cfPure™ Cell-Free DNA Extraction Kit

Isolation of cfDNA from 1 - 5 ml of sample using KingFisher™ Flex Magnetic Processor 24DW

Catalog Number: K5011610, K5011625

Product Description

Biochain's new cfPure Cell-Free DNA Extraction Kit has been designed to isolate circulating DNA from human plasma and serum. The cfPure utilizes Silica coated magnetic bead technology allowing for scalability and easy automation. The cfPure Cell-Free DNA extraction Kit can be used to isolate cfDNA from up to 24 samples of 1 - 5 ml of plasma or serum using the KingFisher™ Flex Magnetic Processor with 24 Deep Well Head. This guide describes the use of the cfPure kit with the KingFisher™ Flex Magnetic Processor 24DW to process samples of 1 - 5 ml .

Kit Contents and Storage

CfPure cfDNA Extraction Kit , 100ml (K5011610)

Item	Amount	Storage
cfPure Lysis/Binding Buffer	1 x 115 ml	Room Temp.
cfPure Wash Buffer	2 x 55 ml	
cfPure Elution Buffer	1 x 6 ml	
cfPure Magnetic Bead Solution	2 x 1.33 ml	

CfPure cfDNA Extraction Kit , 250ml (K5011625)

Item	Amount	Storage
cfPure Lysis/Binding Buffer	3 x 95 ml	Room Temp.
cfPure Wash Buffer	5 x 55 ml	
cfPure Elution Buffer	1 x 15 ml	
cfPure Magnetic Bead Solution	5 x 1.33 ml	

Equipment and Reagents to be Supplied by User

Item	Source
Equipment	
Multi-channel micropipettors	Any
Adjustable Micropipettors	Any
Vortexor	Any
Magnetic Particle Processor	
KingFisher™ Flex Magnetic Particle Processor	Thermofisher 5400630
Magnetic Head	
24 Deep-Well Plates for KingFisher™ Flex Magnetic Particle Processor	Thermofisher 24074440
Deep-Well Plates	
KingFisher™ Flex 24 deep well plate, sterile	Thermofisher 95040490
Tip Combs	
King Fisher Flex 24 Deep Well Tip Comb and Plate	Thermofisher 97002610
Consumables	
Aerosol-resistant pipette tips	Any
Nonstick, RNase-free Microfuge tubes (1.5ml)	Any
MicroAmp™ Clear Adhesive Film	Any
Reagent Reservoirs	Any
Reagents	
Ethanol, 200 proof (Absolute)	Any
SDS, 20% Solution (Only required for Proteinase K treament)	Any
Proteinase K solution (20mg/ml) (Only required for Proteinase K treament)	BioChain Z5050002

Download KingFisher™ Flex Program

1.On cfPure Webpage scroll down to Manual Section.

2. Click cfPure_4-5ml_Flex and/or cfPure_1-2ml_Flex to download program to your computer

3. Refer to KingFisher™ Flex manual for instructions for installing program on the instrument

Important Notes

Starting Material: Both fresh and frozen plasma can be used with the Cell-Free DNA isolation protocol. Fresh plasma, however, tends to have higher yields.

Streck Cell-Free DNA BCT Tube(s): Plasma from blood collected with Streck Cell-Free DNA BCT Tube(s) must go through a Proteinase K treatment prior to Cell-Free DNA isolation to ensure optimal yields. Forgoing Proteinase K treatment may decrease yields by 50%.

Protocol

Prior to Initial Use

The Lysis/Binding and Wash Buffer are shipped as a concentrate. If precipitate is present in either solution, incubate solution at 37°C for 30 minutes. 100% EtOH must be added to both solutions before the first use. Once EtOH is added, these buffers are stable for one year if stored at 4°C. Be sure to close the bottle tightly for long term storage.

For 100 ml kit (K5011610)

- Add 23 ml of fresh 100% ethanol to each bottle of Lysis/Binding Buffer and mix by inverting gently
- Add 51 ml of fresh 100% ethanol to each bottle of Wash Buffer and mix by inverting gently
- Prepare fresh 80% ethanol solution prior to each extraction

For 250 ml kit (K5011625)

- Add 19 ml of fresh 100% ethanol to each bottle of Lysis/Binding Buffer and mix by inverting gently
- Add 51 ml of fresh 100% ethanol to each bottle of Wash Buffer and mix by inverting gently
- Prepare fresh 80% ethanol solution prior to each extraction

Proteinase K Treatment

If samples were collected using a **Streck Cell-Free DNA BCT tube(s)**, Proteinase K treatment is required to ensure optimal yields. If blood was not collected with **Streck Cell-Free DNA BCT tube(s)** proceed to the Lysis/Binding step.

Plasma	Proteinase K	20% SDS Solution
x (x=ml of plasma)	**0.015 x**	**0.050 x**
1 ml	**15 µl**	**50 µl**
2 ml	**30 µl**	**100 µl**
4 ml	**60 µl**	**200 µl**
5 ml	**75 µl**	**250 µl**

1. Add the appropriate amount of plasma to an appropriately sized tube(s)
2. Add 15 µl of Proteinase K (20 mg/ml) for every 1 ml of plasma used
3. Add 50 µl of 20% SDS solution for every 1 ml of plasma used
4. Mix by inverting gently 5 times
5. Incubate at 60°C for 20 minutes
6. After incubation place tube(s) on ice for 5 minutes to cool tube(s) to room temperature

Plate Set up for 1 or 2 ml samples

Set up 24 Deep Well Plates by adding appropriate reagents according to table below

Plate ID	Plate Type	Plate Position	Reagent	Volume per well	
				1 ml	2 ml
Lysis/Binding Plate	24 DW Plate	1	cfPure Lysis/Binding Buffer	1.25 ml	2.5 ml
			cfPure Magnetic Bead Solution	25 µl	50 µl
Wash Plate 1	24 DW Plate	2	cfPure Wash Buffer	1 ml	
Wash Plate 2	24 DW Plate	3	cfPure Wash Buffer	1 ml	
Wash Plate 3	24 DW Plate	4	80% Ethanol	2 ml	
Wash Plate 4	24 DW Plate	5	80% Ethanol	1 ml	
Elution Plate	24 DW Plate	6	cfPure Elution Buffer	50 - 100 µl	
Tip Comb	24 DW Plate	7	Place a 24 Deep-Well Tip Comb in Plate		

Plate Set up for 4 or 5 ml samples

Set up 24 Deep Well Plates by adding appropriate reagents according to table below

Plate ID	Plate Type	Plate Position	Reagent	Volume per well	
				4 ml	5 ml
Lysis/Binding Plate 1	24 DW Plate	1	cfPure Lysis/Binding Buffer	2.5 ml	3.125 ml
			cfPure Magnetic Bead Solution	50 µl	62.5 µl
Lysis/Binding Plate 2	24 DW Plate	2	cfPure Lysis/Binding Buffer	2.5 ml	3.125 ml
			cfPure Magnetic Bead Solution	50 µl	62.5 µl
Wash Plate 1	24 DW Plate	3	cfPure Wash Buffer	1 ml	
Wash Plate 2	24 DW Plate	4	cfPure Wash Buffer	1 ml	
Wash Plate 3	24 DW Plate	5	80% Ethanol	2 ml	
Wash Plate 4	24 DW Plate	6	80% Ethanol	1 ml	
Elution Plate	24 DW Plate	7	cfPure Elution Buffer	50 - 100 µl	
Tip Comb	24 DW Plate	8	Place a 24 Deep-Well Tip Comb in Plate		

- Gently shake Lysis/Binding Plate(s) to mix the reagents
- If extracting cfDNA from a 2 ml sample add enitre sample to a well on Lysis/Binding Plate
- If extracting cfDNA from a 4 or 5 ml sample add half of sample to a well on Lysis/Binding Plate 1 and the other half of sample to the same well on Lysis/Binding Plate 2

Instrument Set up

- Place 24 Deep-Well magnetic head on to machine according the manuals protocol
- Select cfPure_4-5ml_Flex on the instrument for 4 or 5 ml extraction or cfPure_1-2ml_Flex for 1 or 2 ml extractions
- Start the run and follow on screen prompts to load processing plates in their respective positions
- At the end of the run remove elution plate from machine and cover plate or transfer eluate to new tubes
- Isolated cfDNA is ready for immediate use or can be stored at -20°C