A&A BIOTECHNOLOGY
innovating life science

Manual

Blood Mini Plus

Increased efficiency kit for genomic DNA purification from blood samples.
Sample size: up to 1000 µl of fresh or frozen blood.

catalog #	size
022-50PN	50 isolations
022-250PN	250 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

	022-50PN		022-250PN		
component	**quantity**	**cat #**	**quantity**	**cat #**	**storage**
minicolumns	50 pcs	K-K01-50	250 pcs	K-K01-250	15–25 °C
2 ml tubes	100 pcs	K-PGR-100	500 pcs	K-PGR-500	15–25 °C
RA activation solution	22 ml	K-RA-22	110 ml	K-RA-110	15–25 °C
RW binding solution	10 ml	K-RW-10	42 ml	K-RW-42	15–25 °C
LE erythrocyte lysis solution	30 ml	K-LE-30	140 ml	K-LE-140	15–25 °C
BL lysis buffer	12 ml	K-BL-12	55 ml	K-BL-55	15–25 °C
W10 wash solution	28 ml	K-W10-28	140 ml	K-W10-140	15–25 °C
W11 wash solution	50 ml	K-W11-50	250 ml	K-W11-250	15–25 °C
Tris elution buffer (10 mM, pH 8,5)	30 ml	K-TRIS-30	110 ml	K-TRIS-110	15–25 °C
Proteinase K	1.1 ml	K-PRK-11A	5 x 1.1 ml	K-PRK-11A	2-8 °C*

* Proteinase K can be stored at 15-25 °C for up to 12 months.

Additional equipment and reagents

Necessary

- 1.5 ml, 2 ml sterile Eppendorf tubes
- Incubator or thermoblock
- Vortex
- Microcentrifuge

Column preparation

Before starting the isolation procedure, it is important to activate the columns.

1. Add **400 µl** of **RA** activation solution directly onto the minicolumn.
2. Incubate for **5 min** at **room temp**.
3. Centrifuge for **2 min** at **10 000-15 000 RPM**.
4. Discard the filtrates.
5. Place the minicolumns into **the same** tubes.

Material preparation

Blood (fresh or frozen, up to 200 µl)

1. Transfer **200 µl** of **blood** to a 1.5 ml Eppendorf tube (not included).

   Note. For blood volume less than 200 µl, add Tris buffer to a total volume of 200 µl.
2. Follow point 1. of the isolation protocol.

Blood (fresh or frozen, 200 µl-1 ml)

1. Transfer the appropriate amount of **blood** to a 2 ml tube (not included).
   Add **half** of **the volume** of **LE** solution, e.g. add **250 µl** of **LE** solution to 500 µl of blood.
2. Mix by inverting the tube until solution becomes completely transparent.

   Note. The color change occurs in about 3 minutes.
3. Centrifuge for **3 min** at **10 000-15 000 RPM**.
4. Discard supernatant. Add **200 µl** of **Tris** buffer to the pellet (leukocyte cells) and resuspend cells by pipetting.
5. Follow point 1. of the isolation protocol.

For a larger volume of blood samples we recommend Genomic Midi AX (cat. # 895-20) or Genomic Maxi AX (cat. # 995-10).

Isolation protocol

1. Add **200 µl** of **BL** lysis buffer and **20 µl** of **Proteinase K**.
2. Vortex the sample for **10 s** and incubate for **10 min** at **50 °C** with shaking.

   Note. If an automatic continuous shaking is not available, mix the samples by inverting the tubes a few times.
3. Add **150 µl** of **RW** binding buffer.
4. Vortex the samples intensively for **10 s**, briefly centrifuge to remove the material remaining on lids of the tubes. Apply samples onto the activated minicolumns.
5. Centrifuge for **1 min** at **10 000 RPM**.

   Attention. If the lysate does not flow through the column, additional centrifugation for 1 min at maximum speed is recommended.
6. Remove the minicolumns from the tubes. Discard the filtrates.
   Transfer the minicolumns to **new** 2 ml tubes (included).
7. Add **500 µl** of **W10** wash solution.
8. Centrifuge for **1 min** at **10 000 RPM**.
9. Remove the minicolumns from the tubes. Discard the filtrates.
   Transfer the minicolumns to **new** 2 ml tubes (included).
10. Add **500 µl** of **W11** wash solution. Mix by inverting the tubes a few times.

    Note. Mixing is intended to remove residual wash buffer from the inner walls of the column.
11. Centrifuge for **1 min** at **10 000 RPM**.
12. Remove the minicolumns from the tubes. Discard the filtrates.
    Dry the rims of the tubes from any leftover wash solution. Turn the tube upside down and gently touch it against a paper towel.
    Transfer the minicolumns into t**he same** tubes.

13. Add **400 µl** of **W11** wash solution.

14. Centrifuge for **1 min** at **10 000-15 000 RPM**.

15. Transfer the minicolumns to **new** 1.5 ml tubes (not included).

16. Add **100 µl** or **200 µl** of **Tris** buffer.

    Note. For 200 µl blood volume add 100 µl of Tris buffer. For larger volume add 200 µl of Tris buffer.

17. Incubate for **2 min** at **room temp.**

18. Centrifuge for **1 min** at **10 000-15 000 RPM**.

19. Remove the minicolumns and store the tubes with purified DNA at **4 °C or -20 °C** until further use.

Additional information

The final DNA eluate may contain trace amounts of particles from the column membrane. The particles do not affect the quality of the isolated DNA. However, they may be of significance for spectrophotometric readings (ODR 230/260). Before spectrophotometric analysis, it is recommended to centrifuge the eluate for 1 min at maximum speed and take a sample from the top layer of the centrifuged solution.

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

BL 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

RW binding buffer

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
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

W10 wash solution

H225 Highly flammable liquid and vapor.
H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
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

W11 wash solution

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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