AxyPrep Midi and Maxi Plasmid Kits

For the rapid purification of larger amount of plasmid from bacterial cultures

Kit contents, storage and stability

AxyPrep Plasmid Midiprep Kit

Cat. No.	AP-MD-P-10	AP-MD-P-25
Kit size	10 preps	25 preps
Midiprep column	10	25
1.5 ml Microfuge tube	20	50
Plastic wrench	1	1
RNase A	120 μl	270 μl
Buffer S1	55 ml	125 ml
Buffer S2	55 ml	125 ml
Buffer S3K	55 ml	125 ml
Buffer B	55 ml	125 ml
Buffer W1	80 ml	2×100 ml
Buffer W2 concentrate	36 ml	72 ml
Eluent	6 ml	20 ml
Protocol manual	1	1

AxyPrep Plasmid Maxiprep Kit

Cat. No.	AP-MX-P-10	AP-MX-P-25
Kit size	10 preps	25 preps
Maxiprep column	10	25
RNase A	270 μl	700 μl
Buffer S1	115 ml	285 ml
Buffer S2	115 ml	285 ml
Buffer S3K	115 ml	285 ml
Buffer B	115 ml	285 ml
Buffer W1	160 ml	350 ml
Buffer W2 concentrate	2×36 ml	150 ml
Eluent	25 ml	60 ml
Protocol manual	1	1

Except for the RNase A (after addition to Buffer S1), all other reagents are stable for a period of at least 12 months from the date of receipt when stored under ambient conditions. Please avoid exposure to direct sunlight or extremes in temperature. Buffer S2 contains SDS which may precipitate if exposed to cold temperatures. If this occurs, simply warm with a 37°C source and gently agitate to resuspend. To preserve RNase activity, the RNase A is suspended in a solution containing a high

concentration of ammonium sulfate. On occasion, a precipitate may form. If this occurs, the precipitate is easily dissolved in Buffer S1 and the RNase activity is unaffected.

RNase A: 50 mg/ml. Stable at room temperature for up to 6 months. Recommend -20°C for long-term storage. If a precipitate is present, use an aliquot of Buffer S1 to resuspend and transfer to the Buffer S1 bottle.
Buffer S1: Bacterial resuspension buffer. Store at 4°C after addition of RNase A.
Buffer S2: Bacterial lysis buffer. Store at room temperature.
Buffer S3K: Neutralization buffer. Store at room temperature.
Buffer B: DNA binding buffer. Store at room temperature.
Buffer W1: Wash buffer. Store at room temperature.
Buffer W2 concentrate: Desalting buffer. Before the use of the kit, add as much ethanol as indicated on the bottle and mix well. Store at room temperature. Either 100% or 95% denatured ethanol can be used.
Eluent: 2.5 mM Tris-HCl, pH8.5. Store at room temperature.

Introduction

These kits are based upon a modified SDS-alkaline lysis of bacterial cells in combination with selective binding of the plasmid DNA to a special AxyPrep purification column. The yield of plasmid for the Midiprep and Maxiprep kits is up to 100 μg and 500 μg respectively. The protocols provide a simple, rapid and efficient method for the isolation of highly purified plasmid DNA. The entire preparation process can be completed in 30 minutes for the Midiprep, and 45 minutes for the Maxiprep. Plasmid prepared by this method can be used immediately for many routine applications requiring highly purified plasmid DNA, such as mammalian cell transfection, DNA sequencing, restriction digestion, *in vitro* transcription, library screening, ligation and bacterial transformation.

Caution

Buffer S2 contains NaOH, a caustic reagent. Buffers S3K, Buffer B and Buffer W1 contain chemical irritants. When working with these buffers, always wear suitable protective clothing such as safety glasses, laboratory coat and gloves. Be careful and avoid contact with skin and eyes. In case of such contact, wash immediately with water. If necessary, seek medical assistance.

Equipment and consumables required

- 100% or 95% (denatured) ethanol
- Microcentrifuge capable of 12,000×g (for Midi-prep only)
- Centrifuge capable of 6,000×g
- Swinging bucket rotor capable of accommodating 50 ml conical tubes
- 50 ml conical centrifuge tubes or 50 ml high-speed centrifuge tube
- AxyVac Vacuum Manifold (#AP-VM or AP-VM-II)
- Vacuum regulator
- Vacuum source capable of –25-30 inches Hg (approximately -850-1,000 mbar or -12-15 psi)

Preparation before experiment

1) Add RNase A to Buffer S1. Mix well and store at 4°C.
2) Add the amount of ethanol specified on the Buffer W2 label to the Buffer W2 concentrate. Either 100% or 95% (denatured) ethanol can be used.
3) Check Buffer S2 for precipitation before each use. If precipitation occurs, incubate at 37°C to dissolve the precipitate and chill to room temperature. After use, the bottle should be closed immediately in order to avoid neutralization of NaOH by CO_2 in the air.
4) Pre-chill Buffer S3K and Buffer B to 4°C.
5) Pre-warming water or Eluent at 65°C will improve elution efficiency.

Bacterial cultures

Inoculate a small-scale overnight LB culture containing the appropriate antibiotic with a single colony isolated from a freshly streaked plate. This overnight culture will then be used to inoculate a large-scale culture which will be used in the plasmid purification procedure. The following day, use approximately 250-500 μl of the small-scale LB culture to inoculate a large-scale culture, grown in LB, LBG (Luria-Bertain broth + 2% glycerol) or 2×YT media. If a rich broth is preferable, we recommend either LBG or 2×YT for bacterial growth. TB is not recommended for plasmid propagation.

Protocols

A. Plasmid Midiprep

The protocol is designed to produce up to 100 μg of highly purified plasmid DNA from 30-100 ml (depending upon the copy number of the plasmid) of bacterial culture grown in LB medium. The culture volume should be reduced to half or less when rich media, such as LBG or 2×YT are used.

1. Collect 30 ml of overnight LB culture for the preparation of the high-copy plasmid, or 100 ml of overnight LB culture for the preparation of the low-copy plasmid. Centrifuge at 3,000×g for 8 minutes to pellet the bacteria. Discard the supernatant. Invert the centrifuge tube on a paper towel for 1 minute to drain off residual medium.

   IMPORTANT: The culture volume MUST be reduced to half or less when bacteria are grown in rich medium to avoid processing too many bacteria. Excess bacteria will reduce the lysis efficiency of the bacterial cells by Buffer S2 and result in low yield and incomplete purification of the plasmid DNA.

2. Resuspend the bacterial pellet with 4.5 ml of Buffer S1.

   Note: Make sure that RNase A has been added into Buffer S1.

   Note: Make sure that the bacterial pellet is completely resuspended with no residual aggregates. Incompletely ressuspension will reduce lysis efficiency, plasmid yield and purity.

3. Add 4.5 ml of Buffer S2, and mix by gently inverting tube 6-8×.

   Note: Buffer S3K must be added within 5 minutes.

   Note: Vigorous shaking will cause shearing of genomic DNA and result in the contamination with genomic DNA. Do not vortex.

Note: After use, the Buffer S2 bottle should be closed immediately in order to avoid neutralization of the NaOH by ambient CO_2.

Note: Check Buffer S2 for precipitation before each use. If precipitation occurs, incubate at 37°C to dissolve the precipitate and equilibrate to room temperature prior to use.

4. Add 4.5 ml of Buffer S3K (pre-chilled to 4°C) and mix by gently inverting the tube 10×. Let stand at room temperature for 5 minutes. Centrifuge at ≥6,000×g(4°C) for 10 minutes.
   Note: Avoid vigorous shaking. Do not vortex.

5. Transfer the supernatant from Step 4 to a 50ml conical centrifuge tube, then add 4.5 ml of Buffer B (pre-chilled to 4°C) and mix by gently inverting the tube 10×.
6. Attach the AxyVac Vacuum Manifold base to a vacuum pump. Position the manifold top with luer-type fittings on the manifold base. Attach a Midiprep column to a complimentary fitting on the manifold top. Make sure that the column is firmly seated.
7. Transfer the mixture buffer from Step 5 to the Midiprep column. Switch on the vacuum source and adjust to –25-30 inches Hg. Continue to apply vacuum to the column until no lysate remains. Do not turn the vacuum off.
   Note: -25-30 inches Hg is equivalent to approximately -850-1,000 mbar or -12-15 psi.
8. Add 7 ml of Buffer W1. Draw the solution through the Midiprep column.
9. Add 8 ml of Buffer W2 and draw through the Midiprep column.
   Note: Make sure that ethanol has been added into Buffer W2 concentrate.

10. Use the Plastic wrench to detach the end component from the Midiprep column assembly and place it into a 1.5 ml Microfuge tube (provided). Add 300 μl of Buffer W2 and centrifuge at 12,000×g for 2 minutes.
11. Transfer the end component to a fresh 1.5 ml Microfuge tube (provided). Add 300 μl of distilled water or Eluent to the center of the membrane. Let it stand at room temperature for 1 minute and centrifuge at 12,000×g for 1 minute to elute the plasmid DNA.
    Note: Pre-warming water or Eluent to 65°C will often improve elution efficiency.

12. **Option:** Eluting again with 0.2 ml of distilled water or Eluent will increase DNA yield. Add 0.2 ml of distilled water or Eluent to the center of membrane. Stand it at room temperature for 1 minute. Centrifuge at 12,000×g for 1 minute.

B. Plasmid Maxiprep

The protocol is designed to produce up to 500 μg of highly purified plasmid DNA from 120-300 ml (depending upon the copy number of the plasmid) of bacterial culture grown in LB medium. The culture volume should be reduced to half or less when rich media, such as LBG or 2× YT are used.

1. Collect 120 ml of overnight LB culture for the preparation of the high-copy plasmid or 250 ml of overnight LB culture for the preparation of the low-copy plasmid. Centrifuge at 3,000×g for 10 minutes to pellet the bacteria. Discard the supernatant. Invert the tube on a paper towel for 1 minute to drain off residual medium.

   IMPORTANT: The culture volume MUST be reduced to half or less when bacteria are grown in rich medium to avoid processing too many bacteria. Excess bacterial will reduce the lysis efficiency of bacterial cells by Buffer S2 and result in low yield and incomplete purification of the plasmid DNA.

2. Resuspend the bacterial pellet with 10 ml of Buffer S1.

   Note: Make sure that RNase A has been added into Buffer S1.

   Note: Make sure that the bacterial pellet is completely resuspended with no residual aggregates. Incompletely ressuspension will reduce lysis efficiency, plasmid yield and purity.

3. Add 10 ml of Buffer S2 and mix by gently inverting tube for 8-10×.

   Note: Buffer S3K must be added within 5 minutes.

   Note: Vigorous shaking will cause shearing of genomic DNA and result in the contamination with genomic DNA. Do not vortex.

   Note: After use, the Buffer S2 bottle should be closed immediately in order to avoid neutralization of the NaOH by ambient CO_2.

   Note: Check Buffer S2 for precipitation before each use. If precipitation occurs, incubate at 37°C to dissolve the precipitate and equilibrate to room temperature prior to use.

4. Add 10 ml of Buffer S3K (pre-chilled to 4°C) and mix by gently inverting tube for 10-12×. Let stand at room temperature for 5 minutes. Centrifuge at 8,000×g (4°C) for 10 minutes.

   Note: Avoid vigorous shaking. Do not vortex.

5. Transfer the supernatant from Step 4 to a 50ml conical centrifuge tube, then add 10 ml of Buffer B (pre-chilled to 4°C) and mix by gently inverting the tube 10-12×.
6. Attach the AxyVac Vacuum Manifold base to a vacuum source. Position the manifold top with luer-type fittings on the manifold base. Attach a Maxiprep column to a complimentary fitting on the manifold top. Make sure that the Maxiprep column is firmly seated.
7. Transfer the mixture buffer from Step 5 to the Maxiprep column. Switch on the vacuum source and adjust to –25-30 inches Hg. Continue to apply vacuum to the column until no lysate remains. Do not turn the vacuum off.

   Note: -25-30 inches Hg is equivalent to approximately -850-1,000 mbar and -12-15 psi.

8. Add 12 ml of Buffer W1 and draw the solution through the Maxiprep column.
9. Add 14 ml of Buffer W2 along the wall of the Maxiprep column, and draw through the Maxiprep column.

   Note: Make sure that ethanol has been added into Buffer W2 concentrate.

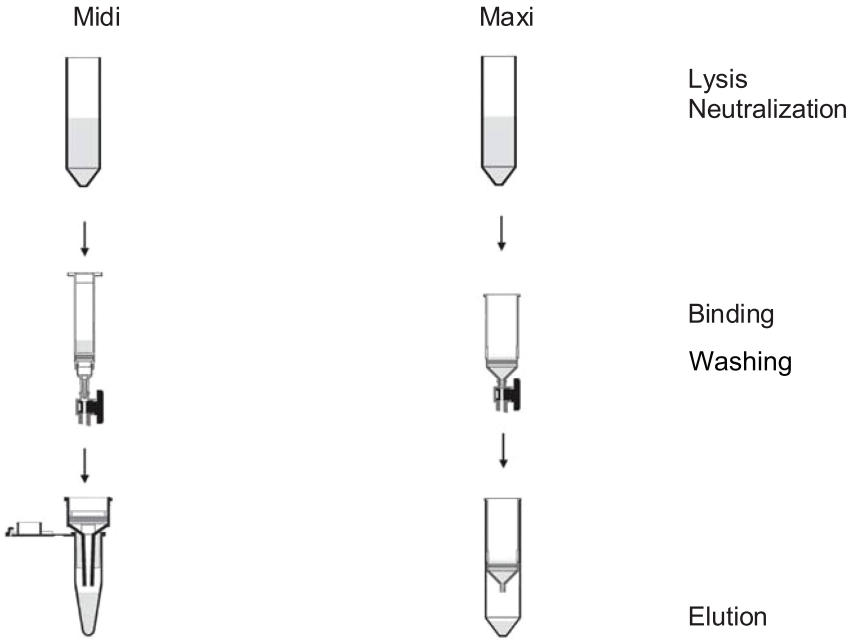
10. Transfer the Maxiprep column into a 50 ml conical centrifuge tube. Add 4 ml of Buffer W2 and centrifuge at ⩾6,000×g for 5 minutes.

    Option: Attach the Maxiprep column to a fitting on the vacuum manifold. Apply vacuum for 10 minutes to remove residual wash solution.

11. Transfer the Maxiprep column into a new 50 ml centrifuge tube. Add 1.5 ml of distilled water or Eluent to the center of membrane. Allow to stand at room temperature for 5 minutes. Centrifuge at ⩾6,000×g for 5 minutes to elute the plasmid DNA.
    Note: Pre-warming water or Eluent to 65°C will often improve elution efficiency.

12. **Option:** Eluting again with 0.75 ml of distilled water or Eluent will increase about 30% of DNA yield. Add 0.75 ml of distilled water or Eluent to the center of membrane. Stand it at room temperature for 1 minute. Centrifuge at ⩾6,000×g for 5 minutes.

Overview



Troubleshooting

1. Little or no plasmid DNA recovered

Plasmid did not propagate efficiently

Restreak fresh plates from glycerol stocks. Be sure that appropriate antibiotics are present and fresh. If using ampicillin, consider replacing with carbenicillin. If necessary, repeat the transformation of bacteria with fresh plasmid. Try a different bacterial host strain.

Incomplete bacterial lysis

Generally attributable to processing too many bacteria or using outdated Buffer S2 in which the NaOH has been compromised through repeated exposure to ambient CO_2. Reduce the culture volume by 50% and repeat the purification to determine if bacterial overload is the cause. Use fresh Buffer S2.

Redissolve by warming to 37°C if precipitation occurs in Buffer S2.

Cell resuspension incomplete

After adding Buffer S1, use vigorous vortexing to ensure complete resuspension of the bacterial pellet. Visually inspect before proceeding with the addition of Buffer S2.

Premature elution of plasmid during Buffer W2 wash step

Make sure that 95-100% ethanol has been added to the Buffer W2 concentrate, and in the correct amount. If uncertain, replace with new stock of Buffer W2. Do not use 70% ethanol (common to many labs) to dilute Buffer W2 concentrate. 95% denature ethanol is 95% ethanol, plus 5% combined isopropanol and methanol and is completely satisfactory for use.

Failure of plasmid to elute

Occasionally, excessive drying of the membrane and bound plasmid may result in diminished elution efficiency. Try decreasing the amount of time that the plate membranes are dried under vacuum prior to elution. Warm the Eluent (or distilled water) to 65°C and allow the column or plate to sit for 5 minutes after the addition of the Eluent before centrifugation.

2. Low DNA quality

Highly purified plasmid DNA will generally exhibit an $A_{260/280}$ = 1.7-1.9. A reading <1.7 generally indicates protein contamination and a reading >1.9 generally indicates RNA contamination. While technically suboptimal in purity, plasmid preps outside the range of 1.7-1.9 will usually perform quite well in many applications, with the possible exception of mammalian cell transfection. In the event that an inordinately low or high $A_{260/280}$ reading is accompanied by poor performance in an application, the following guidelines should be used to determine the source cause of the impurity.

a. Low $A_{260/280}$

Plasmid preps with depressed spectrophotometric readings may also exhibit high background on agarose gels and poor performance in certain enzymatic reactions. This problem is usually attributable to the following:

- Processing too many bacteria
- Incomplete resuspension (Buffer S1)
- Incomplete lysis (Buffer S2)
- Incomplete neutralization (Buffer S3K)

b. High $A_{260/280}$

Plasmid preps with elevated $A_{260/280}$ readings may also exhibit RNA smears or bands on an agarose gel. Residual RNA contamination is usually attributable to the following:

- Failure to add RNase A to Buffer S1
- Buffer S1 too old or RNase A activity compromised
- Processing too many bacteria
- Incomplete resuspension
- Incomplete lysis

3. Plasmid band smeared on gel

A smeared plasmid band usually indicates enzymatic degradation of the plasmid within the bacterial host or during the purification process. This is usually attributable to:

- Use of an endA+ bacterial host
- Excessively long growth of bacterial culture
- Excessively long storage/handling of the harvested bacteria
- Improper storage of harvested bacteria (too warm)
- Incomplete lysis of bacteria (Buffer S2 step)
- Incomplete neutralization of bacterial lysate (Buffer S3K step)

4. Multiple bands on gel

It is quite common to see multiple bands within a single lane when a plasmid sample is run on an agarose gel. These bands represent different forms of the plasmid. Usually, one of the bands is clearly predominant and this is the supercoiled form of the plasmid. Usually, there are 1-3 bands above the supercoil band, indicating plasmid species with slower electrophoretic mobility. These are usually the nicked and dimeric forms of the plasmid (or different combinations thereof). Occasionally, there may be a faint band which runs slightly ahead of the supercoil. This is referred to as the “irreversibly denatured” plasmid and is a biproduct of alkaline lysis. This form of the plasmid is refractory to many/most enzymatic reactions, including restriction and sequencing. The presence of the irreversibly denatured plasmid may become excessive if the plasmid is exposed to denaturing conditions (Buffer S2) for too long a period of time before the addition of Buffer S3K.

5. High background on gel

The background material which stains weakly with ethidium bromide is usually a combination of bacterial debris and bacterial genomic DNA/RNA. Its presence may be attributable to bacterial death and lysis prior to purification or simply processing too many bacteria and overwhelming the ability of the protocol to assimilate debris. Alternatively, incomplete mixing of Buffers S2 and Buffer S3K may also result in the carryover of debris onto the plate.

- Excessively long growth of bacterial culture (cell death and lysis)
- Excessively long storage/handling of the harvested bacteria
- Improper storage of harvested bacteria
- Processing too much bacterial culture
- Incomplete lysis of bacteria (Buffer S2 step)
- Incomplete neutralization of bacterial lysate (Buffer S3K step)

6. Genomic DNA contamination

- Excessively long growth of bacterial culture (cell death and lysis)
- Processing too much bacterial culture
- Excessive agitation after the addition of Buffer S2
- Excessive agitation after the addition of Buffer S3K and Buffer B
- Incomplete lysis of bacteria (Buffer S2 step)
- Incomplete neutralization of bacterial lysate (Buffer S3K step)
- Excessively long exposure to Buffer S2 (too long before addition of Buffer S3K)

7. RNA contamination

While limited amounts of residual bacterial RNA are generally not problematic in many applications, the presence of RNA may be viewed as an indication that certain aspects of the procedure have been compromised. The most likely reasons for the incomplete removal of bacterial RNA are:

- Failure to add RNase A to Buffer S1
- Buffer S1 dated or improperly stored (RNase activity compromised)
- Processing too much bacterial culture (overwhelming the RNase A)
- Incomplete resuspension and mixing during Buffer S1 and Buffer S2 steps

8. DNA does not perform well (general)

Failure of the plasmid to perform in enzymatic reactions is usually indicative of either the presence of an inhibitory contaminant such as salt or ethanol or modification of the plasmid. Occasionally, plasmids propagated through several generations may undergo deletions. It may be necessary to confirm the sequence composition of the plasmid when no other causative factor is apparent.

- Contaminating salt present
- Contaminating ethanol present
- Excessively long exposure to denaturing conditions before neutralization with Buffer S3K
- Nuclease contamination, plasmid degradation
- Deletions