A&A BIOTECHNOLOGY
innovating life science

Manual

Cell-free AX DNA Mini

Kit for circulating free DNA purification from plasma. Procedure with DNA precipitation.
Sample size: 100 - 500 µl of plasma.

catalog #	size
063-25	25 isolations
063-100	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

Advantages

- High-purity cfDNA for downstream applications, minimization of cellular DNA contamination.
- Fast and simple isolation procedure.
- Ability to suspend isolated DNA in any volume of sterile water or buffer.

Specification

form	minicolumn
binding capacity	10 µg DNA
sample size	100 - 500 µl
elution volume	precipitation
downstream applications	sequencing, qPCR

Description

Cell-free AX Kit is dedicated for isolation of cell free DNA from serum/plasma, including cell free fetal DNA (cffDNA) from maternal serum/plasma and circulating tumor DNA (ctDNA). Due to high quality of isolated DNA it can be used for a wide spectrum of applications, e.g. PCR, real-time PCR, next-generation sequencing. Analysis of certain cfDNA sequences enables non-invasive prenatal testing, detection and monitoring of cancer or autoimmune diseases.

Contents

	25 isolations		**100 isolations**		
component	**quantity**	**catalog #**	**quantity**	**catalog #**	**storage**
Spin 10AX columns	25 pcs	K-0006-25	100 pcs	K-0006-100	2-8 °C
2 ml tubes	25 pcs	K-PGR-25	100 pcs	K-PGR-100	15-25 °C
2 ml precipitation tubes	25 pcs	K-PRP-25	100 pcs	K-PRP-100	15-25 °C
L1.4 lysis solution	15 ml	K-L14-15	55 ml	K-L14-55	15-25 °C
K2CF wash solution	35 ml	K-K2CF-35	135 ml	K-K2CF-135	15-25 °C
K3 elution solution	12 ml	K-K3-12	45 ml	K-K3-45	15-25 °C
PM precipitation mix	10 ml	K-PM-10	40 ml	K-PM-40	15-25 °C
Tris buffer (10 mM Tris-HCl, pH 8.5)	2 ml	K-TRIS-2	5 ml	K-TRIS-5	15-25 °C
Proteinase K	600 µl	K-PRK-600B	2 x 1,1 ml	K-PRK-11A	2-8 °C

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- 70% ethanol
- vortex
- microcentrifuge
- incubator or thermoblock set to 50 °C
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Optional

- TE buffer (cat. # 297-100)
- sterile water (cat. #. 003-075, 003-25)

References

1. Orzińska A., Purchla-Szepioła S., Krzemienowska M., Stefanska-Kazmierczak A., Dąbrowski S., Dębska M., Kopeć I., Uhrynowska M., Guz K. *Comparison of non-invasive prenatal testing of a fetal antigen genotype using different isolation methods*, Vox Sanguinis International Journal of Blood Transfusion Medicine, 2020; 115:s.295
2. Jarmuzek P, Wawrzyniak-Gramacka E, Morawin B, Tylutka A, Zembron-Lacny A. *Diagnostic and Prognostic Value of Circulating DNA Fragments in Glioblastoma Multiforme Patients.* Int J Mol Sci. 2024 Apr 11;25(8):4221. doi: 10.3390/ijms25084221

Material preparation (plasma fresh or frozen)

1. Transfer **100 - 500 µl** of plasma to a 1.5 ml tube (not included).
2. Add **an equal volume** of **L1.4** lysis solution and **20 µl** of **proteinase K**.
3. Vortex for **20 s**.
4. Incubate for **20 min** at **50 °C**. Vortex the sample from time to time.
5. Follow point 1. of the isolation protocol.

Isolation protocol

1. **Attention.** The maximum volume of sample applied to the column at one time must not exceed **700 µl**.

   Apply the samples onto the **Spin 10AX** columns.
2. Centrifuge for **1 min** at **8000 RPM (6000 x g)**. Discard the filtrates.
3. If the initial sample volume exceeded **700 µl,** apply the remaining samples onto the **Spin 10AX** columns and centrifuge for **1 min** at **8000 RPM (6000 x g)** again. Discard the filtrates.
4. Place the Spin 10AX columns into **new 2 ml tubes** (K-PGR-25 included).
5. Add **600 µl** of **K2CF** wash solution.
6. Centrifuge for **1 min** at **8000 RPM (6000 x g)**.
7. Discard the filtrates. Place the Spin 10AX columns into **the same** tubes.
8. Add **600 µl** of **K2CF** wash solution.
9. Centrifuge for **1 min** at **8000 RPM (6000 x g)**.
10. Discard the filtrates. Place the Spin 10AX columns into **2 ml precipitation tubes** (K-PRP-25 included).

11. Add **200 µl** of **K3** elution solution.

12. Keep the samples for **2 min** at **room temp.**

13. Centrifuge for **1 min** at **8000 RPM (6000 x g)**.

14. Add **200 µl** of **K3** elution solution.

15. Keep the samples for **1 min** at **room temp.**

16. Centrifuge for **1 min** at **8000 RPM (6000 x g)**.

17. Remove the Spin 10AX columns.

18. **Attention.** PM precipitation mix contains a precipitation enhancer and it should be intensively mixed before use by vigorous hand shaking.

    Add **320 µl** of PM precipitation mix to the eluted DNA.

19. Mix the samples by inverting the tubes a few times and centrifuge for **10 min** at **10 000 RPM (9000 x g)**.

20. Carefully discard supernatant. Be careful not to remove the DNA pellet at the bottom of the tube.

    Attention. When pouring out the supernatant, it is very easy to lose the DNA pellet. For safety, it is recommended to pour the supernatant into the prepared tube so the pellet can be recovered.

21. Add **500 µl** of **70% ethanol** (not included).

22. Mix the sample and centrifuge for **5 min** at **10 000 RPM (9000 x g)**.

23. Carefully discard supernatant. Air dry the DNA pellet for **10 min** at **room temp**.

    Note. If there are any leftovers (small droplets) of alcohol on the tube walls they should be removed with sterile cotton buds.

24. Dried DNA pellets can be dissolved in the desired volume of **Tris** buffer, **TE** buffer or **sterile water** (not included).

    Note. The blue color of DNA precipitate enables visual confirmation of the DNA dissolution process.

25. Store the DNA at -20 °C until later use.

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

L1.4 lysis 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

K2CF 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.

DANGER

K3 elution 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.

DANGER

PM precipitation mix

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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