GSure® Plasmid Mini Prep Kit

#G4613 100 preparations

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

Procedure:

1. Pellet down overnight grown cells in a microfuge tube by centrifugation with a tabletop centrifuge at maximum speed for 1minute.
2. Resuspend harvested bacterial cells in 250µl 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. Add 250µl Buffer GSPB2 and and mix by inverting the tube 4–6 time. G sure plasmid DNA isolation buffers contain 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 5 minutes
4. Add 350µl 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.
5. Centrifuge for 10 minutes at 13,000 rpm (~8500xg) in a table-top microcentrifuge. A compact white pellet will form.
6. Apply the supernatants from step 5 to the Gmini Spin Column by decanting or pipetting. ***Avoid mixing of cell debris with the supernatant*** *as* this may clog Gmini spin Column thus lowering the DNA yield.
7. Centrifuge at 13,000 rpm (~8500xg) for 30–60 s. Discard the flow-through.
8. Wash Gmini Spin Column by adding 600µl Membrane Wash Buffer and centrifuging for 30–60 s as previously.
9. Discard the flow-through.
10. Repeat washing step.
11. Discard the flow-through, and centrifuge for an additional 2 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.***

12. Place the Gmini Spin Column in a clean 1.5 ml microcentrifuge tube (not provided). To elute DNA, add 50µl Nuclease-free Water(provided) to the center of each Gmini Spin Column, let stand for 1 minute, and centrifuge for 1 minute at maximum speed (~8500Xg) on a table top microcentrifuge.
13. Discard the column and collect the eluted DNA present in microcentrifuge tube.

Kit Contents:

GSPB1 Buffer	**: 30ml**
GSPB2 Buffer	**: 30ml**
GSPB3 Buffer	**: 40ml**
Membrane Wash Buffer	**: 60 ml**
Gmini Spin Column	**: 50 pcsX2**
Nuclease-free Water	**: 3mlX2**

Reconstitution of Membrane Wash Buffer:

Before using the kit for first time, add 90ml 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.

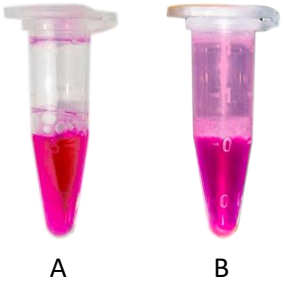
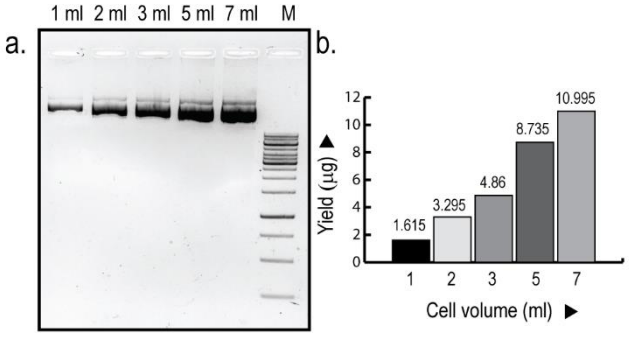
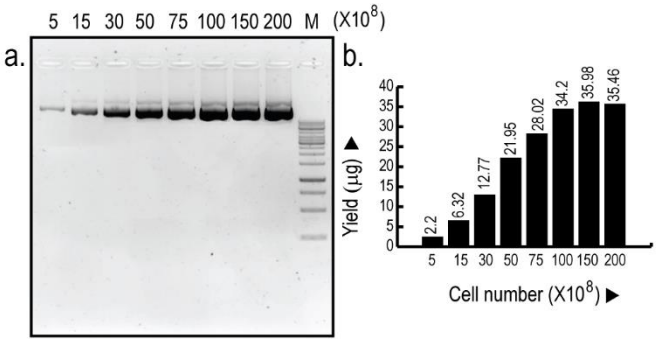


Figure1. sheerPINK:

sheerPINK is a proprietary item of GCC biotech (INDIA) Pvt. ltd. which utilizes a unique strategy to understand the cell lysis and renaturation step. sheerPINK is a chromogenic indicator added in the GCC plasmid isolation buffers which gives a clear idea about the progress of the cell lysis reaction. Addition of buffer GSPB2 with cells suspended in buffer GSPB1, will turn the solution pink. Initially a viscous pink suspension will be visible (A), progressively, after completion of cell lysis, this will turn in a clear pink solution (B). After the addition of buffer GSPB3, color will be disappeared depicting complete neutralization of lysis step. Traces of pink indicate incomplete neutralization, further vigorous mixing by inverting the tube will resolve the issue representing a cloudy white appearance of the lysate.





7kb plasmid DNA isolated from different amount of overnight grown cells. DNA eluted with 50µl nuclease free water. 1µl of each DNA electrophoresed on 1% agarose gel.

Graphical representation of DNA yield. Deep blue bar represents total amount of DNA recovered and light blue represents amount of DNA present in ccc form. Amount calculated by absolute quantity measurement of total DNA yield vs amount of ccc form present by Image LabTM software. 260:280 and 260:230 absorbance ratio of samples varies from 1.82-1.86 and 2.01-2.09 respectively.