Plasmid DNA MaxiPrep Kit

Cat No: DN1031 **Volume:** 10 preps

For research use only

Content	DN1031
RNase A(10mg/ml)	600 µlx2
Solution I	100 ml
Solution II	100 ml
Solution III	120 ml
Wash Buffer DW	40 ml x 3
Wash Buffer DPB	120 ml
Elution Buffer (10 mM Tris-HCl, pH 8.5)	20 ml
Spin Columns	10 each

Description

Plasmid Maxiprep Kit is designed for rapid and cost-effective large-scale preparation of high quality plasmid DNA from recombinant E.coli cultures. The kit utilizes an exclusive silica-based membrane technology in the form of a convenient spin column. Each spin column can recover 400-1,000 µg of plasmid DNA from 100 ml overnight bacterial culture. The kit can be successfully used for efficient purification of any size plasmids and cosmids. The actual plasmid yield and optimal culture volume depend on the plasmid copy number and medium used for cultivation.

The method is based on silica-membrane spin column, silica particles bound DNA selectively athigh salt concentration and low pH, while protein and other contaminants are wash to remove. The pure DNA is eluted with TE buffer or water. This method requires few manipulations, and is both faster and easier to perform than other organic-based extraction methods. The purified DNA is suitable for all common molecular biology procedures, including restriction digestion, cloning, sequencing, etc.

Applications

Purified DNA is free from contaminants and enzyme inhibitors, and typically has A260/A280 ratios between 1.7 and 1.9, and is suitable for applications such as:

- •Restriction digestion
- •PCR
- •DNA Sequencing
- •in vitro transcription

Feature.

- •Fast - procedure takes only 40 min.
- •High yield : 100 ml suspension yield 400-1,000 µg plasmid DNA of high copy
- •High purity - Purified DNA is ready for downstream application such as PCR, restriction digestion.

Storage

Store RNase A at -20℃, other reagents can be store at room temperature for up to 1 year. Any precipitate forms in the buffers during storage, it should be redissolved by incubating the buffers at 37°C before use. After addition of RNase A, the Resuspension Solution can be used for 6 months when stored at 4°C

on the labels that ethanol is added. Store both wash buffers with ethanol at room temperature.

• All purification steps should be carried out at room temperature.

• All centrifugations should be carried out in a table-top microcentrifuge at>12000 g 14,000-10,000)rpm, depending on the rotor type

Growth of Bacterial Cultures

.1Pick a single colony from a freshly streaked selective plate to inoculate 1-5 ml of LB medium supplemented with the appropriate selection antibiotic. Incubate for 16-12hours at 37°C while shaking at 200-250 rpm. Use a tube or flask with a volume of at least 4 times the culture volume.

.2Harvest the bacterial culture by centrifugation at 8000 rpm (6800 x g) in a microcentrifuge for 2 min at room temperature. Decant the supernatant and remove all remaining medium.

Plasmid type and copy number

The Plasmid DNA Maxiprep kit allows purification of all types and sizes of plasmid DNA. Use a high copy number plasmid to obtain a good yield of plasmid DNA. High copy-number plasmids typically yield 2-6 µg DNA/ml LB culture grown overnight. Typical yields from low copy number plasmids are highly dependent upon culture conditions and vector/host strain combinations. If you are using a low copy number plasmid, you will need to use a higher volume of bacterial culture.

The table below lists the volumes of bacterial culture required for Midiprep and Maxiprep plasmid DNA purification depending on the plasmid copy number used.

Plasmid type	Maxiprep
High copy number plasmid	100-200ml
Low cope number plasmid	250-500ml

Protocol

.1Grow transformed E. coli in LB medium.

.2Pellet 100 ml (high copy number plasmid) or 250–500 ml (low copy number plasmid) of an overnight culture. Centrifuge at ~9,000 x g for 5 min at room temperature. Discard the flow-through.

.3Add 7.5 ml Solution I containing RNase A to the pellet and vortex until homogeneous. Incubate the lysate at room temperature for 5 to 10 minutes.

.4Add 7.5 ml Solution II. Mix gently by inverting the capped tube 12 times. Do not vortex. Incubate the lysate at room temperature for 3 to 5 minutes.

.5Add 10 ml Solution III. Mix immediately by inverting the capped tube until the mixture is homogeneous. Do not vortex.

.6Centrifuge at ~12,000 x g for 12 min at room temperature.

.7Carefully remove and load the supernatant from Step 6 onto the spin column. Incubate for 5 minutes at room temperature. Centrifuge at ~12,000 x g for 2 min. Discard the flow-through.

.8Wash the column once with 10 ml Wash Buffer DPB. Centrifuge at ~12,000 x g for 2min. Discard the flow-through.

.9Wash the column with 10 ml Wash Buffer DW, Centrifuge at ~12,000 x g for 2 min. Discard the flow-through. Repeat Step 9 again.

.10Centrifuge the column at ~12,000 x g for 4 minute to remove any residual Wash Buffer DW. Discard the Wash Tube with the flow-through. Air dry the column for 5minutes.

.11Place the Spin Column in a clean 50-ml Recovery Tube. Add 1.5 to 2 ml of preheated Eluent Buffer to the center of the column. Incubate the column for 5 minute at room temperature. Centrifuge at ~12,000 x g for 2 minutes. The Recovery Tube contains your purified plasmid DNA. Discard the column. Store the plasmid DNA at -20℃.

DNA Quantitation

Perform DNA quantitation using UV absorbance at 260 nm. UV Absorbance

.1Prepare a dilution of the DNA solution. Mix well. Measure the absorbance at 260 nm (A260) of the dilution in a spectrophotometer (using a cuvette with an optical path length of 1 cm) blanked against the dilution buffer.

.2Calculate the concentration of DNA using the formula:

DNA (µg/ml) = A260 × 50 × dilution factor

For DNA, A260 = 1 for a 50 µg/ml solution measured in a cuvette with an optical path length of 1 cm.

Before Starting for MaxiPrep Procedures

•Add RNase A to the Solution I according to instructions on the label. Mix well. Mark on the label that RNase A is added. Store at 4°C.

• If the Solution II contains salt precipitates, warm the buffer in a 37°C water bath until the solution clears.

• Add 1.5 volume of 96 - 100% ethanol to 1 volume of Wash Buffer DW, Mix well. Mark