VAHTS Serum/Plasma Circulating DNA Kit

N902

Version 23.1

Product Description

VAHTS Serum/Plasma Circulating DNA Kit is suitable for purification of high-quality circulating cell-free DNA (cfDNA) from 200 µl - 2 ml of cell-free serum, plasma and other body fluid samples, based on superparamagnetism magnetic particle purification technology. This kit is optimized for low molecular weight nucleic acid, and has the advantages of high recovery rate and purity with reliable reproducibility. The quality of the obtained cfDNA is stable and reliable，which can be directly used in qPCR and the library construction of second-generation sequencing and other conventional experiments. This kit can be used with automated nucleic acid extraction instruments for simply and quickly large-scale extraction, which can greatly reducing the workload of the operator and the artifical error during the experiment.

Components

	Components	N902-02 (200 rxns)
BOX 1	VAHTS Particles G	8 ml
	Proteinase K	4 × 35 mg
	Proteinase Dissolve buffer	8 ml
BOX 2	Buffer SDS	20 ml
	Buffer B	160 ml
	Buffer W1	120 ml
	Buffer W2	2 × 40 ml
	Elution Buffer	20 ml

Storage

BOX 1, store at 2 ~ 8℃. Adjust shipping method according to different destinations. Dissolved Proteinase K must be stored at -30 ~ -15℃.
BOX 2, store at 15 ~ 25℃ and transport at room temperature.

Applications

It is applicable for 200 µl - 2 ml plasma, serum and other samples.

Notes

For research use only. Not for use in diagnostic procedures.

1. To avoid a decrease in Proteinase K activity, newly prepared Proteinase K should be used. Proteinase K cannot be stored at room temperature for a long time, and repeated freezing and thawing should be avoided. It is recommended that the dissolved Proteinase K should be divided into small parts and stored at -30 ~ -15℃ after use.
2. VAHTS Particles G can't be frozen or centrifuged, otherwise, it may cause irreversible damage to beads.
3. VAHTS Particles G must be vortexed more than 20 sec to mix thoroughly before use.
4. For the first use, please add absolute ethanol to Buffer W2 according to the label, mark the bottle body and cap and mix well before use.
5. For plasma stored in cell-free DNA storage tubes, the 60℃ incubation step cannot be omitted during sample preparation.
6. Alcohol residue should be avoided when the magnetic beads are dried, otherwise the cfDNA recovery efficiency will be affected. Usually, there is no reflection on the surface of magnetic beads pellet and no ethanol odor means the beads are dried well.
7. It is recommended to leave 2 - 3 µl of liquid when transferring the supernatant in the last step, to aviod absorbing the micro-beads and affect the subsequent experiments.
8. Serum/plasma samples used for extraction should be as fresh as possible and repeated freezing and thawing should be avoided,which may lead cfDNA degradation and lower cfDNA recovery.
9. To set the automatic extraction process, please refer to the manual process and the recommended procedures of automated nucleic acid extraction instruments.

Preparation

Pre-start preparation:

1. Dissolution of Proteinase K: Add 1.75 ml Protease Dissolve Buffer to powdered Proteinase K. Mix upside down for 10 - 15 times to fully dissolve Proteinase K, store at -20℃.
2. For N902-02, add 160 ml absolute ethanol to each bottle of Buffer W2 before using, mix thoroughly and store at room temperature.

For different volume of plasma, add each component according to the volume in the table below: (for high-sensitivity procedures)

Components	500 µl	1 ml	2 ml
VAHTS Particles G	30 µl	60 µl	120 µl
Proteinase K (Dissolved)	25 µl	50 µl	100 µl
Buffer SDS	75 µl	150 µl	300 µl
Buffer B	625 µl	1.25 ml	2.5 ml
Buffer W1	500 µl	1 ml	2 ml
Buffer W2	500 µl	1 ml	2 ml
Elution Buffer	30 - 50 µl	50 - 100 µl	100 - 150 µl

▲The sample compatibility range of this kit is 200 µl - 2 ml, and the volume of each component can be adjusted according to relationship of the actual starting volume.

Experiment Process

Step 1: Plasma Preparation

1.Gently mix the whole blood upside down, transfer to a centrifuge tube, and centrifuge at 1,800 rpm (300 × g) for 20 min at room temperature.
2.Separate the supernatant plasma into a new centrifuge tube.
3.Centrifuge again at 7,900 rpm (6,000 × g) for 5 min at room temperature to completely remove residual blood and cells debris.

Step 2: Sample Treatment

This protocol takes the starting volume of 500 µl as an example to extract cell-free DNA from serum and plasma samples. For other starting volumes (1 ml/2 ml), please refer to the preparation list. Please select the corresponding extraction process according to the type of blood collection tube.

- Fast process (applicable to fresh blood collected by ordinary EDTA anticoagulation tube)

1. Add 25 µl Proteinase K and 30 µl VAHTS Particles G to a 2 ml centrifuge tube.
2. Add 500 µl serum or plasma , shake and mix for 5 sec.
3. Add 625 µl of Buffer B to the above samples, shake and mix for 10 - 15 min at room temperature, mix upside down for several times during shaking, briefly centrifuge and collect. Proceed directly to Step 3: cfDNA Extraction.

- High-sensitivity process (suitable for plasma stored in cell-free DNA storage tubes, with efficiently reduce the potential DNA-protein cross-links)

1. Add 500 µl serum or plasma to a 2 ml centrifuge tube.
2. Add 25 µl Proteinase K and 75 µl Buffer SDS, shake and mix for 5 sec, then incubate at 60℃ for 10 - 20 min.
3. Add 625 µl Buffer B and 30 µl VAHTS Particles G to the above samples, shake and mix for 6 min at room temperature, mix upside down for several times during shaking, briefly centrifuge and collect. Proceed directly to Step 3: cfDNA Extraction.

Step 3: cfDNA Extraction

1. Place the centrifuge tube onto the magnetic stand for 3 - 5 min. After the solution is clear, carefully remove the supernatant.
2. Add 500 µl Buffer W1, vortex for 15 sec, briefly centrifuge and collect.
3. Place the centrifuge tube onto the magnetic stand for 3 - 5 min. After the solution is clear, carefully remove the supernatant.
4. Add 500 µl Buffer W2 (before the first use, make sure the absolute ethanol is added according to “Preparation 2”), vortex for 15 sec, briefly centrifuge and collect.
5. Place the centrifuge tube onto the magnetic stand for 3 - 5 min. After the solution is clear, carefully remove the supernatant.
6. Repeat steps 4 - 5 once.
7. Centrifuge briefly, put the centrifuge tube onto the magnetic stand and carefully discard all of the supernatant.
8. Air dry the beads at room temperature for 5 - 10 min until there is no reflection on the surface of beads pellet.
9. Add 30 - 50 µl Elution Buffer or eluents such as sterilized water, vortex to disperse magnetic beads. Incubate the tubes at room temperature for 3 - 5 min, and briefly vortex for 1 - 2 times to accelerate the dissolution of DNA.
10. Place the centrifuge tube onto the magnetic stand for 3 min. Transfer the DNA solution to a new 1.5 ml centrifuge tube.