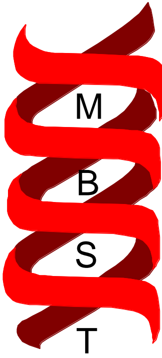
Molecular Biological System Transfer



Plasmid Isolation Kit
Minipreparation

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Introduction

The MBST Plasmid Isolation Kit provides a fast and simple method for the Isolation of pure plasmid. The Isolation Purification requires neither phenol and chloroform extraction nor ethanol precipitation.

Plasmid isolation is grounded on a selective binding of nucleic acids to a silica-based membrane in the presence of chaotropic salts. Non-nucleic acids elements or oligonucleotids do not bind to the membrane and will be removed by centrifugation.

The plasmid DNA is purified of residual contaminants by passing the wash buffer twice through the column containing membrane. The plasmid DNA is then eluted in the elution buffer, and is ready for use for enzymatic reactions.

Warning: For research use only. The Products listed in this manual are not for diagnostic or therapeutic use in humans or animals.

Plasmid Minipreparation Protocol

- Prepare a 70°C water bath

-Add 26 ml of ethanol (96-100%) to the wash buffer (WB) before using the first time. The WB buffer is stable for several months at room-temperature when properly stored (tightly closed).

-If a precipitate has formed in the homogenization buffer (HB), dissolve it by incubation at 37°C.

- Homogenization buffer must be stored at 4ºC.

1) Centrifuge 1.5 to 5 ml E.coli overnight cultures grown in LB-medium at 7500 g for 1 min at 4ºC.

2) Discard the supernatant and re-suspend bacterial pellet in 200 µl of homogenization buffer (HB). Re-suspend the pellet exactly without vortex. If necessary transfer the re-suspended bacteria in the 1.5 ml sterile tube.

3) Add 200 µl of lysis buffer (LB) and mix promptly by inverting the tube several times (4-6 times). Do not vortex. Avoid the shearing of the genomic DNA. The solution should become slightly clear. Lysis buffer should be closed immediately to avoid acidification from CO_2 in the air. Do not allow the lysis reaction to proceed for more than 5 min.

4) Add 200 µl of chilled neutralization buffer (NB), mix immediately by gently inverting the tubes for 6 times. Precipitation is enhanced by incubating on ice for 15 min. A flocculants white precipitate will be formed.

5) Centrifuge by high speed (12000 g) for 10 min. at 4ºC. The cell debris will form a compact pellet.

6) Apply the supernatant from step 5 to the sterile tube and re-centrifuge the supernatant at 12000 x g for 10 min. at 4ºC.

7) Apply the supernatant from step 6 to the sterile tube and add 250 µl 100% ethanol, mix thoroughly by vortexing

8) Apply the mixture from step 7 to the spin column^{MBST}, close the cap and centrifuge at 8.000 x g for 1 min.

9) place the spin column^{MBST} in a clean 1.5 ml tube and discard the tube containing the infiltrate.

10) Add 500 µl of wash buffer (WB) to the spin column^{MBST} , centrifuge at 8.000 x g for 1 min. and discard the tube containing the infiltrate.

11) Repeat step 10 once more

12) Place the spin column^{MBST} in a clean tube and centrifuge at full speed for a further 2 min to remove the ethanol completely. Place the spin column^{MBST} in a sterile 1.5 ml tube and discard the tube containing the infiltrate.

13) Add 50 µl of elution buffer (EB) preheated to 70°C to the spin column^{MBST} . Incubate at room temperature for 3 min. then centrifuge at 8.000 x g for 1 min.

14) For increased concentration, transfer the 50 µl of the step 13 to the same spin column^{MBST} and centrifuge again for 1 min in the tube from step 13.

Quantitation and determination of purity of DNA:
DNA concentration should be estimated by agarose gel electrophoresis or determined in eluates at 260nm in a spectrophotometer blanked against elution buffer using the following formula:
[DNA (μg/ml)] = A^{260} x D (dilution factor) x 40
Ratio of absorbance at 260 nm to absorbance 280nm determines the purity of DNA. $A^{260/280}$ is for pure DNA between 1.7 to 1.9.

Components

The MBST Plasmid Isolation Kit contains sufficient reagents for 50 PCR product purifications.

Components:

Component	Amount
Homogenization buffer	12 ml
Lysis buffer	12 ml
Neutralization buffer	12 ml
Wash buffer	26 ml
Elution buffer	10 ml
RNase A	Powder
Columns^{MBST} s	50

Elution buffer is:
10 mM Tris-Cl pH 7.4 and 1 mM EDTA pH 8.0

Add 1 ml Homogenization buffer to the tube containing RNase A. Dissolve thoroughly and apply the solution into the Homogenization buffer container. Homogenization buffer must be stored at 4ºC and all other buffers and columns^{MBST} should be stored at room temperature.

Additional material required:

Ethanol (96%-100%)
1.5 ml Tubes

Cautions:
The researcher is requested to handle with the chemical reagents in this kit with absolute care.

Please wear gloves and avoid contact with eyes, skin, and clothing.