Plasmid Fast Midiprep Kit

(High quality Plasmid Purification Kits for Transfection)

User Manual

Version 6.0

Catalog No. 11510F/11520F/11500F

Storage Conditions: Room Temperature

For Research Use Only

Principle

The m.biotech High quality Plasmid Purification Kits for Transfection procedure is based on a modified alkaline lysis method, followed by binding of plasmid DNA onto membrane in the presence of low-salt and pH conditions. RNA, proteins, dyes, and low-molecular-weight impurities are removed by a medium-salt wash. Plasmid DNA is eluted in a high-salt buffer and the concentrated and desalted by isopropanol precipitation. No expensive equipment such as ultracentrifuges and HPLC, or toxic reagents, such as phenol and ethidium bromide are required.

Culture volume

Do not exceed the maximum recommended culture volumes given at the beginning of each protocol. Using larger culture volumes will lead to an increase in biomass and can affect the efficiency of alkaline lysis, leading to reduced yield and purity of the preparation. The protocol for High quality Plasmid Purification Kits for Transfection is optimized for use with cultures grown in standard Luria Bertani (LB) medium, grown to a cell density of approximately 3–4 x 109 cells per ml. We advise harvesting cultures after approximately 12–16 hours of growth, which typically is the transition from logarithmic into stationary growth phase. It is best to assess the cell density of the culture, and if that is too high, reduce the culture volumes accordingly or increase the volumes of Buffer T1, Buffer T2, Buffer T3. A high ratio of biomass to lysis buffers will result in poor lysis conditions and subsequently low DNA yield and purity. For determination of cell density calibration of each individual spectrophotometer is required to facilitate accurate conversion of OD600 measurements into the number of cells per ml. This can be achieved by plating serial dilutions of a culture onto LB-agar plates in the absence of antibiotics. The counted colonies are used to calculate the number of cells per ml, which is then set in relation to the measured OD600 values.

Convenient stopping points in protocols

The purification procedure can be stopped and continued later by freezing the cell pellets obtained by centrifugation. The frozen cell pellets may be stored at –20°C for several weeks. In addition, the DNA eluted from the Spin- or Ez-Column may be stored overnight at 2–8°C, after which the protocol can be continued.

Important Note

Please read the following notes before starting any of the Plasmid Purification procedures for Transfection.

Before equipment

● Add the RNase A Solution to Buffer T1, mix, store at 2-8℃.

● Check Buffer T2 before use for salt precipitation.

If any precipitated, heat to dissolve (37℃). Wear, gloves when handling these buffer.

● Isopropanol and 70% ethanol.

● In Spin-Column, If remainder should occur

Protocol for Plasmid Fast Midiprep Kit

(Reusable High quality Plasmid Purification Kits for Transfection)

Please read "Important Notes" before starting.

Cat. No.	11510F 10 preps	11520F 25 prep	11500F 100 preps	Storage Condition
Buffer EQ	50 ml	125 ml	250 ml X 2ea	RT
Buffer T1	50 ml	125 ml	125 ml X 4ea	RT
Buffer T2	50 ml	125 ml	250 ml X 2ea	RT
Buffer T3	50 ml	125 ml	250 ml X 2ea	RT
Buffer TU	125 ml	125 ml X 2ea 60 ml X1	250 ml X 4ea 125 ml X 1ea	RT
Buffer EN	50 ml	125 ml	250 ml X 2ea	RT
Buffer TE	5 ml	12 ml	60 ml	RT
RNase A (25mg/ml)	250 ul	600 ul	600 ul X 4ea	4 ℃
Midi Column & Tube	Each 10 ea	Each 25 ea	Each 100 ea	RT
Protocol	1 ea	1 ea	1 ea	-

1. Harvest a 50 ml cultured high-copy-number plasmids or cosmids (>2 µg DNA/ml LB).
   If you wish to stop the protocol and continue later, freeze the cell pellets at -20°C.

2. Apply 5 ml of Buffer EQ to the Midi-Column. Let stand for 1 min.
   Centrifuge at ≥ 4,000 x g in a swing-bucket rotor at room temperature for 1 min or vacuum. OR centrifuge at ≥ 8,000 x g in a fixed-angle rotor for 3 min (*disregard of remainder, next step*). Discard the flow-through.

3. Suspend pelleted bacterial cells in 5 ml of Buffer T1.

4. Add 5 ml of Buffer T2. Mix gently by inverting the capped tube five times. *Do not vortex.*
   Incubate at room temperature for 5 min.

5. Add 5 ml of Buffer T3. Mix immediately by inverting the tube five times. *Do not vortex.*

6. Centrifuge at ≥ 15,000 x g at room temperature for 10 min.
   If centrifugation is done at 4°C, supernatant must be warmed to room temperature before loading on column.

7. Apply the cleared lysate of step 6 into the equilibrated Midi-Column.
   Centrifuge at ≥ 4,000 x g in a swing-bucket rotor at room temperature for 1 min or vacuum. OR centrifuge at ≥ 8,000 x g in a fixed-angle rotor for 3 min (*disregard of remainder, next step*). Discard the flow-through.

8. Apply 12 ml of Buffer TU to the Midi-Column.
   Centrifuge at ≥ 4,000 x g in a swing-bucket rotor at room temperature for 2 min or vacuum. OR centrifuge at ≥ 8,000 x g in a fixed-angle rotor for 3 min (*disregard of remainder, next step*). Discard the flow-through.

9. Place the Midi-Column in a clean 50 ml centrifuge tube (not provided).
   Apply 5 ml of Buffer EN to the Midi-Column. Centrifuge at ≥ 4,000 x g in a swing-bucket rotor at room temperature for 2 min. OR centrifuge at ≥8,000 x g in a fixed-angle rotor for 3 min (*disregard of remainder, next step*).
   Note: For constructs larger than 45–50 kb, prewarming the Buffer EN to 65°C may help to increase yield.

10. Precipitate DNA by adding 0.7 volume isopropanol to the eluted DNA.
Mix and centrifuge at ≥ 15,000 x g at 4°C for 30 min. carefully discard the supernatant.

11. Wash DNA pellet with 2 ml 70% ethanol. Centrifuge at ≥ 15,000 x g at 4°C for 10 min.
Carefully and fully pipet off the ethanol wash without disturbing the pellet.
Air-dry the pellet.

12. Redissolve the DNA in 0.4 ml or a suitable volume of Buffer TE, pH 8.0 or 10 mM Tris-Cl, pH8.5.

Troubleshooting Guiade

This troubleshooting guide may be helpful in solving any problems that may arise.

Problem	Comments and suggestions
Low or no DNA yield	
No DNA in lysate	
a) Plasmid did not Propagate	Check the conditions for optimal growth.
b) Alkaline lysis was Inefficient	If cells have grown to very high densities or a larger amount of cultured medium than recommended was used, the ratio of biomass to lysis reagent is shifted. This may result in poor lysis conditions, because the volumes of Buffer T1, T2, and T3 are not sufficient for setting the plasmid DNA free efficiently. Reduce culture volume or increase volumes of Buffer T1, T2, and T3. Also insufficient mixing of lysis reagents will result in reduced yield.
c) Insufficient lysis for low-copy plasmids	For low-copy plasmid preparations, doubling the volumes of Buffers T1, T2, and T3 may help to increase plasmid yield and quality.
d) Lysate incorrectly prepared	Check Buffer T2 for SDS precipitation resulting from low storage temperatures and dissolve the SDS by warming.
DNA is found in the flow-through of cleared lysate	
a) Plasmid Purification membrane overloaded	If rich culture media, such as TB or 2x YT are used, culture volumes must be reduced. It may be necessary to adjust LB culture volume if the plasmid and host strain show extremely high copy number or growth rates.
b) RNase A digestion omitted	Ensure that RNase A is added to Buffer T1 before use.
c) RNase A digestion Insufficient	Reduce culture volume if necessary. If Buffer T1 containing RNase A is more than 6 months old, add additional RNase A.
d) SDS was in lysate	Buffer T3 before use. If lysate is too viscous for effective mixing of Buffer T3, reduce culture volume or increase volumes of Buffer T1, T2, and T3.
No DNA in eluate	
a) No DNA in the lysate	See section "No DNA in lysate"
b) DNA passed through in the flow-through or wash	See previous two sections.

fraction

Little or no DNA after precipitation

a) DNA failed to precipitate	Ensure that the precipitate is centrifuged at ≥15,000 x g for 30 min. Recover DNA by centrifuging for longer and at higher speeds. Try another isopropanol batch.
b) DNA pellet was lost	Isopropanol pellets are glassy and may be difficult to see. Mark the outside of the tube before centrifugation. Isopropanol pellets may also be loosely attached to the side of the tube, so pour supernatant off gently.
c)DNA was poorly redissolved	Check that DNA is completely redissolved. Be sure to wash any DNA off the walls, particularly if glass tubes and a fixed-angle rotor are used. Up to half of the total DNA may be smeared on the walls. Alternatively, a swinging bucket rotor can be used to ensure that the pellet is located at the bottom of the tube.

Plasmid DNA difficult to redissolve

a) Pellet was overdried	Air-dry pellet instead of using a vacuum, especially if the DNA is of high molecular weight. Redissolve DNA by warming the solution slightly, and allowing more time for redissolving.
b) Residual isopropanol in pellet	Ensure that pellets are washed with 70% ethanol to remove traces of isopropanol. Redissolve DNA by warming the solution slightly, and allowing more time for redissolving. Increase volume of buffer used for redissolving if necessary.
c) Too much salt in pellet	Ensure that isopropanol is at room temperature for precipitation, and wash the pellet twice with room temperature 70% ethanol. Recover DNA by increasing the volume of buffer used for redissolving.
d) Buffer pH was too low	Ensure that the pH of the buffer used for redissolving is ≥8.0, since DNA does not dissolve well in acidic solutions.
e) Resuspension (T1) volume too low	Increase resuspension (T1) volume if the solution above the pellet is highly viscous.

Contaminated DNA/poor-quality DNA

a)Genomic DNA in the eluate	Mixing of bacterial lysate was too vigorous. The lysate must be handled gently after additional Buffer T1, T2 and T3 to prevent shearing of chromosomal DNA. Reduce culture volume if lysate is too viscous for gentle mixing.
b) RNA in the eluate	RNase A digestion was insufficient. Check culture volume against recommended volumes, and reduce if necessary. Check that the RNase A provided with the kit has been used.

	If Buffer T1 is more than 6 months old, add more RNase A.
c) Nuclease contamination	Check buffers for nuclease contamination and replace if necessary. Use new glass- and plasticware, and wear gloves.
d) Lysis time was too Long	Ensure that lysis step (Buffer T2) does not exceed 5 min.
e) Overloaded alkaline lysis	Check the culture volume and yield against the capacity of the Spin-Column. Reduce the culture volume accordingly or alternatively increase the volume of Buffer T1, T2, and T3.
f) Plasmid DNA is nicked/sheared/Degraded	DNA was poorly buffered. Redissolve DNA in Buffer TE, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage.
g) Shearing during Redissolving	Redissolve DNA gently, without vortexing or vigorous pipetting. Avoid using small pipet tips.
h) Particles in redissolved DNA	Centrifuge the DNA solution and transfer supernatant to a new tube. The particles have no affect on DNA quality. Alternatively, use UltraHiFast kits containing Precipitatior, which filters the eluate.
Poor DNA performance	
a) Too much salt in pellet	Ensure that isopropanol is at room temperature for precipitation, and wash the pellet twice with room temperature 70% ethanol. Precipitate the DNA again to remove the salt.
b) Residual protein	Check culture volume against the recommended volumes and reduce if necessary. Ensure that the bacterial lysate is cleared properly by centrifugation at ≥15,000 x g for 45 min, or using a Cartridge.
Extra DNA bands on analytical gel	
a) Dimer form of plasmid	Dimers or multimers of supercoiled plasmid DNA are formed during replication of plasmid DNA. Typically, when purified plasmid DNA is electrophoresed, both the supercoiled monomer and dimer form of the plasmid are detected upon ethidium bromide staining of the gel. The ratio of these forms is often host dependent.
b) Plasmid has formed Denatured supercoils	This species runs faster than closed circular DNA on a gel and is resistant to restriction digestion. Do not incubate cells for longer than 5

	min in Buffer T2. Mix immediately after addition of Buffer T3.
c) Possible deletion mutants	Some sequences are poorly maintained in plasmids. Check for deletions by restriction analysis. Cosmid clones, in particular, should always be prepared from freshly streaked, well-isolated colonies, since cosmids are not stable in *E. coli* for long periods of time.

Blocked Spin-Column

a) Lysate was turbid	Ensure that the lysate is clear before it is loaded onto the column. Ensure that Buffer T3 is chilled before use. Check g-force and centrifugation time. Alternatively, clear the lysate using a Cartridge. To clear a blocked Spin-Column, positive pressure may be applied (e.g., by using a syringe fitted into a rubber stopper with a hole).

Cartridges

Cartridge clogs during filtration

a)Too large culture volume used	Use no more than the culture volume recommended in the protocol.
b) Inefficient mixing after addition of Buffer T3	Mix well until a fluffy white material has formed and the lysate is no longer viscous.
c) Mixing too vigorous after addition of Buffer T3	After addition of Buffer T3 the lysate should be mixed immediately but gently. Vigorous mixing disrupts the precipitate into tiny particles which may clog the Cartridge.
d) Cartridge was not loaded immediately after addition of Buffer T3	After addition of Buffer T3 the lysate should be poured immediately into the Cartridge. Decanting after incubation may disrupt the precipitate into tiny particles which may clog the Cartridge.
e) Cartridge was agitated during incubation	Pour the lysate into the Cartridge immediately after addition of Neutralization Solution and do not agitate during the 10 min incubation. Agitation causes the precipitate to be disrupted into tiny particles, instead of forming a layer.
f) Incubation after addition of Buffer T3 on ice instead of at RT	Ensure incubation is performed at room temperature Buffer T3 on ice instead of in the Cartridge. Precipitate flotation is more efficient at room temperature than on ice.
g) Incubation time after addition of Buffer T3 too short	Incubate with Buffer T3 as indicated in the protocol. If the precipitate has not risen to the top after the 10 min incubation, carefully run a sterile pipet tip or sterile spatula around the cartridge wall to dislodge the precipitate before continuing with the filtration.

Lysate not clear after filtration

a) Precipitate was forced through the Catridge	Filter until all of the lysate has passed through the Midi or Maxi Cartridge, but do not apply extreme force. Approximately 12 ml (Midi) or 25 ml (Maxi) of the lysate are typically recovered.

Precipitator Modules

DNA does not perform well

a) Eluate contains residual alcohol	Ensure that the membrane is dried by pressing air through the Precipitator at least twice. Dry the outlet nozzle of the Precipitator with absorbent paper.

Precipitator clogs during use

a) Too much DNA applied to the Precipitator	Do not load eluate from several columns on the Precipitator.
b) Precipitator Midi Module was used for precipitation of eluate from a column	Use the size of Precipitator corresponding to the Spin-Column being used.
c) Ethanol was used for precipitation instead of isopropanol	Use of ethanol instead of isopropanol for precipitation leads to a finer precipitate that can clog the module.

Precipitator casing breaks, causing leakage

a) Excessive exposure of Precipitator to alcohol	Prolonged incubation with alcohol may weaken the joint between upper and lower part of the Precipitator. Complete steps 15~20 within 10~15 min.
b) Precipitator attached with excessive force	Do not apply excessive force, bending, or twisting when attaching the Precipitator to the syringe.
c) Precipitator inlet was bent during processing	Do not stress the inlet by resting one edge of the Precipitator on a hard surface (e.g., the edge of a sink) and depressing the syringe plunger. Always apply gentle, even, pressure perpendicularly to the Precipitator.

Customer & Technical Services

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