GSure® Plasmid MIDI Kit

#G4617 10 preparations

Storage: GSPB1 at 4°C on arrival; Rest at Room Temperature

Procedure:

1. Pellet down overnight grown cells in a 50ml falcon tube by centrifugation at 5000 rpm 4^0C for 10 minutes. To harvest 200ml cells, one can collect cells in 4 different tubes or else in the same falcon tube by repeated centrifugation.
2. Resuspend harvested bacterial cells in 5ml Buffer GSPB1 (**GSPB1 needed to be stored at 4°C on arrival and shaken very thoroughly before use**). Resuspension should be done by vigorous vortexing, for better efficiency, tap vortex to resuspend the cells. No cell clumps should be visible after complete resuspension of the pellet.
3. When processing with 4 different tubes for same isolation, resuspend cell pellet in first tube and transfer the solution in the next one, resuspend again and do the same for rest of the samples.
4. After completion of resuspension of total cell pellet in GSPB1, incubate the tube in ice for 10 minutes.
5. Add 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.
6. Incubate the tube at room temperature for 15 minutes. G sure plasmid DNA isolation buffer contains a chromogenic indicator (**sheer PINK**) 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 minutes.
7. Add 7.5ml 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 colour represents incomplete neutralization.
8. Incubate the tube in ice for not less 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.
9. Centrifuge the tube for 15 minutes at 10,000 rpm (~8500xg) in a bench top cold centrifuge at 4^0C. A compact white pellet will form at the bottom of the tube.
10. Collect the supernatant from step 9 in a fresh 50ml falcon tube, If possible, use cheese cloth to filter the supernatant while transferring. ***Avoid mixing of cell debris with the supernatant*** *as* this may clog Gmaxi spin Column thus lowering the DNA yield.
11. Supernatant collected at this stage could be stored at 4^0C for 16 hr.
12. Apply the supernatants from step 10 to the GMaxi Spin Column by decanting.
13. Centrifuge the column at 10000 rpm (~8500xg) for 5 minutes. Discard the flow-through.
14. Wash GMaxi Spin Column by adding 15ml of Buffer Membrane Wash Buffer and centrifuging for 5 minutes as mentioned previously.
15. Discard the flow-through.
16. Repeat washing step.
17. Discard the flow-through, and centrifuge the GMaxi spin column for an additional 5 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.***

18. Place the Gmini SpinColumn in a clean 50ml falcon tube (not provided). To elute DNA, add 1ml-5ml Nuclease-free Water to the center of GMaxi Spin Column, For complete elution of membrane bound DNA minimum 1ml of Nuclease-free Water is required to wet the membrane properly, Let stand for 2 minutes, and centrifuge for 5 minutes at maximum speed (~8500Xg) on a bench top cold centrifuge.
19. Discard the column and collect the eluted DNA present in centrifuge tube.

Kit Contents:

GSPB1 Buffer	**: 60ml**
GSPB2 Buffer	**: 60ml**
GSPB3 Buffer	**: 90ml**
Membrane Wash Buffer	**: 140ml**
GMaxi Spin Column	**: 10pcs.**
Nuclease-free Water	**: 50ml**

Reconstitution of Membrane Wash Buffer:

Before using the kit for first time, add 210ml of absolute ethanol (molecular biology grade) with the provided Buffer. Mix thoroughly by shaking. Once ethanol added, tighten the cap properly after each use.