A&A BIOTECHNOLOGY
innovating life science

Manual

Cell-free AX DNA

Kit for circulating free DNA purification from plasma.
Sample size: 4 ml of plasma.

catalog #	size
054-50	50 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

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Advantages

- High-purity cfDNA for downstream applications, elimination of cellular DNA contamination.
- Fast and simple isolation procedure.
- Maximum sample volume - 4 ml.
- Ability to suspend isolated DNA in any volume of sterile water or buffer.

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

	50 isolations		
component	**quantity**	**catalog #**	**storage**
Genomic Mini AX columns	50 pcs	K-AX01-50	4-8 °C
2 ml tubes	50 pcs	K-PRP-50	room temp.
L1.4 lysis solution	220 ml	K-L14-220	room temp.
K1 equilibrating solution	55 ml	K-K1-55	room temp.
K2CF wash solution	220 ml	K-K2CF-220	room temp.
K3 elution solution	60 ml	K-K3-60	room temp.
PM precipitation mix	45 ml	K-PM-45	room temp.
Tris buffer (10 mM Tris-HCl, pH 8.5)	6 ml	K-TRIS-6	room temp.
Proteinase K	6 ml	K-PRK-6	4-8 °C

Additional equipment and reagents

Necessary

- 15 ml Falcon tubes
- 2 ml Eppendorf tubes
- 70% ethanol
- Incubator or thermoblock set to 50 °C
- Vortex
- Microcentrifuge

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

Important notes

If at any step of the isolation the flow of solution through the column is stopped, the column should be centrifuged in a tilting rotor for **2 min** at **2 000 RPM**.

Isolation protocol

1. Transfer **4 ml** of plasma to a 15 ml Falcon tube (not included).
2. Add **4 ml** lysis solution **L1.4** and **80 µl proteinase K.**
3. Mix the sample by inverting the tube and incubate for **20 min** at **50 °C** with mixing.

   Attention. If automatic mixing is not available, mix the samples by inverting the tubes a few times.
4. During incubation prepare the Genomic Mini AX columns placed inside 15 ml Falcon tubes.
   Apply **1 ml** of K1 equilibrating solution. Wait for the solution to flow through the column.
5. Apply **4 ml** of the sample onto the equilibrated Genomic Mini AX column.
   Wait for the solution to flow through the column.
6. Discard the solution from a 15 ml Falcon tube and transfer again the Genomic Mini AX column to the same 15 ml tube.
7. Add the rest of the sample onto the Genomic Mini AX column.
   Wait for the solution to flow through the column.
8. Discard the solution from a 15 ml Falcon tube and transfer again the Genomic Mini AX column to the same 15 ml tube.
9. Add 4 ml of K2CF wash solution.
   Wait for the solution to flow through the column.
10. Add **100 µl** of **K3** elution solution.
    Wait for the eluate to flow through the column.
11. Transfer the Genomic Mini AX column to a **2 ml** precipitation tube (included).

    Note. The column drop director possesses proper fitting that allows easy attachment to the precipitation tube.
12. Add **1 ml** of **K3** elution solution. Wait for the eluate to flow through the column.

Remove the Genomic Mini AX column.

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

Add **800 µl** of PM precipitation mix to the eluted DNA.

14. Mix the sample by inverting the tube a few times and centrifuge for **10 min** at **10 000 RPM.**

Information. The light-blue DNA pellet should be visible at the bottom of the precipitation tube.

15. 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.

16. Add **500 µl** of **70% ethanol** (not included).
Mix the sample and centrifuge for **3 min** at **10 000 RPM.**

Note. The light-blue DNA pellet should be visible at the bottom of the precipitation tube.

17. 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.

18. Air dry the DNA pellet for **5 min** at **room temp.** upsite- down.

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

19. 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.

20. 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.

WARNING

K1 equilibrating 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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