A&A BIOTECHNOLOGY
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*Manual*

# Plasmid Mini AX Gravity

Gravity flow increased efficiency kit for high-copy plasmid DNA purification.

| catalog # | size |
|---|---|
| 015-100 | 100 isolations |

For research use only.

**Guarantee**
A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

# Table of Contents

# Specification

| | |
|---|---|
| **form** | minicolumn |
| **binding capacity** | 20 µg of DNA |
| **sample size** | up to 3 ml of bacterial culture |
| **elution volume** | from 120 µl |
| **elution solution** | E buffer |

# Contents

| component | size | storage |
|---|---|---|
| **Micro AXB** columns | 100 pcs | 2–8 °C |
| **Gravity tubes** | 100 pcs | 15–25 °C |
| **L1** cell suspension solution | 35 ml | 2–8 °C |
| **L2** lysis solution | 35 ml | 15–25 °C |
| **L3** neutralizing solution | 35 ml | 15–25 °C |
| **K1** equilibrating solution | 60 ml | 15–25 °C |
| **K2P** first wash solution | 70 ml | 15–25 °C |
| **W2** second wash solution | 60 ml | 15–25 °C |
| **E** elution buffer | 10 ml | 2–8 °C |
| **N** neutralizing buffer | 1 ml | 15–25 °C |
| **T** solution | 400 µl | 15–25 °C |

# Additional equipment and reagents

## Necessary

- Centrifuge
- 5 ml sterile Falcon tubes
- 1.5 ml sterile Eppendorf tubes

## Optional

- Gravity flow rack (cat.# 008-1)

# Important notes

- Kit contains the LySee color system for easy optical control of alkaline lysis progress (page 7).
- SDS detergent is a component of L2 lysis solution and precipitates at low temperatures. Whenever the L2 lysis solution is not clearly transparent it must be warmed at 40 °C to form a thoroughly clear solution.

# Protocol

1. Centrifuge up to **1-3 ml** of overnight bacterial culture.

2. Discard the supernatant. Suspend the bacterial pellet in **300 µl** of **L1** cell suspension solution.

   **Note**. During the pellet bacterial suspension, the solution will change color from a transparent deep pink to opaque light pink. The suspension is completed with complete disappearance of the pellet at the bottom tube.

3. Add **300 µl** of **L2** lysis solution and gently mix. Keep for **3 min** at **room temp**.

   **Note.** After the addition of L2 lysis solution, gently mix the tube so as not to cause fragmentation of the chromosomal DNA. Gently mix the tube by inverting a few times. The mixture should change appearance and color. After 3 min of incubation, the lysate must be completely clear and uniformly raspberry. If not, mix the lysate a few times and incubate again for 3 min at room temp.

4. Add **300 µl** of **L3** neutralizing solution and gently mix until the disappearance of the raspberry color of the lysate.

   **Note.** After the addition of L3T neutralizing solution followed by the rapid precipitation of the potassium salts (SDS), chromosomal DNA and certain proteins. After mixing, the tube contents should change the color to yellowish. No traces of raspberry color indicates complete neutralization and successful ending of the alkaline lysis.

5. Centrifuge for **10 min** at **10 000 RPM**.

6. During incubation prepare the Micro AXB columns by securely attaching them to the top of receiving Gravity tubes and placing them upright in the rack.

Reference photo:
placing the columns and receiving tubes in the Gravity flow rack.

7. Apply **500 µl** of **K1** equilibrating solution onto the Micro AXB column.
Wait for the solution to flow through the column.

It is a good practice to apply the K1 solution to the column wall to avoid accidental blockage of the column flow by an air bubble between the membrane and the K1 solution.

The column is ready for use when the solution stops dripping from the capillary.

8. After centrifugation, collect the clarified supernatant and apply it onto the equilibrated **Micro AXB** column.
Wait **4 min** for the lysate to flow through the column by gravity.

The flow rate strongly depends on DNA concentration in the sample. The more DNA, the slower the flow rate.
As soon as the lysate stops dripping, proceed to the next step.
Lysate flow rate troubleshooting - page 8.

**Note:** applies to points 8-10 of the isolation protocol

- In the case of DNA isolation from a smaller number of samples (up to 10), it should be observed whether the lysate has completely passed through the column. When the solution stops dripping from the capillary, proceed to the next step in the isolation protocol.
- In the case of DNA isolation from a larger number of samples (over 10), we recommend waiting up to 4 min, instead of observing the process in individual columns.

9. Apply **700 µl** of **K2P** first wash solution onto the **Micro AXB** column.
Wait for the solution to flow through the column

10. Apply **500 µl** of **W2** second wash solution onto the **Micro AXB** column.
Wait for the solution to flow through the column

11. Before using E buffer, it is recommended to do a functionality test - page 8.

Apply **40 µl** of **E** elution buffer onto the **Micro AXB** column.
Keep for **2 min** at **room temp.**

E elution buffer loses activity upon prolonged contact with air. Always close the E elution buffer vial tightly directly after use. Store E elution buffer at 2-8 °C.

The purpose of this step is to decrease the total volume of eluate, since the column void volume is about 40 µl.

12. Prepare the 1.5 ml elution tubes (not included).
Apply **2 µl** of **N** neutralizing buffer onto the bottom of each tube.

DNA neutralization - page 8.

13. Transfer the Micro AXB columns to the prepared elution tubes.

14. Before using E buffer, it is recommended to do a functionality test - page 8.

Apply **40 µl** of **E** elution buffer onto the **Micro AXB** column.

E elution buffer loses activity upon prolonged contact with air. Always close the E elution buffer vial tightly directly after use. Store E elution buffer at 2-8 °C.

15. Centrifuge for **30-60 s** at **5000 RPM**.

16. Remove the **Micro AXB** column. Close the tube with purified DNA and store until later use.

# LySee color system

The LySee color system enables an easy and convenient visual control of alkaline lysis. The visual control system prevents common handling errors of incomplete cell resuspension, inefficient cell lysis and incomplete precipitation of unwanted cell components.

## Resuspension and lysis

The addition of the transparent purple L1 color cell suspension solution to the bacterial cell pellet makes the bacterial cell pellet easy to localize (fig 1). During the suspension of the bacterial cell pellet, the solution turns opaque light pink (fig 2). The suspension is completed with the complete disappearance of the pellet at the bottom of the tube. After the addition of L2 lysis solution and incubation, lisate turns transparent raspberry. Cell lysis is completed when the solution will turn homogeneously transparent raspberry (fig 3).

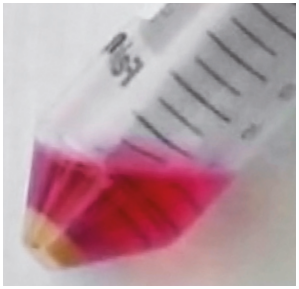


fig 1

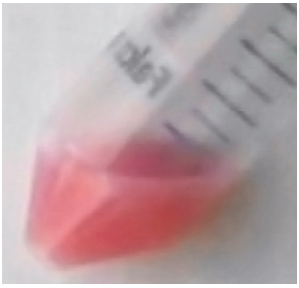


fig 2

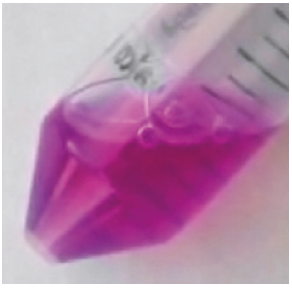


fig 3

## Neutralization and precipitation

The addition of the L3 neutralizing solution causes rapid precipitation of potassium salts (SDS), chromosomal DNA and some proteins (fig 4). After mixing, the solution turns yellowish (fig 5). No traces of raspberry color indicates complete neutralization and successful ending of alkaline lysis (fig 6).

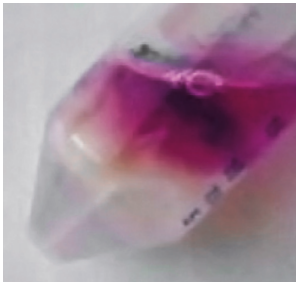


fig 4

fig 5

fig 6

# Lysate flow rate troubleshooting

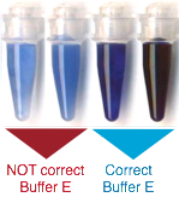
| problem | reason | solution |
|---|---|---|
| very slow rate of lysate through the Micro AXD column | highly concentrated DNA in the sample | - place the Micro AXD column into an Eppendorf tube and centrifuge.<br>- for the next isolation, reduce the amount of sample by half. |
| air bubbles in the receiving tube capillary | the Micro AXD column is not not securely attached to the receiving tube | - “tightening” the Micro AXD column.<br>- reattach the column in luer-like fitting simultaneously by pressing the column down and twisting. |

# DNA neutralization

E elution buffer is strongly alkaline and may cause DNA degradation upon freezing. Thus it is necessary to use a N neutralizing buffer. We recommend adding the N neutralizing buffer to the elution tube before the elution step. If the N neutralizing buffer was not added before the elution step it can be added directly before freezing DNA samples. The use of N neutralizing buffer enables secure DNA storage conditions at 10 mM TrisHCl, pH 8.5.

# E buffer functionality test

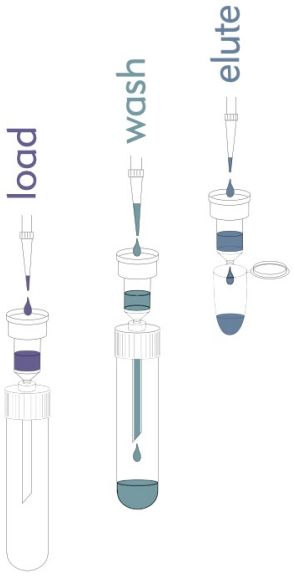
E buffer has a critical influence on DNA elution efficiency and thus overall DNA purification yield. The kit contains T solution which enables testing of the E buffer correct functionality.




**Typically it is suggested to perform such a test in the following cases:**
- E buffer was not used for a long period of time (at least 2 months).
- E buffer was stored at room temp. for a long period of time (at least 2 weeks).
- E buffer vial was not closed tightly.

**Procedure:**
Transfer 20 µl of E buffer to PCR tubes; add 2 µl of T solution; mix the sample, wait 2 min. Compare the mixture color with the reference color guide.

# Gravity flow technology



# Safety Information

WARNING

### L2 lysis solution

H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335 May cause respiratory irritation.
P261 Avoid breathing dust.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P342+P311 If experiencing respiratory symptoms call a Poison Center or doctor/physician.

WARNING

### K1 equilibrating solution

H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

### E elution buffer

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a Poison Center or doctor/physician.

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