

MEGA-spin™ Agarose Gel Extraction Kit

Cat. No.

17183

250 columns

DESCRIPTION

DNA fragments for probe DNA or ligation must be separated and purified from other DNA fragments. MEGA-spin™ employs a column method to purify target DNA in excised agarose gel. The column method uses a highly concentrated salt solution to keep the target DNA bound to the column membrane. The binding reaction occurs due to the disruption of the organized structure of water molecules and the interaction with the nucleic acids. Thus the adsorption to the specifically pretreated membrane is favored. Since the binding process is specific for nucleic acids, the bound material can be separated and purified from impurities e.g. salts and proteins, with simple washing step. Nucleic acids elute from the column membrane in a low salt buffer or water.

MEGA-spin™ is designed to extract and purify DNA of 100bp to 10kb from standard or low-melt agarose gels in TAE or TBE buffer. Furthermore, the kit guarantees a high yield of purification up to 70-90%. DNA fragments isolated with MEGA-spin™ Agarose Gel Extraction Kit are efficiently ligated into plasmid cloning vectors or specifically labeled using either random primed labeling or nick translation. No inhibition of digestion with restriction endonucleases is observed.

APPLICATIONS

MEGA-spin™ Agarose Gel Extraction Kit is designed for the efficient isolation of DNA fragments from TAE or TBE agarose gels. The kit is applicable for DNA isolation from standard agarose gels as well as low melting point agarose gels

KIT CONTENTS :

| | |
|------------------------------------|-------------|
| 1. Agarose lysis buffer | 180ml |
| 2. Washing buffer | 50ml |
| : Add 200ml of the absolute EtOH. | |
| 3. Elution buffer | 20ml |
| 4. MEGA-spin™ column (Blue) | 250 columns |
| : Columns containing the membrane. | |
| 5. Collecting tube | 250 tubes |
| : Polypropylene tube for 2ml. | |

CONSIDERATIONS BEFORE USE

1. Selective binding

The MEGA-spin™ system combines the convenience of spin-column method with selective binding properties of a silica-gel membrane. Special buffers provided with the kit are optimized for efficient recovery of DNA and removal of contaminants in each specific application. DNA bind to the silica-membrane in presence of high salt, while contaminants pass through the column. Impurities are efficiently washed away.

2. Efficient recovery of large fragments of DNA

The efficiency of DNA recovery from agarose gels is related to its molecular weight. As the size of the DNA fragments increases, the yield progressively decreases. The kit gives reasonable yields of DNA fragments less than 7-10 kb in length (70-90%).

3. Efficient recovery of small amounts of DNA

The smaller the amount of DNA applied to the gel, the lower the yield of purified fragments. This kit is useful for recovering bands that contain less than 300-500 ng of DNA.

STORAGE CONDITION

Store at room temperature. The term of validity is marked on the box.

ADDITIONAL REQUIRED EQUIPMENT

Agarose (iNTRON, 32032); scalpel
Gel running buffer: TAE buffer or TBE buffer Electrophoresis Sterile
Absolute ethanol
Standard tabletop microcentrifuge
Microcentrifuge tubes, sterile (1.5ml)
TE buffer (10 mM Tris-HCl, 0.1 mM EDTA; pH 8.0 - 8.5)

PROTOCOL

1. Separate the DNA of interest in an agarose gel of suitable concentration. Use either TAE or TBE running buffer.
2. After electrophoresis, cut out the interesting DNA fragment with a sharp scalpel or razor blade. Carefully take as much agarose gel as possible.
Note : If sliced agarose gel put into Agarose lysis buffer, the total volume may be increased. Thus, highly concentrated Agarose lysis buffer is diluted, and results low elution efficiency. Therefore, minimize the size of the gel slice by removing extra agarose
3. Weigh the gel slice in a 1.5ml tube. Add 3 volumes of Agarose lysis buffer to 1 volume of gel (300µl per 100 mg of agarose gel).
Note : Add 300µl of Agarose lysis buffer to each 100mg of gel. If more than 2% of agarose gel, add more Agarose lysis buffer.
4. Incubate at 50-55 °C for 5-10min (or until the gel slice has completely dissolved). To help dissolve gel, mix with vortex every 23min during the incubation.
Note : Completely solubilize agarose For >2% agarose gel, increase incubation time.
5. While waiting for the incubation in step 4, insert a column into collection tube.
6. To bind DNA, apply the sample to the column, and centrifuge for 1min. Discard the flow-through after centrifuging and place the spin column back in the same 2ml collection tube.
Note : The maximum volume of the column reservoir is 800µl. For sample volumes of more than 800µl, simply load and spin again.
7. Add 700µl of Washing buffer to the column and centrifuge for 1 min at 13,000rpm. Discard the flow-through after centrifuging and place the spin column back in the same 2ml collection tube.
Note : If the DNA will be used for salt sensitive applications, such as blunt-end ligation and direct sequencing, repeat the step 7 using 500µl of Washing buffer.
8. Discard the filtrate and centrifuge the column for additional 1min to dry the membrane. Completely remove residual ethanol.
9. Put the column into a clean and sterile centrifuge tube. Add 50µl of Elution buffer or distilled water to the upper reservoir of the column, and let it stand for 1min. Then, centrifuge the tube assembly at 13,000 rpm for 60sec.
Note : It is suggested to use at least 20µl of the Elution buffer to obtain best result.