the tube.

9. Collect the supernatant from previous step in a fresh 15ml centrifuge tube, if possible, use cheese cloth to filter the supernatant while transferring. Avoid mixing of cells debris with the supernatant as this may clog GMidi spin Column thus lowering the DNA yield. Supernatant collected at this stage could be stored at 4^{O}C for 16 hr.

10. Apply the supernatants from step 9 to the GMidi Spin Column by decanting.

11. Centrifuge the column at 4000 rpm (~2500xg) for 5 minutes. Discard the flow-through.

12. Wash GMidi spin column once by adding 4ml GSETWB (for endo-toxin removal) and incubate it for 3 minutes and centrifuge the column at 4000rpm for 2 minutes and discard the flow-through.

13. Wash GMidi spin column by adding 4ml Membrane Wash Buffer and incubate it for 3 minutes and centrifuge the column at 4000rpm for 2 minutes and discard the flow-through. Repeat the previous washing step once.

14. Discard the flow-through, and centrifuge the GMidi spin column for an additional 10 minutes to remove residual wash buffer from membrane.

> **This step is extremely important to ensure complete removal of ethanol. Presence of ethanol in purified plasmid DNA may inhibit successive enzymatic reactions.**

15. Place the GMidi Spin Column in a clean 15 ml falcon tube (not provided). To elute DNA, add 0.2ml-1ml Nuclease free water (provided) to the center of GMidi Spin Column. For complete elution of membrane bound DNA minimum 0.2ml of Nuclease free water is required to wet the membrane properly. Let it stand for 5 minutes, and centrifuge for 10 minutes at maximum speed (~2500Xg) on a bench top cold centrifuge. While eluting with 200ul of nuclease free water, a second elution with the same amount of water will recover residual amount of DNA left over the membrane.

16. Discard the column and collect the eluted DNA present in centrifuge tube.

17. Trouble-shooting for concentrated DNA yield

18. Elute the DNA with 1ml of nuclease free water.

19. Aliquot 330ul of eluted DNA in 3 microfuge tubes.

20. Add 33ul of 3M Ammonium Acetate. Vortex the tube vigorously.

21. Add 1050ul of absolute ethanol (preferably chilled) and vortex.

22. Incubate the tube at -200C for 15min.

23. Centrifuge the tube at 14,000xg for 10 min.

24. Wash the pelleted nucleic acid with 70% ethanol (chilled), twice.

25. Air-dry the pellet and dissolve in desired amount of nuclease free water.

Kit Contents:

GSPB1 Buffer	:	70ml
GSPB2 Buffer	:	70ml
GSPB3 Buffer	:	100ml
GSETWB Buffer	:	75ml
Membrane Wash Buffer	:	120ml
GMidi Spin Column	:	25pcs.
Nuclease Free Water	:	25ml

Reconstitution of GSETWB Buffer:

Before using the kit for first time, add 60ml of absolute ethanol (molecular biology grade) with the provided Buffer. Mix thoroughly by shaking. Once ethanol added, tighten the cap properly after each use.

Reconstitution of Membrane Wash Buffer: Before using the kit for first time, add 180ml of absolute ethanol (molecular biology grade) with the provided Membrane Wash Buffer. Mix thoroughly by shaking. Once ethanol added, tighten the cap properly after each use.