

cfPure™ Cell-Free DNA Extraction Kit

Isolation of cfDNA using KingFisher™ Flex Magnetic Processor 96DW

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 96 samples of 600 µl of plasma or serum using the KingFisher™ Flex Magnetic Processor with 96 Deep Well Head.. This guide describes the use of the cfPure kit with the KingFisher™ Flex Magnetic Processor 96DW to process samples of 600 µl or less.

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 96DW	Thermofisher 5400630
Deep-Well Plates	
96 Deep-Well Plates for KingFisher™ Flex Magnetic Particle Processor	Thermofisher 95040460
Standard Plates	
96 Standard Plates for KingFisher™ Flex Magnetic Particle Processor	Thermofisher 97002540
Tip Combs	
96 Deep-Well Tip Combs for KingFisher™ Flex Magnetic Particle	Thermofisher 97002534

Item	Source
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 treatment)	Any
Proteinase K solution (20mg/ml) (Only required for Proteinase K treatment)	BioChain Z5050002

Download KingFisher™ Flex Program

1. On cfPure Webpage scroll down to Manual Section.
2. Click cfPure_600ul_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
600 µl	9.0 µl	30 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

Set up 96 Plates by adding appropriate reagents according to table below

Plate ID	Plate Type	Plate Position on Instrument	Reagent	Volume per well
Lysis/Binding Plate 1	96 Deep-Well Plate	1	cfPure Lysis/Binding Buffer	375 µl
			cfPure Magnetic Bead Solution	7.5 µl
Lysis/Binding Plate 2	96 Deep-Well Plate	2	cfPure Lysis/Binding Buffer	375 µl
			cfPure Magnetic Bead Solution	7.5 µl
Wash Plate 1	96 Deep-Well Plate	3	cfPure Wash Buffer	1 ml
Wash Plate 2	96 Deep-Well Plate	4	80% Ethanol	1 ml
Wash Plate 3	96 Deep-Well Plate	5	80% Ethanol	500 µl
Elution Plate	96 Standard Plate	6	cfPure Elution Buffer	30 - 50 µl
Tip Comb	96 Deep-Well Plate	7	Place a 96 Deep-Well Tip Comb in Plate	

- Gently shake Lysis/Binding Plate 1 and 2 to mix the reagents
- Add 300 µl of plasma sample to the wells of Lysis/Binding Plate 1 and 2
-If Proteinase K treatment was used add 320 µl to each well

Instrument Set up

- Place 96 well Deep-Well magnetic head on to machine, and select cfPure_600ul_Flex on the instrument
- 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 and cover immediatly
- Isolated cfDNA is ready for immediate use