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Manual

Bead-Beat Micro AX Gravity

Versatile, increased efficiency gravity flow kit for genomic DNA purification from various sources. Procedure with mechanical lysis.

catalog #	size
106-20	20 isolations
106-100	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

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component	20 isolations	100 isolations	storage
Micro AXD columns	20 pcs	100 pcs	2–8 °C
Gravity tubes	20 pcs	100 pcs	15–25 °C
Bead-beat tubes (zirconia / silica beads)	20 pcs	100 pcs	15–25 °C
LSU lysis buffer	24 ml	120 ml	15–25 °C
K1G equilibrating solution	12 ml	55 ml	15–25 °C
W1G first wash solution	14 ml	70 ml	15–25 °C
W2 second wash solution	12 ml	60 ml	15–25 °C
E elution buffer (without EDTA)	5 ml	20 ml	2–8 °C
N neutralizing buffer	500 µl	1 ml	15–25 °C
T solution	100 µl	400 µl	2–8 °C
Proteinase K	600 µl	2 x 1.1 ml	4–8 °C

The binding capacity of the column is 20 µg.

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- Beadbeater (BioSpec, MP Biomedicals)
- Incubator or thermoblock set to 50 °C
- Centrifuge, vortex

Optional

- DTT (cat. # 2010-5, 2010-25, 2010-10P) (for DNA isolation from yeast)
- Sterile water (cat. # 003-075, 003-25)
- RNAse (cat. # 1006-10, 1006-50)
- Gravity flow rack (cat. # 008-1)

Important information

- 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.

Isolation protocol

1. Material samples:
   - **Bacterial, mold, yeast liquid culture:** centrifuge **1-2 ml** of samples for **5 min** at **10 000-12 000 RPM** and discard supernatant.
   - **Bacterial, mold, yeast solid culture:** up to **100 mg**
   - **Plant fragments:** up to **100 mg**
   - **Tissue samples:** up to **20 mg**
   - **Biological environmental samples:** up to **200 mg**
   - **Other biological samples:** up to **50 mg**

2. Add **1 ml** of **LSU** lysis buffer and **20 µl** of **proteinase K**.

   For DNA isolation from yeast it is recommended to add 10 µl of 1M DTT solution (not included).

3. Transfer the mixture to a Bead-beat tube containing zirconia/silica beads.
   Transfer the sample tube to Beadbeater and run it for **30-60 s** at **maximum power**.

4. Incubate for **15-30 min** at **50 °C**. Vortex the sample a few times.

   The incubation step can be performed in Eppendorf Thermomixer or analogous equipment at 1400 RPM and 50 °C.

   RNA digestion (optional): add 5 µl of RNAse (10 mg/ml solution) (not included). Mix and incubate for 5 min at room temp.

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

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

6. Apply **500 µl** of **K1G** equilibrating solution onto the **Micro AXD** column.
   Wait for the solution to flow through the column.

   It is a good practice to apply the K1G solution to the column wall to avoid accidental blockage of the column flow by an air bubble between the membrane and the K1G solution.

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

7. After incubation, centrifuge the sample for **5 min** at **12 000 RPM**.

8. Collect the clarified supernatant and apply it onto the equilibrated Micro AXD column.
Wait **10 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 6.

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 10 min, instead of observing the process in individual columns.

9. Apply **600 µl** of **W1G** first wash solution onto the **Micro AXD** column.
Wait for the solution to flow through the column.

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

11. Before using E buffer, it is recommended to do a functionality test - page 6.

Apply **60 µl** of **E** elution buffer onto the **Micro AXD** column.
Keep for **5 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 60 µl.

12. Prepare the 1.5 ml elution tubes (not included).
Apply **5 µl** of **N** neutralizing buffer onto the bottom of each tube.

DNA neutralization - page 6.

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

14. Before using E buffer, it is recommended to do a functionality test - page 6.

Apply **120 µl** of **E** elution buffer onto the **Micro AXD** column.
Wait **10 min** for the buffer to flow through the column by gravity.

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.

After 10 min check that the buffer has passed through the column. If not, this indicates a very large amount of DNA in the sample. In this case, it is recommended to centrifuge the sample (column within tube) for 30-60 s at 5000 RPM.

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

Lysate flow rate troubleshooting

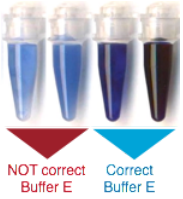
problem	reason	solution
very slow rate of lysate through the Micro AXD column	highly concentrated DNA in the sample	- place the Micro AXD column into an Eppendorf tube and centrifuge. - for the next isolation, reduce the amount of sample by half.
air bubbles in the receiving tube capillary	the Micro AXD column is not not securely attached to the receiving tube	- “tightening” the Micro AXD column. - 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

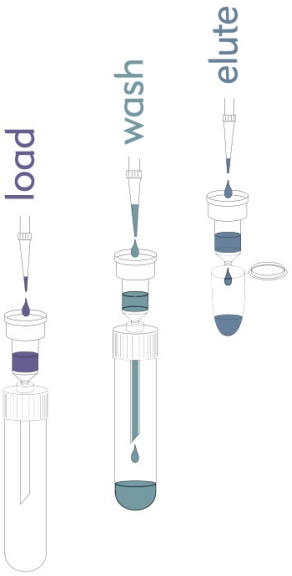
E buffer has a critical influence on DNA elution efficiency and thus overall DNA purification yield. The kit contains T solution which enables testing of the E buffer correct functionality.



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

 DANGER	**Proteinase K** 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	**LSU lysis buffer** 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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