AxyPrep Plasmid Miniprep Kit

For the rapid purification of plasmid DNA from bacterial cultures

Kit contents, storage and stability

Cat. No.	AP-MN-P-4	AP-MN-P-50	AP-MN-P-250
Kit size	4 preps	50 preps	250 preps
Miniprep column	4	50	250
2 ml Microfuge tube	4	50	250
1.5 ml Microfuge tube	4	50	250
RNase A	10 µl	30 µl	150 µl
Buffer S1	2 ml	15 ml	75 ml
Buffer S2	2 ml	15 ml	75 ml
Buffer S3	2.5 ml	21 ml	105 ml
Buffer W1	2.8 ml	28 ml	135 ml
Buffer W2 concentrate	2.4 ml	24 ml	2×72 ml
Eluent	1 ml	5 ml	25 ml
Protocol manual	1	1	1

With the exception of the RNase A (after addition to Buffer S1), all reagents are stable for a period of at least 12 months from the date of receipt when stored under ambient conditions. 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℃ 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: Resuspension buffer. Store at 4°C after addition of RNase A.

Buffer S2: Lysis buffer. Store at room temperature.

Buffer S3: Neutralization buffer. Store at room temperature.

Buffer W1: Wash buffer. Store at room temperature.

Buffer W2 concentrate: Desalting buffer. Before using the kit, add ethanol according to instructions on the bottle label. Either 100% or 95% denatured ethanol can be used. Store at room temperature.

Eluent: 2.5 mM Tris-HCl, pH 8.5. Store at room temperature.

Introduction

The AxyPrep Plasmid Miniprep Kit is based upon a modified SDS-alkaline lysis of bacterial cells in combination with selective binding of the plasmid DNA to a special Miniprep column. Each column has a binding capacity of at least 20 µg. The protocol provides a simple and reliable method to achieve the rapid isolation of highly purified plasmid DNA. The protocol has been optimized for bacterial cultures grown in LB (Luria-Bertani) broth, but can also be used for cultures grown in rich broths, such as LBG (LB + 2% glycerol) and 2×YT. TB (Terrific Broth) is not recommended for plasmid purification. Each column can process up to 4 ml of bacterial culture grown in LB or up to 2 ml of culture grown in rich broth. The entire procedure can be completed within 20 minutes. The highly purified plasmid DNA is eluted in a small volume of Tris buffer eluent or deionized water and can be used immediately for many routine applications, such as DNA sequencing, restriction digestion, in vitro transcription, library screening, ligation and transformation.

Caution

Buffer S2 contains NaOH which is a caustic reagent. Buffer S3 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 eyes and skin. In the case of such contact, wash immediately with water. If necessary, seek medical assistance.

Equipment and consumables required

- Benchtop microcentrifuge capable of 12,000×g
- AxyVac vacuum manifold (catalog #AP-VM) or comparable model with luer-type fittings
- Vacuum source capable of –25-30 inches Hg
- Vacuum regulator
- 100% or 95% (denatured) ethanol

Preparation before experiment

1) Before using the kit, add the RNase A to Buffer S1. Mix well and store at 4°C.
   Note: If a precipitate is present, use a small volume of Buffer S1 to resuspend the RNase A and then transfer to the Buffer S1 bottle.

2) Add the volume of ethanol specified on the bottle label to the Buffer W2 concentrate and mix well. 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 then equilibrate 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-warming Eluent to 65°C may improve elution efficiency.

AxyPrep Plasmid Miniprep Vacuum Protocol

Any vacuum manifold with complementary fittings can be used with the Miniprep columns. A negative pressure of –25-30 inches Hg is required. It is advisable to place a trap between the vacuum manifold and the pump or vacuum source to avoid contamination. -25-30 inches Hg is equivalent to approximately -850-1,000 mbar and -12-15 psi.

1. Collect 1-4 ml of overnight LB culture. Centrifuge at 12,000×g for 1 minute to pellet the bacteria. Decant or pipette off as much of the supernatant as possible.
   Note: When using rich broths such as LBG or 2×YT, reduce the culture volume by half. Excessive bacteria will reduce lysis efficiency, resulting in low yield and reduced purity of the plasmid DNA. Do not exceed 2 ml of bacterial culture grown in rich broth.

2. Resuspend the bacterial pellet in 250 µl of Buffer S1 by vortexing. Please be sure that the bacteria are completely resuspended before proceeding.
   Note: Be sure that RNase A has been added into Buffer S1.

3. Add 250 µl of Buffer S2, and mix by gently inverting the tube 4-6×. Do not vortex.
   Note: Vigorous shaking or vortexing will cause shearing of the bacterial genomic DNA and result in the contamination of the plasmid DNA.
   Note: After use, the Buffer S2 bottle should be closed immediately in order to avoid neutralization of NaOH by ambient CO_2.
   Note: Buffer S3 (Step 4, below) must be added within 5 minutes.

4. Add 350 µl of Buffer S3, and mix by gently inverting 6-8×. Centrifuge at 12,000×g for 10 minutes to clarify the lysate. Do not vortex.
   Note: Vigorous shaking or vortexing will result in contamination with genomic DNA.

5. Attach the vacuum manifold to the vacuum source. Insert the required number of AxyPrep Plasmid Miniprep columns into the fittings on the vacuum manifold. Transfer the clarified supernatant from Step 4 into the Miniprep column. Switch on the vacuum source and adjust the regulator to achieve a negative pressure of –25-30 inches Hg. Allow the vacuum to continue until no liquid remains in the column(s).

6. **Optional step: Buffer W1 Wash**
   Washing with Buffer W1 is required only in cases where the plasmid has been propagated in an endA+ bacterial strain, such as the JM series and HB101. These strains often exhibit high levels of endonuclease activity which will degrade the plasmid DNA. Omit this step if XL1-Blue or DH5α are used.
   Proceed to Step 7 if an endA- bacterial strain is used.
   Add 500 µl of Buffer W1 to each Miniprep column. Allow the vacuum to continue until no liquid remains in any of columns.

7. Pipette 700 µl of Buffer W2 along the wall of the column(s) to remove residual salt. Turn the vacuum on and adjust the regulator to –25-30 inches Hg. Continue to apply vacuum until no fluid remains in the column(s). Repeat this wash step with a second 700 µl aliquot of Buffer W2.
8. Transfer the Miniprep column into a 2 ml Microfuge tube (provided) and centrifuge at 12,000×g for 1 minute to purge residual Buffer W2 from the binding membrane.
9. Transfer the Miniprep column into a clean 1.5 ml Microfuge tube (provided). To elute the purified plasmid DNA, add 60~80 µl of Eluent (or deionized water) to the center of the membrane. Let it stand for 1 min at room temperature. Centrifuge at 12,000×g for 1 minute.

AxyPrep Plasmid Miniprep Spin Protocol

1. Collect 1-4 ml of overnight LB culture. Centrifuge at 12,000×g for 1 minute to pellet the bacteria. Decant or pipette off as much of the supernatant as practical.

   Note: When using rich broths such as LBG or 2×YT, reduce the culture volume by half. Excessive bacteria will reduce lysis efficiency, resulting in low yield and reduced purity of the plasmid DNA. Do not exceed 2 ml of bacterial culture grown in rich broth.

2. Resuspend the bacterial pellet in 250 µl of Buffer S1 by vortexing. Please be sure that the bacteria are completely resuspended before proceeding.

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

3. Add 250 µl of Buffer S2, and mix by gently inverting the tube for 4-6×. Do not vortex.

   Note: Vigorous shaking or vortexing will cause shearing of the bacterial genomic DNA and result in the contamination of the plasmid DNA.

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

   Note: Buffer S3 (Step 4, below) must be added within 5 minutes.

4. Add 350 µl of Buffer S3, and mix by gently inverting 6-8×. Centrifuge at 12,000×g for 10 minutes to clarify the lysate. Do not vortex.

   Note: Vigorous shaking or vortexing will result in contamination with genomic DNA.

5. Place a Miniprep column into an uncapped 2 ml Microfuge tube (provided). Transfer the clarified supernatant from Step 4 into the Miniprep column. Transfer the Miniprep column and 2 ml Microfuge tube to microcentrifuge and spin at 12,000×g for 1 minute.
6. **Optional step: Buffer W1 Wash**

   Washing with Buffer W1 is required only in cases where the plasmid has been propagated in an endA+ bacterial strain. These strains often exhibit high levels of endonuclease activity which will degrade the plasmid DNA.

   Proceed to Step 7 if an endA- bacterial strain is used.

   Pipette 500 µl of Buffer W1 into each Miniprep column. Centrifuge at 12,000×g for 1 minute.

7. Pipette 700 µl of Buffer W2 into each Miniprep column. Centrifuge at 12,000×g for 1 minute.

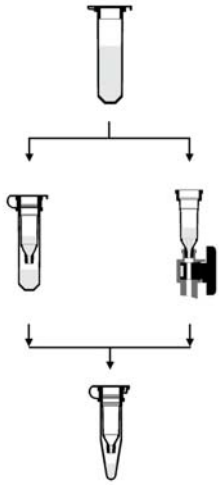
   Note: Make sure that the volume of ethanol specified on the bottle label has been added to the Buffer W2 concentrate.

8. **Optional Step:** Discard the filtrate from the 2 ml Microfuge tube. Place the Miniprep column back into the 2 ml Microfuge tube. Add 700 µl of Buffer W2 to the Miniprep column and centrifuge at 12,000×g for 1 minute.

   Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.

9. Discard filtrate from the 2 ml Microfuge tube. Place the Miniprep column back into the 2 ml Microfuge tube. Centrifuge at 12,000×g for 1 minute.
10. Transfer the Miniprep column into a clean 1.5 ml Microfuge tube (provided). To elute the purified plasmid DNA, add 60~80 µl of Eluent (or deionized water) to the center of the membrane. Let it stand for 1 min at room temperature. Centrifuge at 12,000×g for 1 minute.

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 this is the cause by over-used bacteria.

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

Check to be sure that 95-100% ethanol has been added to the Buffer W2 concentrate, and in the correct amount. If unsure, 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. It 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 deionized water) to 60°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. In the event that an inordinately low or high $A_{260/280}$ reading is accompanied by poor performance, the above 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 S3)

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
- Incomplete lysis of bacteria (Buffer S2 step)
- Incomplete neutralization of bacterial lysate (Buffer S3 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 S3.

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 S3 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 S3 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 S3
- Incomplete lysis of bacteria (Buffer S2 step)
- Incomplete neutralization of bacterial lysate (Buffer S3 step)
- Excessively long exposure to Buffer S2 (too long before addition of Buffer S3)

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. This is fairly common with cosmids. 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 S3
- Nuclease contamination, plasmid degradation
- Deletions

9. Sequencing-related problems (fluorescent capillary)

- Complete sequencing failure
  Check the DNA yield, the sequencing reaction setup including the running conditions, and correct concentration. Try using less DNA in the sequencing reaction.
- Low signal
  Increase the number of cycles to 45-60 for the sequencing reactions or increase the amount of template DNA used. 400-500 ng of plasmid or cosmid should be optimal for most sequencing reactions.
- Short read length
  This may indicate the presence of a contaminant (usually salt) which is injurious to the DNA polymerase used in the sequencing reaction. Salt contamination will also interfere with electrokinetic uptake of labeled fragments into the capillaries during sequencing and this can result in shortened read lengths and/or reduced signal intensities. Alternatively, the amount of plasmid template may be insufficient. However, depending on the source and length of the insert DNA, it may be difficult to achieve the long sequence reads that are routinely obtained with standard short inserts or high copy number plasmids. Sequencing large template DNAs can also sometimes be problematic, even if ultrapure quality DNA is used.
  Be sure that the Buffer W2 wash step is performed correctly to avoid salt contamination. Increase the number of cycles to 45-60 for the sequencing reactions. If necessary, use gel-filtration or ultrafiltration diagnostically to desalt a limited number of plasmid samples to verify salt contamination. Try increasing the amount of plasmid used in the sequencing reactions by 50%-100%.

For technical inquiries about AxyPrep Kits, please contact Axygen Biosciences at *support.axyprepkits@axygenbio.com*

Warranty/Disclaimer

AxyPrep Kits are designed for R&D and general laboratory use only. Axygen Biosciences makes no claims regarding the performance of these kits for clinical or diagnostic applications. Axygen Biosciences warrants that this kit will perform as indicated for the specified application for a period of up to 12 months from the date of receipt when stored in the specified manner and used according to the instructions provided. In using this product, the customer agrees that Axygen Biosciences shall not be held liable for any direct or indirect damages, including, but not limited to, personal injury, property damage or lost profits (or other economic loss) resulting from the use or inability to use this product. In the event that this product fails to perform in the specified manner, remedial measures on the part of Axygen Biosciences shall be limited to the replacement of this product and will be implemented at the discretion of Axygen Biosciences.