KURABO

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Isolation of cell-free DNA Quick Guide

cfDNA from Plasma

QuickGene cfDNA Isolation Kit (CF-L)

In this Quick Guide, the protocol for isolation of cell-free DNA from plasma is a digest from the Handbook of QuickGene cell-free kit (CF-L) and the Operation Manual of QuickGene-Mini8L. * Before using, please read the Operation Manual.

Wear protective gloves and safety goggles during the experiments.

step1 Preparations

In order to isolate the target cell-free DNA, please prepare the following items.

1 Preparations

QuickGene-Mini8L	10 ml pipette or pipette aid	Tube mixer
Cell-free DNA kit (CF-L) 		
High grade ethanol (>99%)		
1.5 ml microtube (for DNA collection)		
15 or 50 ml centrifuge tube (for lysate preparation)		
Micropipettes (P-1000)		
Water bath (56℃)		
Protective gloves		
Safety goggles		

2 Preparations of Reagents

◆ **Protease (ECF)**

Add 3.3 ml nuclease-free water into the vial containing lyophilized Protease, leave it for 30 minutes or more at room temperature with occasionally stirring it. Dissolve it completely.

(Reconstituted ECF is stable for 2 months when stored at 4℃. More than 2 months, Dividing the solution into aliquots and storage at -80℃ is recommended.)

◆ **Lysis Buffer (LCF)**

Mix thoroughly before use. If the precipitates are formed, dissolve fully by incubating at 37℃.

◆ **Wash Buffer (WCF)**

Add 160 ml ethanol (>99%) into the bottle and mix well.

After adding the ethanol, close the cap and store at room temperature.

◆ **Elution Buffer (CCF)**

Use CCF for elution of cfDNA.

Continue to step.2

step2 Protocol

In order to gain the target yield of cfDNA, please follow the protocol below.

1 Set the temperature of the water bath at 56℃

2 Set the consumables to QuickGene-Mini8L

Regarding setting of the consumables, please refer to the Operation Manual of QuickGene-Mini8L.

3 Prepare Lysate

1) Add 300 µl of ECF (previous dissolved in nuclease-free water) into bottom of a 15 ml centrifuge tubes.
2) Add 2 ml of a plasma sample. (After adding the sample, immediately process to 3)

Carry out 3) and 4) one sample continuously.

3) Add 2.5 ml LCF, and mix the sample with shaking upside-down intensely 10 times immediately.

Mix the sample with shaking upside-down thoroughly, and mix ECF, plasma sample and LCF well.
Next step is mixing the solutions by vortex mixer.
If you don't have a vortex mixer at the speed of 2,500 rpm or more, please mix upside-down completely in this step.

4) Mix with vortex mixer at the maximum speed (2,500 rpm or more) for 15 seconds.

In case mixing is insufficient, the yield of DNA might decrease or the cartridge (CA) might clog.

5) Incubate with water bath at 56℃ for 5 minutes.

Carry out 6) and 7) one sample continuously.

6) Add 1.2 ml of ethanol (>99%), and mix the sample with shaking upside-down intensely 10 times immediately.
7) Mix with vortex mixer at the maximum speed (2,500 rpm or more) for 15 seconds.

In case mixing is insufficient, the yield of DNA might decrease or the cartridge (CA) might clog.

4 Complete the lysis

Perform the isolation operation within 30 minutes, after completing the lysis.

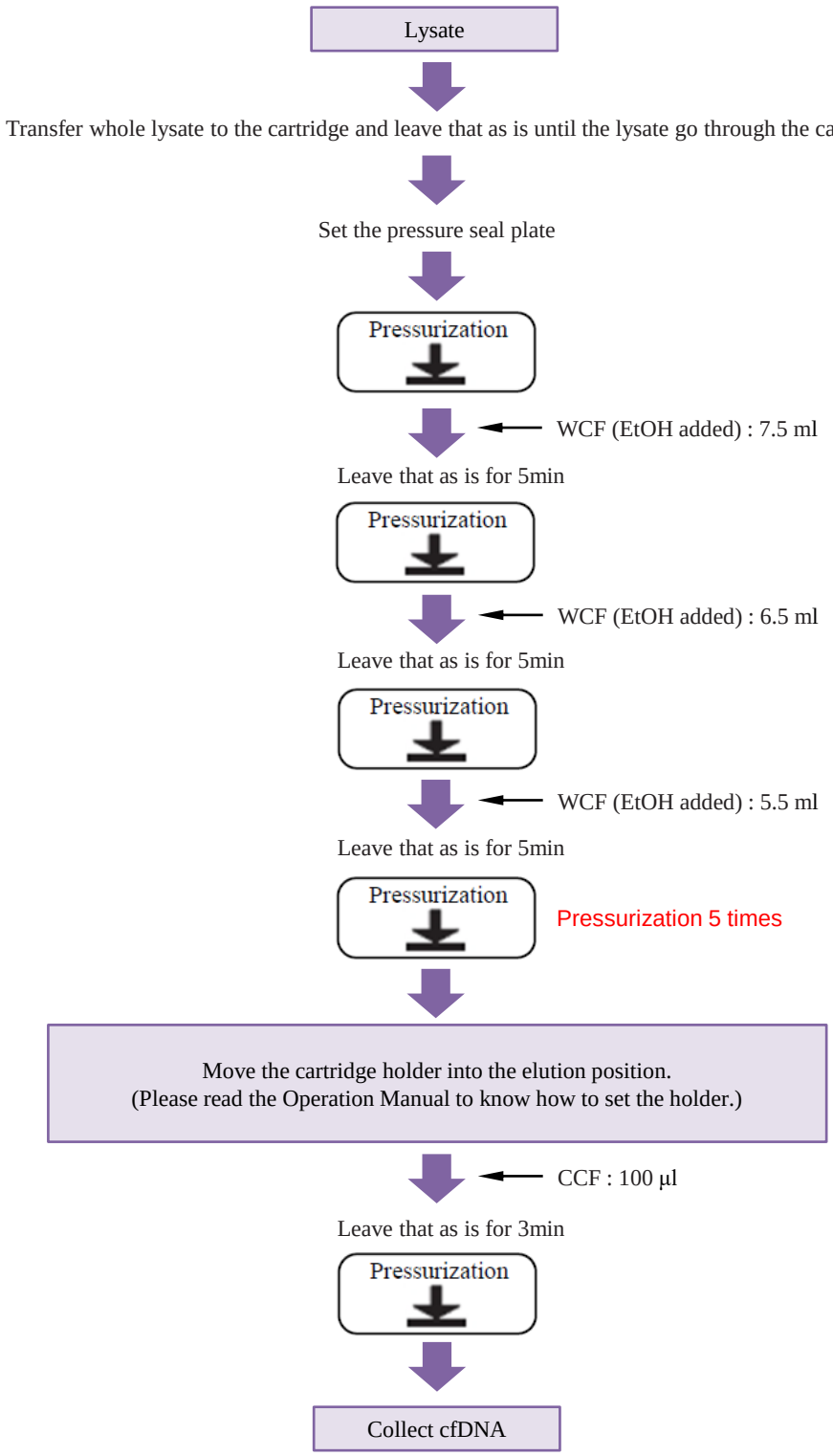
Continue to step.3

step3 Isolation protocol with QuickGene-Mini8L

Use QuickGene-Mini8L to isolate cfDNA.

QuickGene-Mini8L Workflow
The Pressurization mark in the workflow indicates the following operations.

1. Set holder into system. ※Please read the Operation Manual to know how to set the holder.
2. Rotate pressurizing switch toward the front side to start pressurizing.
3. Make sure that there is no residual liquid in the cartridge and return the pressurizing switch to original position.
4. Move the holder to pressurize the next row. Repeat 2. and 3.
5. Pull out holder from system.



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