Molecular Biological System Transfer

Rapid DNA Isolation Kit from Swab

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Introduction

The MBST DNA extraction kit provides a fast and simple method for the isolation of DNA from eukaryotic cells.

Purification requires neither phenol and chloroform extraction nor ethanol precipitation.

DNA purification is grounded on a selective binding of nucleic acids to a silica-based membrane in the presence of chaotropic salts. Non-nucleic acids elements do not bind to the membrane and will be removed by centrifugation. DNA is then purified of residual contaminants by passing the wash buffer twice through the membrane. DNA is then eluted in the elution buffer, and is ready for use for enzymatic reactions such as restriction analyses or PCR.

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

DNA Isolation Protocol from Swab

Prepare a 60°C water bath

- Add 1.1 ml of distilled water to the proteinase K and mix well. The proteinase K solution should be stored at –20°C. It is recommended to store proteinase K in aliquotes of 120 – 220 µl at –20°C.

If a precipitate has formed in lysis buffer, dissolve it by incubation at 50°C

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

1- transfer 100 µl Elution buffer into a sterile tube and place the swab into the tube. (Some swabs have a head that can be snapped off and left in the tube. If you have this type of swab, go ahead and break it off into the buffer.)

2- add 300 µl lysis buffer.

3- add 20 µl proteinase K into the tube, mix by vortexing and incubate for 1 h 60ºC

4- add 580 µl binding buffer to the tube mix by vortexing and incubate for 10 min at 70ºC

5- place the spin column MBST A in a clean tube and transfer the swab into the spin column, centrifuge for 1 min at 8000 x g

6- transfer the solution from the tube from step 5 into the solution of step 4

7- add 440 µl ethanol (100%) into the tube and mix thoroughly

8- apply the half of the mixture from step 7 into the spin column MBST B and centrifuge at 8.000 x g for 1 min. Remove the infiltrate and apply the other half of the mixture into the same column and centrifuge again for 1 min. at 8.000 x g. Place the spin column MBST B in a new eppendorf tube

9- add 500 µl of wash buffer (WB) to the spin column MBST B and centrifuge at 8.000 x g for 1 min. Place the spin column MBST B in a clean eppendorf tube and discard the tube containing the infiltrate

10- place the spin column MBST B in a clean eppendorf tube

11- again add 500 µl of wash buffer (WB) to the spin column MBST B, centrifuge at 8.000 x g for 1 min. and discard the tube containing the infiltrate

12- place the spin column MBST B in a clean eppendorf tube and centrifuge at full speed for a further 2 min to remove the ethanol completely. Place the spin column MBST B in a clean eppendorf tube and discard the tube containing the infiltrate

13- add 50 - 100 µl of elution buffer preheated to 70°C to the spin column MBST B, incubate at room temperature for 3 min., then centrifuge at 8.000 x g for 1 min.

14- repeat step 13 with the same elution buffer 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 50
Ratio of absorbance at 260nm to absorbance 280nm determines the purity of DNA. $A_{260/280}$ is for pure DNA between 1.7 to 1.9.

Components

The MBST DNA extraction kit contains sufficient reagents for 50 DNA preparations.
Components:

Lysis buffer	16 ml
(If a precipitate has formed, dissolve by incubating at 50°C)	
Binding buffer	31 ml
Proteinase K	10 mg
Wash buffer	26 ml
Elution buffer	20 ml
Columns^{MBST} A	50
Columns^{MBST} B	50

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

All buffers and columns^{MBST} should be stored at room temperature. Proteinase K should be stored dry at 4°C and dissolved at -20°C in small aliquots.

Additional material required:
Ethanol (96%-100%)
Microfuge 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.