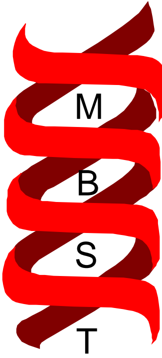
Molecular Biological System Transfer



Rapid Genomic DNA Isolation Kit

Research Institute
Molecular Biological System Transfer
Tehran Iran

Introduction

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

- 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) Add 180 µl of lysis buffer (LB) to the cell pellet (up to 1 x 10^7 cells) or to 100 µl blood and immediately mix thoroughly. In the case of tissue use the same amount of lysis buffer for up to 50 mg tissue materials.

2) Add 20 µl of proteinase K to the sample, mix thoroughly by vortexing and incubate at 55°C for 10 min.

   It is essential that the sample is completely lysed and forms a homogenous solution.

3) Add 360 µl of binding buffer (BB) to the sample, mix by vortexing for ca. 30 sec. and incubate for 10 min at 70°C.

   It is essential that the sample forms a homogenous solution after addition of BB.

4) Add 270 µl of ethanol (96-100%) to the sample and mix thoroughly by vortexing.

It is essential that the sample forms a homogenous solution after addition of ethanol.

5) Place a spin column^{MBST} in a 1.5 ml tube. Apply the mixture from step 4 to the spin column^{MBST}, close the cap and centrifuge at 8.000 x g for 1 min. Place the spin column^{MBST} in a clean 1.5 ml tube and discard the tube containing the infiltrate.

6) Add 500 µl of wash buffer (WB) to the spin column^{MBST} and centrifuge at 8.000 x g for 1 min. Place the spin column^{MBST} in a clean 1.5 ml tube and discard the tube containing the infiltrate.

7) Again 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.

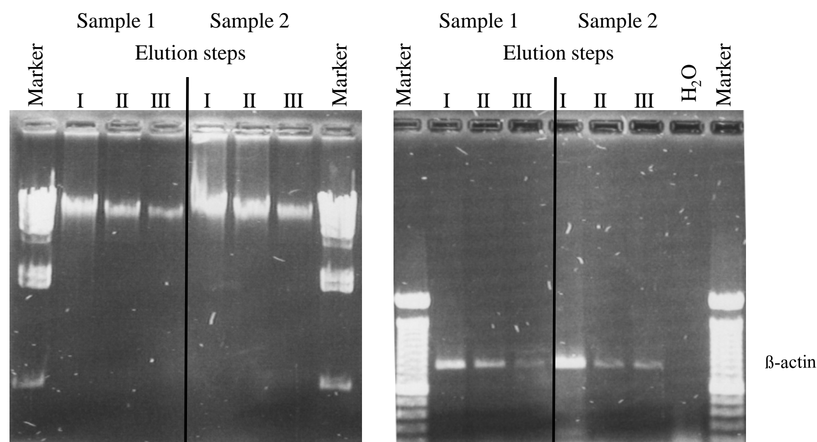
8) Place the spin column^{MBST} in a clean tube and centrifuge at full speed for a further 2 min to remove the ethanol completely. Place the spin column^{MBST} in a clean 1.5 ml tube and discard the tube containing the infiltrate.

9) Add 100-200 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.

10) Repeat step 9 once more in a clean tube. More than 85% of DNA should be eluted in the first and second elution steps.

    By a high concentration of source material the remaining adsorbed DNA on membranes^{MBST} should be eluted in a third and fourth elution steps in clean tubes.

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.



DNA was isolated from ca. 5 x 10^6 (sample 1) or 1 x10^7 (sample 2) mononuclear cells isolated from peripheral blood in three elution steps. Five µl from each eluate was analyzed on 1% agarose gel (left). The DNA was amplified in PCR using primers derived from ß-actin (right). For each reaction 1 µl was used.

Components

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

Lysis buffer (If a precipitate has formed, dissolve by incubating at 50°C)	10 ml
Binding buffer	20 ml
Proteinase K	10 mg
Wash buffer	26 ml
Elution buffer	20 ml
Spin columns	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.