A&A BIOTECHNOLOGY
innovating life science

Genomic Micro AX Blood Gravity 96-well

Gravity flow kit for genomic DNA purification from blood. Form: 96-well plates. Sample size: up to 100 µl of fresh or frozen blood.

192 isolations
cat. # 101-192

960 isolations
cat. # 101-960

Table of Contents

Kit contents

element	quantity		store at
	cat # 101-192	cat # 101-960	
P96 purification plates	2 pcs	10 pcs	+4 to +8 °C
R96 receiving plates	6 pcs	30 pcs	room temp.
L96 lysis plates	2 pcs	10 pcs	room temp.
E96 elution plates	2 pcs	10 pcs	room temp.
self-adhesive aluminium foil	2 pcs	10 pcs	room temp.
self-adhesive transparent 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 elution buffer	40 ml	5 x 40 ml	+4 to +8 °C
N neutralizing buffer	2 x 1000 µl	3 x 1000 µl	room temp.
T solution	400 µl	2 x 400 µl	room temp.
Proteinase K	5 x 1100 µl	5 x 4.5 ml	+4 to +8 °C

Necessary equipment and materials not included in kit

- material for DNA isolation
- Incubator working at 50 °C (chamber incubator is recommended)
- centrifuge (optionally)

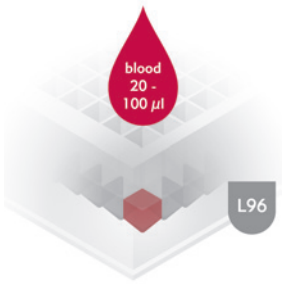
Notes

E elution buffer

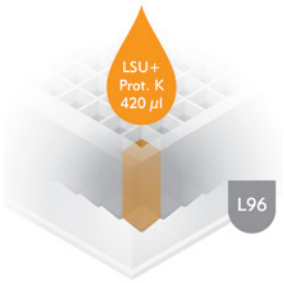
E elution buffer loses activity by prolonged contact with air. Always close the E elution buffer container tightly directly after use.

Isolation Protocol

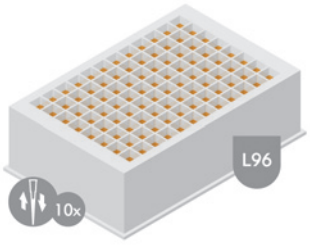
1. Transfer **20-100 µl** of each blood sample to **L96** lysis plate.



2. Add **420 µl** of **LSU** lysis buffer and **Proteinase K** mixture. Mixture should be prepared in 20:1 ratio (20 parts of LSU and 1 part of Proteinase K)
   (!) Mixture should be prepared with 10 – 15 % excess. 46 ml of mixture should be enough to process whole 96-well plate.

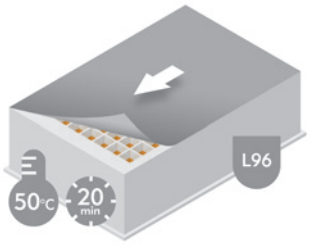


3. Mix **10 times** by pipetting



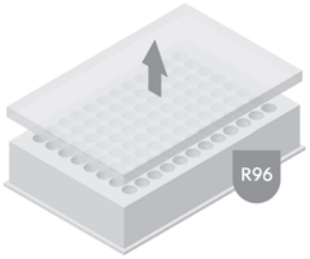
4. Seal **L96** lysis plates with self-adhesive aluminium foil. Incubate for **10 min** at **50 °C**. Do not prolong the incubation time.

   (!) We recommend using chamber incubator to eliminate condensation on the self-adhesive aluminium foil.

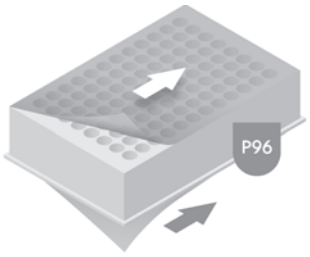


5. During incubation, prepare **P96** purification plates:

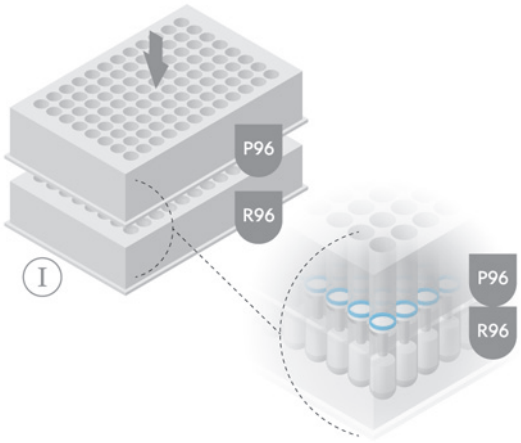
- remove lid from **R96** receiving plate



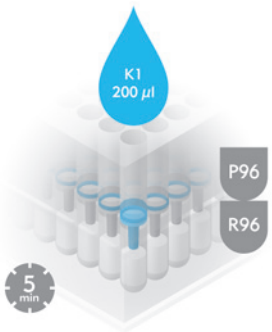
- remove transparent foil from the top and bottom of **P96** purification plate



- Gently place **P96** purification plate on **R96** receiving plate. Avoid applying pressure.

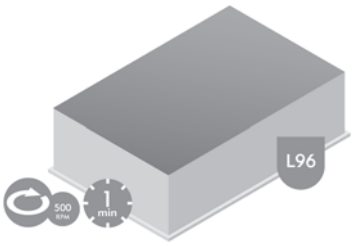


- apply **200 µl** of **K1G** equilibrationg solution onto **P96** purification plate. Wait for **5 min.** until solution flows through.



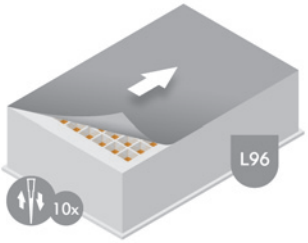
6. After incubation, centrifuge **L96** lysis plate for **1 min** at **500 x g**.

   (!) See "Sample condensation" in "Additional information" chapter



7. Gently remove self-adhesive aluminium foil from **L96** lysis plate. Mix samples by 10-times pipetting.

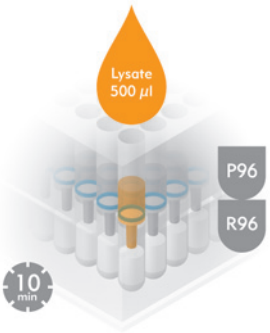
   Thorough mixing is crucial for DNA isolation yield.



8. Apply **500 µl** of **lysates** onto pre-equilibrated **P96** purification plates. Wait up to **10 min** until lysate flows through.

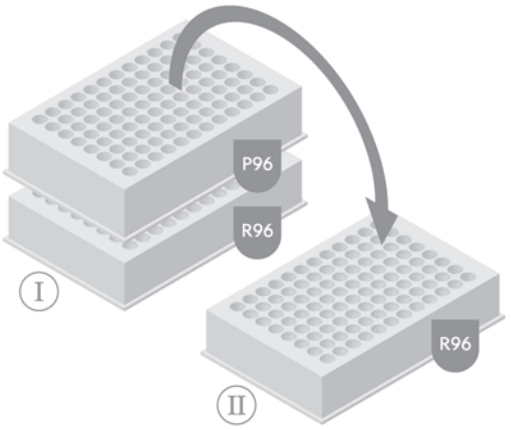
   Flow rate depends on DNA concentration in the sample. In general the higher DNA concentration in the lysate, thle slower flow rate.

   After 10 min inspect the plate and ensure all the lysates passed through P96 purification plate completely.



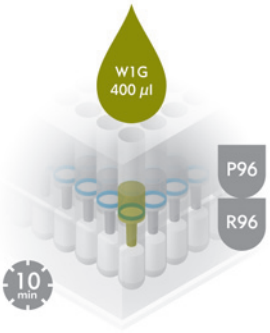
9. Transfer **P96** purification plate to new **R96** receiving plate.

   This step can be done by the gripper of a liquid handler.



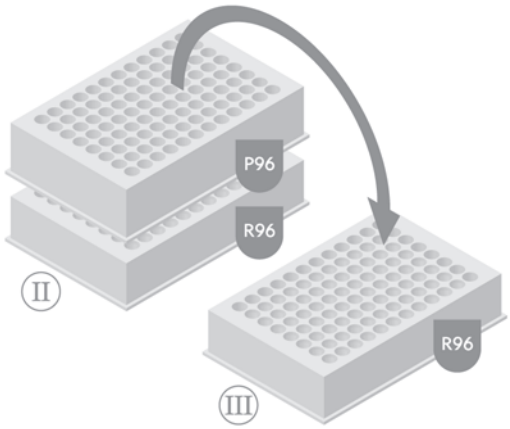
10. Add **400 µl** of **W1G** first wash solution. Wait for **10 min** until solution flows through.

    After 10 min inspect the plate and ensure all the solution passed through P96 purification plate completely.



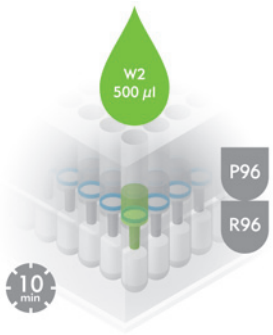
11. Transfer **P96** purification plate to new **R96** receiving plate.

    This step can be done by the gripper of a liquid handler.

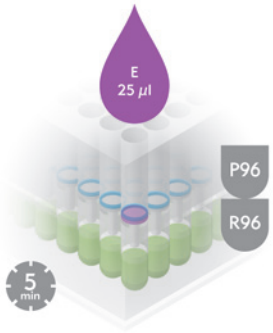


12. Add **500 µl** of **W2** second wash solution. Wait for **10 min** until solution flows through.

    After 10 min inspect the plate and ensure all the solution passed through P96 purification plate completely.

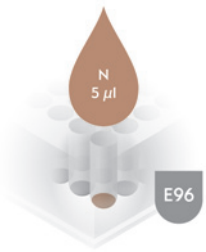


13. Add **25 µl** of **E** elution buffer. Wait for **5 min**.



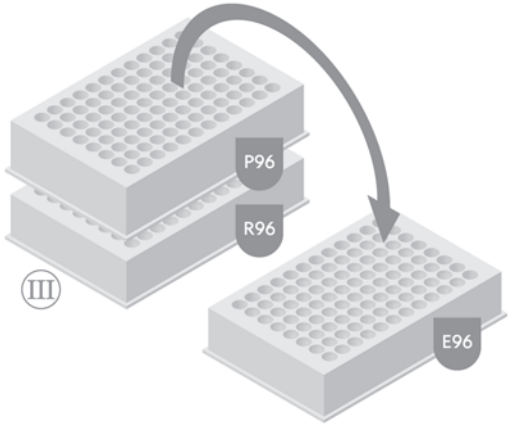
14. Prepare **E96** elution plate. Add **5 µl** of **N** neutralizing buffer.

    (!) This step can be skipped if You are not planing to freeze DNA samples after isolation. See "DNA sample neutralization" in "Additional information" chapter.



15. Transfer **P96** purification plate to **E96** elution plate.

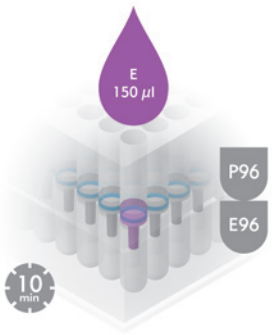
    This step can be done by the gripper of a liquid handler.



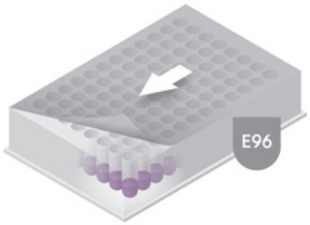
16. Elute DNA by adding **150 µl** of **E** elution buffer onto **P96** purification plate. Wait for **10 min** until solution flows through.

    After 10 min inspect the plate and ensure all the buffer passed through P96 purification plate completely.

    If buffer did not passed through after 10 min see "DNA elution troubleshooting" in "Additional information" chapter.



17. Discard **P96** purification plate. Seal the **E96** elution plate containing isolated DNA with self-adhesive transparent foil.



Additional information

Sample condensation

We recommend short centrifugation of L96 lysis plate after incubation to remove sample condensation from self-sdhesive aluminium foil. In case of chamber incubator, condesation does not occur and centrifugation can be skipped.

E elution buffer functionality test

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

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

- E elution buffer was not used for longer than 2 months.
- E elution buffer container was stored at room temperature for longer than 2 weeks.
- E elution buffer container was not closed tightly.

Testing E elution buffer functionality procedure:

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

E buffer functionality is **correct**

E buffer functionality is **incorrect**

DNA sample 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 elution plate before the elution step (point 14. of isolation protocol). If the N neutralizing buffer was not added at point 14. of isolation protocol, it can be added directly before freezing DNA samples. The use of N neutralizing buffer enables secure DNA storage in freezer.

DNA elutionTroubleshooting

In case the elution buffer did not passed trough P96 purification plate completely after 10 minuntes short centrifugation is required. Transfer the assembled P96 i E96 plates to a swing-out plate rotor capable of acommodation of two plates assembly with total height of minimum 5,8 cm (i. e. Sigma 3K-15 centrifuge with a rotor 11240 with 13145 plate adaptors). Centrifuge for 1 min at 2 000 x g. **While processing odd number of plates always use the counter-weight plate.**

Guarantee

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

- not following the supplied protocol
- the use of equipment and materials other than recommended
- the use of reagents other than recommended or which are not a part of this kit
- the use of expired or improperly stored reagents and plates

Safety information

Proteinase K	 DANGER	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.
LSU lysis buffer	 WARNING	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.
K1G equilibrating solution	 WARNING	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.
E elution buffer	 DANGER	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.