# AxyPrep Bacterial Genomic DNA Miniprep Kit

***For Rapid DNA Purification from Bacterial samples***

## Kit contents, storage and stability

| Cat. No. | AP-MN-BT-GDNA-50 | AP-MN-BT-GDNA-250 |
|---|---|---|
| Kit size | 50 preps | 250 preps |
| AxyPrep columns | 50 | 250 |
| Spin-filter | 50 | 250 |
| 2 ml microfuge tubes | 150 | 750 |
| 1.5 ml microfuge tubes | 50 | 250 |
| RNase A | 20 μl | 100 μl |
| Lysozyme | 60 mg | 2 × 140 mg |
| Buffer S | 10 ml | 50 ml |
| 0.25 M EDTA | 2 ml | 10 ml |
| Buffer G-A | 26 ml | 130 ml |
| Buffer G-B | 26 ml | 130 ml |
| Buffer DV(empty) | 1 | 1 |
| Buffer DV-A | 5 ml | 10 ml |
| Buffer BV | 25 ml | 125 ml |
| Buffer W1 | 28 ml | 135 ml |
| Buffer W2 concentrate | 24 ml | 2 × 72 ml |
| Eluent | 12 ml | 60 ml |
| Protocol manual | 1 | 1 |

*Except for the RNase A (after addition to Buffer S) and the lysozyme, all buffers and 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. 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 S 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 visible, use a small aliquot of Buffer S to dissolve and transfer to the Buffer S bottle.

Lysozyme: Dissolved in 50% glycerin to a final concentration of 50mg/ml. The dissolved lysozyme is stable for 6 months when stored at -20°C.

Buffer S: Bacterial protoplast preparation buffer. After addition of RNase A, mix well and store at 4°C. The dissolved RNase A is stable for 6 months when Buffer S is stored at 4°C.

0.25 M EDTA: Store at room temperature.

Buffer G-A: Lysis buffer. Store at room temperature.

Buffer G-B: Protein-removal buffer. Store at room temperature.

Buffer DV-A: Buffer DV additive. Used for preparation of Buffer DV (refer to Preparation before experiment on page 2 for details). Store at room temperature.

Buffer DV: Phase-partition buffer. Store at room temperature. Prepare by following the instructions described in “Preparation Before Experiment” on page 3 of this manual.

Buffer BV: DNA binding 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 isolation of bacterial genomic DNA by this kit is based upon the efficient release of genomic DNA by a special cell lysis buffer, Buffer G-A. Following this, rapid separation of the genomic DNA from proteins, polysaccharides and lipids is achieved by a unique phase-partition step. Highly purified genomic DNA in the lower phase is then selectively bound to a special AxyPrep column. After washing successively with Buffer W1 and Buffer W2 to remove residual impurities and salt, the purified bacterial genomic DNA is eluted from the AxyPrep column in Tris buffer or water. This kit is suitable for the rapid isolation of up to 20 μg of genomic DNA from $1.0 \times 10^9$ bacterial cells. The purified DNA is predominantly 30 kb in length, and is suitable for a varitey of applications demanding highly purified, high molecular weight genomic DNA, such as PCR, Southern blot analysis, RAPD, AFLP and RFLP, etc.

## Caution

Buffer G-B, Buffer BV and Buffer W1 contain chemical irritants. When working with the buffers, always wear suitable protective clothing such as safety glasses, laboratory coat and gloves. Be careful to 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

- Heated water bath
- Vacuum manifold (Axygen catalog #AP-VAC) or comparable model
- Vacuum regulator
- Vacuum source (-25-30 inches Hg required)
- Microcentrifuge capable of 12,000 × g
- 100% or 95% (denatured) ethanol
- Isobutanol & Isopropanol

## Preparation before experiment

1) Before using the kit, add the amount of ethanol specified on the bottle label to the Buffer W2 concentrate. Either 100% or 95% denatured ethanol can be used.
2) Prepare Buffer DV: Add 2 ml of Buffer DV-A, 125 ml of isopropanol and 75 ml of isobutanol to the 250-ml bottle provided with kit and mix well.
3) Chill Buffer DV at 4°C before experiment.
4) Dissolve lysozyme in 50% glycerine before using the kit.
5) Add RNase A into Buffer S and mix well.

   **Note:** If a precipitate is present, use a small volume of Buffer S to resuspend the RNase A and the transfer to the Buffer S bottle.

6) Switch on the water bath at 65°C.
7) Check Buffer G-A and Buffer G-B for precipitation before each use. If precipitation occurs, warm at 65°C to dissolve the precipitate, then equilibrate to room temperature before use.
8) Pre-warming the Eluent to 65°C will often improve elution efficiency.

## Protocols

*Please read through the entire protocol, including the "Notes", carefully before proceeding. Genomic DNA can be purified from either gram- or gram+ bacterial strains with this procedure.*

### Bacterial Genomic DNA Miniprep vacuum protocol

*A vacuum manifold, such as Axygen's Vacuum manifold with complimentary fittings which can accommodate the AxyPrep column is required for Steps 10-12. –25-30 inches Hg is required. -25-30 inches Hg is approximately equivalent to -850-1,000 mbar and -12-15 psi.*

1. Collect $1.0\times10^9$ bacteria in a 2 ml microfuge tube (provided). Spin at top speed (12,000 × g) for 30 seconds. Discard the supernatant and resuspend the bacterial pellet in 150 µl of Buffer S, containing RNase A.

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

   **Note:** Be sure that the bacterial pellet is homogenously resuspended. Aggregates and clumps will reduce lysis efficiency and yield.

   **Note:** $1.0\times10^9$ bacteria is equivalent to 1 ml of bacterial culture at $OD_{600}$ = 1-1.5

2. Add 20 µl of lysozyme and mix well. Allow to sit at room temperature for 5 minutes before proceeding.

   **Note:** When using gram+ bacteria, incubate at 37°C for 30 minutes, following the addition of lysozyme.

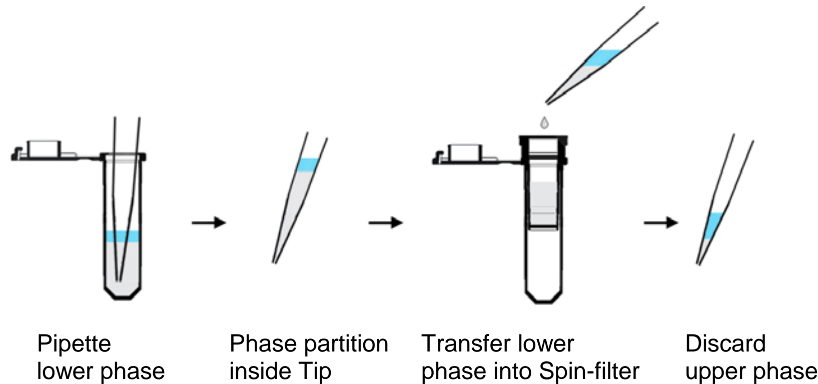
3. Add 30 µl of 0.25 M EDTA (pH 8.0). Mix well and incubate on ice for 5 minutes.
4. Add 450 µl of Buffer G-A and vortex for 15 seconds. Heat in a water bath at 65°C for 10 minutes.
5. Add 400 µl of Buffer G-B, followed by 1 ml of Buffer DV (pre-chilled to 4°C). Mix vigorously. Centrifuge at 12,000 × g for 2 minutes.

   **Note:** Please refer to "Preparation before experiment" on page 3 to prepare Buffer DV.

6. Aspirate off as much of the upper phase as possible, without disturbing the interphase. Discard the upper phase.
7. Add 1 ml of Buffer DV (pre-chilled to 4°C) to the remaining interphase and lower phase. Mix vigorously to achieve homogeneity and centrifuge at 12,000 × g for 2 minutes.
8. Discard the colored upper phase. Transfer the lower phase to a Spin-filter, placed into a 2 ml microfuge tube (provided) and centrifuge at 12,000 × g for 1 minute.

   **Note:** When recovering the lower phase, complete removal of the upper phase is not necessary. Any of the upper phase which is carried over will rapidly shift to the top of the pipette tip and can easily be removed (Refer to the figure below).

   **Note:** If the lower phase is transferred without any contaminating interphase debris, Step 8 can be omitted.




   **Note:** Avoid carryover of upper phase liquid. This will cause inhibition of genomic DNA binding to the AxyPrep column (Step 9, below).

9. Discard the spin-filter. Add 400 μl of Buffer BV to the filtrate and mix well.
10. Attach the vacuum manifold base to a vacuum pump. Transfer the binding mix from Step 9 to the AxyPrep column. Turn on the vacuum source and adjust to –25-30 inches Hg. Draw all solution through the AxyPrep column.
11. Add 500 μl of Buffer W1 and draw the solution through the column.
12. Add 700 μl of Buffer W2 along the wall of AxyPrep column to wash off Buffer W1 on it and draw through the column. Wash once again with 700 μl of Buffer W2.

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

    **Note:** Add Buffer W2 along the tube wall to wash off any residual salt.

13. Place the AxyPrep column into the 2 ml microfuge tube. 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.

14. Transfer the AxyPrep column in a clean 1.5 ml microfuge tube (provided). To elute DNA, add 100-200 μl of water or the Eluent to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12000 × g for 1 minute.

    **Note:** Pre-warming water or Eluent at 65°C will often improve elution efficiency.

**Bacterial Genomic DNA Miniprep spin protocol**

1. Collect $1.0\times10^9$ bacteria in a 2 ml microfuge tube (provided). Spin at top speed (12,000 × g) for 30 seconds. Discard the supernatant and resuspend the bacterial pellet in 150 µl of Buffer S, containing RNase A.

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

   **Note:** Be sure that the bacterial pellet is homogenously resuspended. Aggregates and clumps will reduce lysis efficiency and yield.

   **Note:** $1.0\times10^9$ bacteria is equivalent to 1 ml of bacterial culture at $OD_{600}$ = 1-1.5

2. Add 20 µl of lysozyme and mix well. Allow to sit at room temperature for 5 minutes before proceeding.

   **Note:** When using gram+ bacteria, incubate at 37°C for 30 minutes, following the addition of lysozyme.

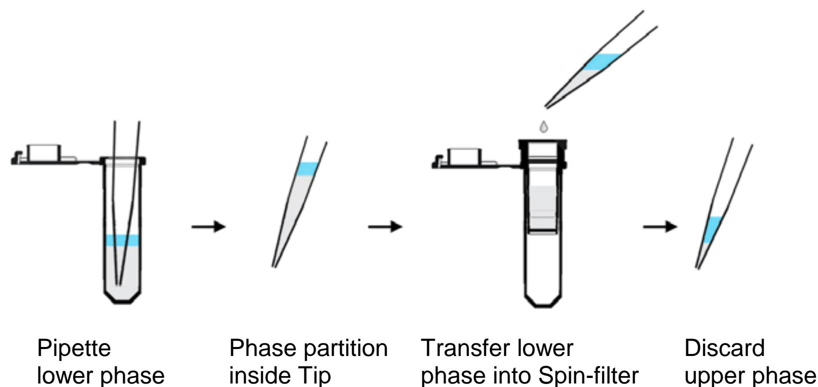
3. Add 30 µl of 0.25 M EDTA (pH 8.0). Mix well and incubate on ice for 5 minutes.
4. Add 450 µl of Buffer G-A and vortex for 15 seconds. Heat in a water bath at 65°C for 10 minutes.
5. Add 400 µl of Buffer G-B, followed by 1 ml of Buffer DV (pre-chilled to 4°C). Mix vigorously. Centrifuge at 12,000 × g for 2 minutes.

   **Note:** Please refer to “Preparation before experiment” on page 3 to prepare Buffer DV.

6. Aspirate off as much of the upper phase as possible, without disturbing the interphase. Discard the upper phase.
7. Add 1 ml of Buffer DV (pre-chilled to 4°C) to the remaining interphase and lower phase. Mix vigorously to achieve homogeneity and centrifuge at 12,000 × g for 2 minutes.
8. Discard the colored upper phase. Transfer the lower phase to a Spin-filter, placed into a 2 ml microfuge tube (provided) and centrifuge at 12,000 × g for 1 minute.

   **Note:** When recovering the lower phase, complete removal of the upper phase is not necessary. Any of the upper phase which is carried over will rapidly shift to the top of the pipette tip and can easily be removed (Refer to the figure below).

   **Note:** If the lower phase is transferred without any contaminating interphase debris, Step 8 can be omitted.




   **Note:** Avoid carryover of upper phase liquid. This will cause inhibition of genomic DNA binding to the AxyPrep column (Step 9, below).

9. Discard the spin-filter . Add 400 µl of Buffer BV to the filtrate and mix well.

10. Place a AxyPrep column to a 2 ml microfuge tube (provided). Transfer the binding mix from Step 9 to the AxyPrep column. Centrifuge at 12,000 × g for 1 minute.
11. Discard the filtrate from the 2 ml microfuge tube. Place the AxyPrep column back to the 2 ml microfuge tube. Add 500 µl of Buffer W1 to the AxyPrep column and centrifuge at 12,000 × g for 1 minute.
12. Discard the filtrate and place the AxyPrep column back to the 2 ml microfuge tube. Add 700 µl of Buffer W2, and centrifuge at 12,000 × g for 1 minute.
    **Note:** Make sure that ethanol has been added into Buffer W2 concentrate.

13. **Optional Step**: Discard the filtrate from the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Add 700 µl of Buffer W2 to the AxyPrep 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.

14. Discard the filtrate. Place the AxyPrep column back into the 2 ml microfuge tube. Centrifuge at 12,000 × g for 1 minute.

15. Transfer the AxyPrep column in a clean 1.5 ml microfuge tube (provided). To elute DNA, add 100-200 µl of water or the Eluent to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12000 × g for 1 minute.
    **Note:** Pre-warming water or Eluent at 65°C will often improve elution efficiency.

## Overview

Add 150 µl of Buffer S containing RNase A
Add 20 µl of lysozyme
Add 30 µl of 0.25 M EDTA
Add 450 µl of Buffer G-A
Add 400 µl of Buffer G-B

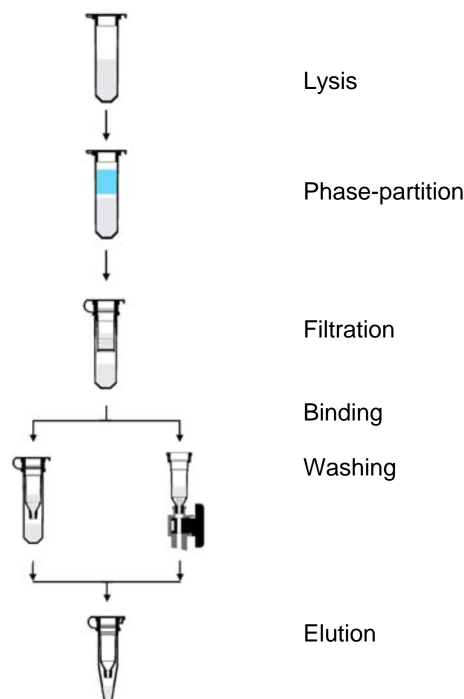
Add 1 ml of Buffer DV
Repeat extraction with Buffer DV

Add 400 µl of Buffer BV

Add 500 µl of Buffer W1
Add 700 µl of Buffer W2

Repeat wash with Buffer W2

Add 100-200 µl of Eluent



## Troubleshooting

### 1. Low or no yield

- Insufficient bacteria processed
- Inefficient lysis
  - Lysozyme inactive (store at -20°C for up to 6 months)
  - Insufficient incubation time with lysozyme
- Bring the upper phase liquid into the lower phase
- DNA not efficiently eluted
  - AxyPrep column membrane overdried during vacuum removal of Buffer W2

### 2. Low $A_{260/280}$

- Too many bacteria processed
- Inefficient lysis with lysozyme
- Contamination with interphase material

### 3. RNA present (elevated $A_{260/280}$)

- Failure to add RNase A to Buffer S
- Buffer S dated
- Incomplete digestion of bacteria by lysozyme

### 4. Genomic DNA appears to be degraded

Depending upon the completeness of degradation, the genomic DNA will either appear as a smear or as a smear trailing in front of a high molecular weight band on an agarose gel. Since no physical measure used during the purification process is sufficient to cause any visually discernable degradation, the most likely source is enzymatic. Many strains of bacteria exhibit high levels of endonuclease activity. These endA+ strains must be lysed rapidly and completely to prevent substantial enzymatic degradation of the intrinsic genomic DNA.

### 5. Genomic DNA performs poorly in enzymatic reactions

- Low DNA concentration
- Salt contamination: insufficient W1 removal
- Ethanol contamination: insufficient centrifugation to remove residual W2

### 6. Clogged spin-filter

- Too many bacteria processed
- Inefficient lysis

### 7. Clogged AxyPrep column

- Too many bacteria processed
- Inefficient lysis