A&A BIOTECHNOLOGY
innovating life science

Plasmid Mini AX Gravity

Gravity flow, Increased efficiency kit for high-copy plasmid DNA purification.
version 0517

100 isolations

Cat. # 015-100

gravity flow

The binding capacity of the plasmid DNA purification column is 20 µg of DNA.

For R&D use only.

A&A BIOTECHNOLOGY, Aleja Zwycięstwa 96/98, 81-451 Gdynia
tel: +48 58 6982194, +48 883 323 761, fax: +48 58 6228578
VAT PL5840254403
info@aabiot.com www.aabiot.com

Kit Contents

Component	Quantity	Store at
Micro AXB columns	100 pcs	+4 to +8 °C
Gravity tubes	100 pcs	Room Temp.
L1 suspension solution	35 ml	Room Temp.
L2 lysis solution	35 ml	Room Temp.
L3 neutralizing solution	35 ml	Room Temp.
K1 equilibrating solution	60 ml	Room Temp.
K2P first wash solution	70 ml	Room Temp.
W2 second wash solution	60 ml	Room Temp.
E elution buffer	10 ml	+4 to +8 °C
N neutralizing buffer	1 ml	Room Temp.
T solution	400 µl	+4 to +8 °C

Equipment and materials necessary for DNA isolation that are not included in kit

1. Material for DNA isolation
2. 1.5 ml sterile Eppendorf tubes
3. Clear PCR tubes (option)
4. Benchtop microcentrifuge

NOTE:
Before you start working, we recommend cleaning the work surface using LabZAP™ product (cat. # 040-500)

A&A Biotechnology provides one year guarantee on this kit
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the protocol supplied
- not recommended use of equipment and materials
- the use of other reagents than recommended or which are not a component of the kit
- the use of expired or improperly stored reagents and columns

Isolation protocol

1. Centrifuge 1-3 ml of overnight bacterial culture.
   Discard the supernatant.
   Suspend the bacterial pellets in 300 µl of L1 suspension solution.

   Carefully re-suspend the bacterial pellet to obtain efficient lysis. Thoroughly resuspended bacterial sample in L1 suspension solution will form a nontransparent light pink suspension without any leftovers of bacterial residue, present at the bottom of the tube.

2. Add 300 µl of L2 lysis solution and mix by inverting the tubes.
   Incubate for 3 min at room temp.

   Carefully mix the tube content upon L2 solution addition, to prevent the chromosomal DNA fragmentation. The mixture should change its appearance and colour along the alkaline lysis progress.
   After 3 minutes of incubation the lysate should turn violet and completely transparent. If not the incubation and mixing time should be prolonged for another 3 minutes.

3. Add 300 µl of L3 neutralizing solution and mix by inverting the tubes.

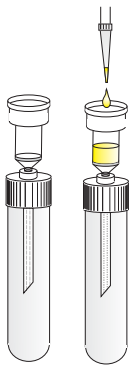
   Mixing will make the sample yellowish. Loss of any traces of violet color indicates completed and successful alkaline lysis.

4. Centrifuge for 10 min at 10 000 RPM.

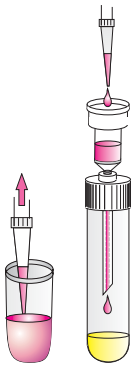
5. During centrifugation prepare the appropriate number of Micro AXB columns. Place each Micro AXB column tip into the fitting on top of the gravity tube cap. Place assembled Micro AXB columns with tubes in a suitable rack.

   Subsequently apply 500 µl of K1 equilibrating solution onto each Micro AXB column.

   The solution should penetrate the column and start dripping down at the bottom of micro-drain by means of gravity. As soon as the solution stops dripping the Micro AXB column is ready for the DNA purification process.



6. Apply the supernatant onto pre-equilibrated Micro AXB columns. Wait until the lysates pass through the columns by gravity. This takes up to 4 min.

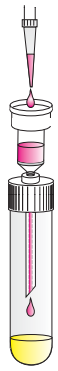


The flow rate strongly depends on DNA concentration in the sample.

As soon as the lysate stops dripping proceed to the next step.

7. Add 700 µl of K2P first wash solution.
Wait until the K2P first wash solution passes through the Micro AXB columns.

8. Add 500 µl of W2 second wash solution.
Wait until the W2 second wash solution passes through the Micro AXB columns.



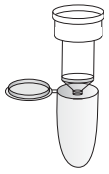
9. Add 40 µl of E elution buffer and wait 2 min.

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

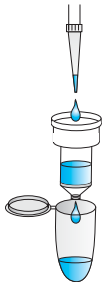
E elution buffer loses activity upon prolonged contact with air. Always close the E elution buffer vial tightly directly after use.

10. Prepare the 1.5 ml elution tubes (not included) and add 2 µl of N neutralizing buffer to the bottom of each tube.

DNA neutralization: See “Additional information” – page 5.



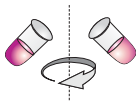
11. Transfer the Micro AXB columns to the prepared elution tubes.



12. Elute the DNA by adding 40 µl of E elution buffer onto the Micro AXB columns.

E elution buffer loses activity upon prolonged contact with air. Always close the E elution buffer vial tightly directly after use.

13. Centrifuge for 30–60 s at 5000 RPM.



14. Remove the Micro AXB columns.
Close the tubes with purified DNA and store at +4 °C to +8 °C.

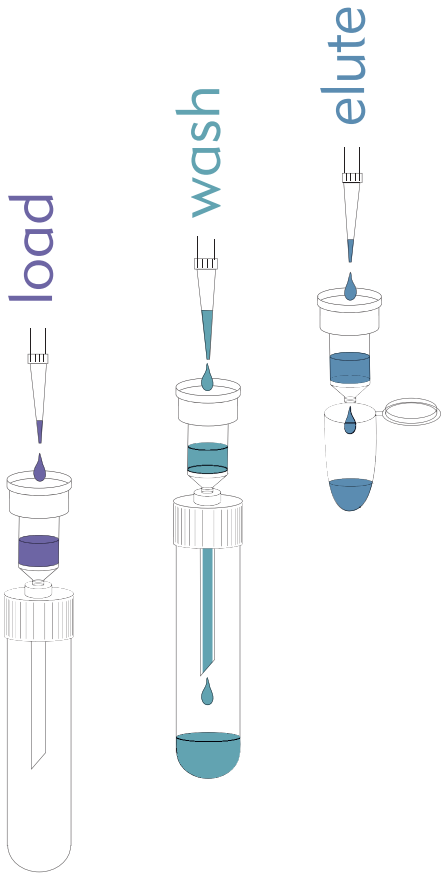
Additional Information

DNA neutralization. The E elution buffer is strongly alkaline and may cause DNA degradation upon freezing. Thus it is necessary to use N neutralizing buffer.
We recommend to add the N neutralizing buffer to the elution tube before the elution step point 10. of isolation protocol).
If the N neutralizing buffer was not added in point 10. of isolation protocol, it can be added directly before freezing DNA samples.
The plasmid DNA prepared in this case can be used directly for applications such as: sequencing reaction, PCR, cloning.
The use of N neutralizing buffer enables secure DNA storage conditions at 10 mM TrisHCl, pH 8.5

Notes

Problem	Reason	Solution
Very slow flow rate of lysate through column.	Highly concentrated DNA in sample.	Place microcolumn in an Eppendorf tube and spin it. At the next isolation reduce the quantity of original sample.
Air bubbles present in the receiving tube capillary.	The gravity microcolumn is not attached tightly to the receiving tube.	Reattach the column in luer-like fitting simultaneous by pressing the column down and twisting.

Gravity flow technology



Products based on Gravity flow technology

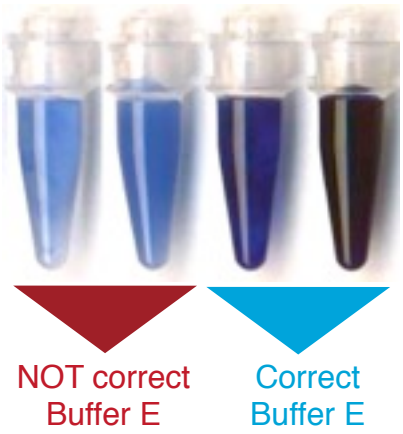
Product	Quantity	Material	Cat. #
Genomic Micro AX Blood Gravity	100 isolations	Blood	101-100
Genomic Micro AX Swab Gravity	100 isolations	Swabs	105-100
Genomic Micro AX Swab Gravity Plus	100 isolations + swab tools	Swabs	105-100P
Genomic Micro AX Bacteria Gravity	100 isolations	Bacteria	102-100
Genomic Micro AX Bacteria+ Gravity	100 isolations	G+ Bacteria	102-100M
Genomic Micro AX Tissue Gravity	100 isolations	Tissue	104-100
Genomic Micro AX Plant Gravity	100 isolations	Plant	103-100
Bead-Beat Micro AX Gravity	20 isolations	Difficult samples	106-20
	100 isolations	Difficult samples	106-100

Buffer E functionality test

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

Typically it is suggested to perform such a test in the following cases:

- the buffer E was not used for a long period of time (at least 2 months)
- the buffer E vial was stored at room temperature for a long period of time (at least 2 weeks)
- the buffer E vial was not closed tightly



Testing the elution buffer E functionality procedure

1. Transfer 20 µl of elution buffer E to clear 200 µl PCR tube
2. Add 2 µl of solution T and mix the sample
3. Wait 2 min and compare the mixture colour with the reference colour guide

Safety information

DANGER

L3 neutralizing solution
H315 Causes skin irritation.
H318 Causes serious eye damage.
H335 May cause respiratory irritation.
P261 Avoid breathing dust.
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.

DANGER

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 (Xn, Harmful, R:22-36/38, S:26-37)
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.

WARNING

K2P first wash 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.