A&A BIOTECHNOLOGY
innovating life science

*Manual*

# Genomic Micro AX Blood Gravity 96-well

Gravity flow kit for genomic DNA purification from blood. Form: 96-well plates.

| catalog # | size |
|---|---|
| 101-192 | 192 isolations |
| 101-960 | 960 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

**Contents** 3

**Additional equipment and reagents** 3

- Necessary 3
- Optional 3

**Important information** 4

**Lysing-enzyme mixture preparation** 4

**Isolation protocol** 4

- DNA neutralization 6
- E buffer functionality test 6

**Safety information** 7

# Contents

| component | 192 isolations | 960 isolations | storage |
|---|---|---|---|
| **P96** purification plate | 2 pcs | 10 pcs | 4-8 °C |
| **R96** receiving plate | 6 pcs | 30 pcs | room temp. |
| **L96** lysis plate | 2 pcs | 10 pcs | room temp. |
| **E96** elution plate | 2 pcs | 10 pcs | room temp. |
| **Protective film** | 2 pcs | 10 pcs | room temp. |
| **Aluminum foil** | 2 pcs | 10 pcs | room temp. |
| **LSU** lysis buffer | 100 ml | 2 x 250 ml | room temp. |
| **K1G** equilibrating solution | 50 ml | 250 ml | room temp. |
| **W1G** first wash solution | 100 ml | 2 x 250 ml | room temp. |
| **W2** second wash solution | 120 ml | 2 x 250 ml | room temp. |
| **E** lution buffer (does not contain EDTA) | 40 ml | 5 x 40 ml | 4-8 °C |
| **N** neutralizing buffer | 1 x 1 ml | 5 x 1 ml | room temp. |
| **T** solution | 400 µl | 2 x 400 µl | 4-8 °C |
| **Proteinase K** | 5 x 1.1 ml | 5 x 4.5 ml | 4-8 °C |

# Additional equipment and reagents

## Necessary

- Sterile bottles, beakers or suitable vessels with a minimum capacity of 100 ml
- Incubator or thermoblock set to 50 °C
- Centrifuge with swing-out rotor for 96-well plates (8 cm high), e.g. Sigma 4-K16 with rotor #09100/09366

## Optional

- Vortex for 96-well plates

# 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 4-8 °C.

# Lysing-enzyme mixture preparation

Lysing-enzyme mixture prepare **in 20:1 proportion for each sample**, i.e.:
**400 µl** of **LSU** lysis buffer and **20 µl** of **proteinase K**.

**Note:**
Lysing-enzyme mixture should be prepared with 10-15% excess so that there is no shortage when adding in step 2. of the isolation protocol. **Prepare approximately 46 ml of lysing-enzyme mixture per plate.**

# Isolation protocol

1. Transfer **20-100 µl blood sample** to **L96** lysis plate.

2. Add **420 µl** of **lysing-enzyme mixture** (LSU lysis buffer + proteinase K).

3. Mix by pipetting (10 times).

4. Stick **the aluminum foil** on the **L96** lysis plate.
Incubate for **10 min** at **50 °C**. Vortex the samples a few times.

   The incubation step can be performed in Eppendorf Thermomixer or analogous equipment at 1400 RPM and 50 °C in order to eliminate water condensation on the aluminum foil.

5. During incubation prepare the plates, which will be used later in the isolation protocol:

   - Remove the **lid** from the **R96** receiving plate.
     The lid protects the sorption cartridges in the wells.
   - Remove the **protective film** from top and bottom of **P96** purification plate.
   - Assemble the **P96** purification plate with the **R96** receiving plate. Plates should be gently assembled, without pressing.

6. Apply **200 µl** of **K1G** equilibrating solution onto wells of the **P96** purification plate.
Wait **5 min** for the solution to flow to the R96 receiving plate.

   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. This does not apply to automatic applications.

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

7. After incubation, transfer the **L96** lysis plate to the swing-out rotor.

**Note:** If an odd number of plates use the counter-plate for centrifugation.

Centrifuge for **1 min** at **500 x g**.

8. Remove the **aluminum foil** from the **L96** lysis plate. Mix by pipetting (10 times).

This is the key step for efficiency of DNA isolation.

9. Apply **500 µl** of **lysate** from the **L96** lysis plate onto the well of the **P96** purification plate.
Wait **10 min** for the lysate to flow to the **R96** receiving plate 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.

10. Carefully separate the plates. Remove the **R96** receiving plate.
Assemble the **P96** purification plate with **a new R96** receiving plate (after removing the lid from it).

This step can be performed by a gripper - a transfer head in a liquid handler.

11. Apply **400 µl** of **W1G** first wash solution onto the well of the **P96** purification plate.
Wait **10 min** for the lysate to flow to the **R96** receiving plate by gravity.

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

12. Carefully separate the plates. Remove the **R96** receiving plate.
Assemble the **P96** purification plate with **a new R96** receiving plate (after removing the lid from it).

This step can be performed by a gripper - a transfer head in a liquid handler.

13. Apply **500 µl** of **W2** second wash solution onto the well of the **P96** purification plate.
Wait **10 min** for the lysate to flow to the **R96** receiving plate by gravity.

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

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

Apply **25 µl** of **E** elution buffer onto the well of the **P96** purification plate.
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 4-8 °C.

The purpose of this step is to decrease the total volume of eluate,

15. Prepare the **E96** elution plate. Apply **5 µl** of **N** neutralizing buffer onto the well of the **E96** elution plate.

DNA neutralization - page 6.

16. Carefully separate the plates. Remove the **R96** receiving plate.
Assemble the **P96** purification plate with **a E96** elution plate.

This step can be performed by a gripper - a transfer head in a liquid handler.

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

Apply **150 µl** of **E** elution buffer onto the well of the **P96** purification plate.
Wait **10 min** for the buffer to flow to the **E96** elution plate 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 4-8 °C.

After 10 min check that the buffer has passed into the E96 elution plate. If not, this indicates a very large amount of DNA in the sample. In this case, it is recommended to centrifuge the sample.

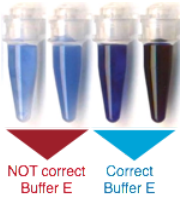
18. Carefully separate the plates. Remove the **P96** purification plate.
Stick the **new protective foil** on the **E96** elution plate. Store the purified DNA at 4 °C until later use.

## 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.

# 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.

A&A BIOTECHNOLOGY
innovating life science

A&A Biotechnology, ul. Strzelca 40, 80-299 Gdańsk, Poland
phone +48 883 323 761, +48 600 776 268
info@aabiot.com, www.aabiot.com