

AxyPrep Plasmid Miniprep Kit

Kit contents, storage and stability

Cat. No.	AP-MN-P-50	AP-MN-P-250
Kit size	50 preps	250 preps
AxyPrep Column	50	250
2 ml microfuge tube	50	250
1.5 ml microfuge tube	50	250
RNase A	30 µl	150 µl
Buffer S1	15 ml	75 ml
Buffer S2	15 ml	75 ml
Buffer S3	25 ml	125 ml
Buffer W1	28 ml	135 ml
Buffer W2 concentrate	24 ml	2 × 72 ml
Eluent	5 ml	25 ml
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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°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. Store at room temperature. 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-Cl, 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 AxyPrep 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 kit can also be used to isolate large recombinant constructs, such as BACs, PACs and P1s but some decrease in elution efficiency may be encountered. 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 use with this kit. 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. Buffers 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
- AxyPrep vacuum manifold (catalog #AP-VAC) 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 CO₂ in the air.
- 4) Pre-warming the Eluent to 60°C may improve elution efficiency.

AxyPrep Plasmid Miniprep Vacuum Protocol

Any vacuum manifold with complimentary fittings can be used with the AxyPrep 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 $12,000 \times g$ 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 $2 \times$ 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 μ l 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 μ l of Buffer S2, and mix by gently inverting the tube for $4-6 \times$. 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 μ l of Buffer S3, and mix by gently inverting $6-8 \times$. Centrifuge at $12,000 \times g$ 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 to the AxyPrep 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 DH5 α are used.

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

Add 500 μ l of Buffer W1 to each AxyPrep column. Allow the vacuum to continue until no liquid remains in any of columns.

7. Pipette 700 μ l 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).

8. Repeat this wash step with a second 700 μ l aliquot of Buffer W2.

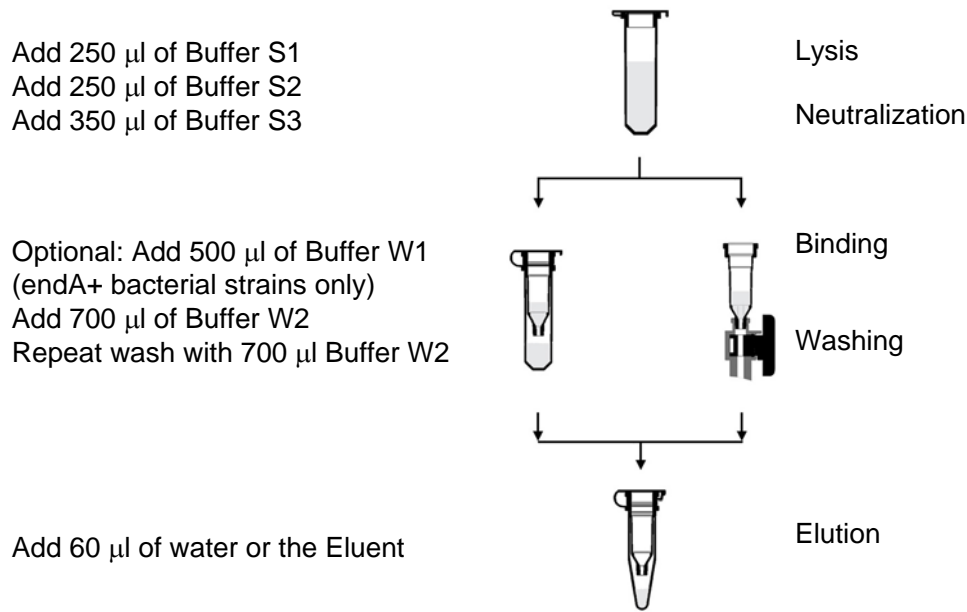
9. Transfer the AxyPrep column into a 2 ml microfuge tube (provided) and centrifuge at $12,000 \times g$ for 1 minute to purge residual Buffer W2 from the binding membrane.
10. Transfer the AxyPrep column to a clean 1.5 ml microfuge tube (provided). To elute the purified plasmid DNA, add 60~80 μ l of Eluent (or deionized H₂O) to the center of the membrane. Let it stand for 1 min at room temperature. Centrifuge at $12,000 \times g$ for 1 minute.

AxyPrep Plasmid Miniprep Spin Protocol

1. Collect 1-4 ml of overnight LB culture. Centrifuge at $12,000 \times g$ 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 $2 \times$ 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 μ l 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 μ l of Buffer S2, and mix by gently inverting the tube for 4-6 \times . 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 CO₂.
Note: Buffer S3 (Step 4, below) must be added within 5 minutes.
4. Add 350 μ l of Buffer S3, and mix by gently inverting 6-8 \times . Centrifuge at $12,000 \times g$ for 10 minutes to clarify the lysate. Do not vortex.
Note: Vigorous shaking or vortexing will result in contamination with genomic DNA.
5. Place the AxyPrep column into an uncapped 2 ml microfuge tube (provided). Transfer the clarified supernatant from Step 4 to the AxyPrep column. Transfer the AxyPrep column and 2 ml microfuge tube to microcentrifuge and spin at $12,000 \times g$ 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 μ l of Buffer W1 into each AxyPrep column. Centrifuge at $12,000 \times g$ for 1 minute.
7. Pipette 700 μ l of Buffer W2 into each AxyPrep column. Centrifuge at $12,000 \times g$ for 1 minute.
8. **Optional Step:** Discard the filtrate from the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Add 700 μ l of Buffer W2 to the AxyPrep column and centrifuge at $12,000 \times g$ 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 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Centrifuge at $12,000 \times g$ for 1 minute.
10. Transfer the AxyPrep column to a clean 1.5 ml microfuge tube (provided). To elute the purified plasmid DNA, add 60–80 μ l of Eluent (or deionized H₂O) to the center of the membrane. Let it stand for 1 min at room temperature. Centrifuge at $12,000 \times g$ 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 CO₂

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 ensure 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. Little or no BAC or P1 DNA recovered.

These large recombinant constructs are usually present as unique copies within the bacterial host and as a result, the yield is usually quite low. In addition, these very large species often exhibit reduced elution efficiency, which further reduces their yield. A single AxyPrep column can be used to consolidate several neutralized lysates and the BACs or P1s they contain. The following modification can be tried:

- a. Process 4-6, 2 ml aliquots of bacterial BAC or P1 culture grown in rich broth (LBG or 2 × YT) through Step 4 in the protocol.
- b. Load each of the neutralized lysates onto a single AxyPrep column in succession.
- c. Follow 1× with Buffer W1 and 3× with Buffer W2 according to the protocol.
- d. Elute using a pre-warmed (65°C) eluent.

3. 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
- S1 too old or RNase A activity compromised
- Processing too many bacteria
- Incomplete resuspension

- Incomplete lysis

4. 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)

5. 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. Within the plasmid prep, this is the dominant form of the plasmid. Usually, there are 1-3 bands above the supercoil band, indicating 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 byproduct 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.

6. 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 segregate this debris from the plasmid. 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)

7. 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)

8. RNA contamination

While limited amounts of residual bacterial RNA are generally not problematic in many applications, the presence of RNA may be viewed as a signal 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

9. 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
- Nuclease contamination, plasmid degradation
- Deletions

10. 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. 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 (cosmids and BACS) can also sometimes be problematic, even if ultrapure quality DNA is used.
Ensure 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%.