For QuickGene-Mini8L

KURABO

Circulating cell-free DNA extraction Quick Guide

QuickGene cfDNA Isolation Kit (QG CF-L)

Ver.20-01

This sheet is the procedure for extracting circulating cell-free DNA from plasma.
Before using the kit, check the reagent safety information by SDS.

Wear a laboratory coat, gloves and safety goggles during experiments.

Step1 Preparations

Prepare the following items for the desired cfDNA isolation.

1 Required materials

- QuickGene-Mini8L
- cfDNA Isolation Kit

- Ethanol (>99%)
- 1.5 ml microtubes (for DNA elution)
- 15ml or 50ml centrifuge tube (for pre-treatment)
- Micropipetter (P-1000)
- Water bath (56ºC)
- Disposable gloves
- Safety goggles

10 ml pipettor or Electric pipettor

Tube mixer

2 Preparation of reagents

◆ECF-01 (Lyophilized pretreatment enzyme)

When using ECF-01, pipette 3.3 ml of nuclease-free water into the vial containing lyophilized protease. Dissolve it completely. Reconstituted ECF-01 is stable for 2 months when stored at 4ºC. Storage at -20ºC will prolong the life of ECF-01, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –20ºC is recommended.

◆LCF-01 (Lysis Buffer)

Mix thoroughly before use. If the precipitates are formed, dissolve them fully by incubating at 37° C. Cool down it to room temperature before use.

◆WCF-01 (Wash Buffer)

Add 160 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use. After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label. Close the cap firmly to prevent volatilizing.

◆CCF-01 (Elution Buffer)

Use CCF-01 for elution of cfDNA.

Next > > Protocol for 2ml of plasma

Step2A Protocol (2 ml of plasma)

Be sure to follow the steps below to obtain the desired yield.

1 Set the water bath to 56ºC.

2 Set consumables in QuickGene-Mini8L.

Please refer to the QuickGene-Mini8L instruction manual for the set of consumables.

3 Preparation of lysate

1) Dispense 0.3 ml of dissolved pretreatment enzyme (ECF-01) to the bottom of a 15 ml centrifuge tube.
2) Add 2 ml of plasma. Perform 3) immediately after adding plasma.

Follow steps 3) and 4) one sample at a time.

3) Add 2.5 ml of Lysis Buffer (LCF-01) and immediately shake vigorously up and down 10 times.

Make sure that the ECF-01, plasma, and LCF-01 are well mixed by shaking.
Vortex in the next step. If the maximum speed of your vortex is 2,500 rpm or less, mix thoroughly with shaking.

4) Vortex for 15 seconds at maximum speed (2,500 rpm or more recommended).

Insufficient mixing can result in reduced DNA yield and clogged cartridges.

5) Incubate for 5 minutes in a water bath set at 56 ºC.

Follow steps 6) and 7) one sample at a time.

6) Add 1.2 ml of ethanol (> 99%) and immediately shake vigorously up and down 10 times.
7) Vortex for 15 seconds at maximum speed (2,500 rpm or more recommended).

Insufficient mixing can result in reduced DNA yield and clogged cartridges.

4 Completion of lysis

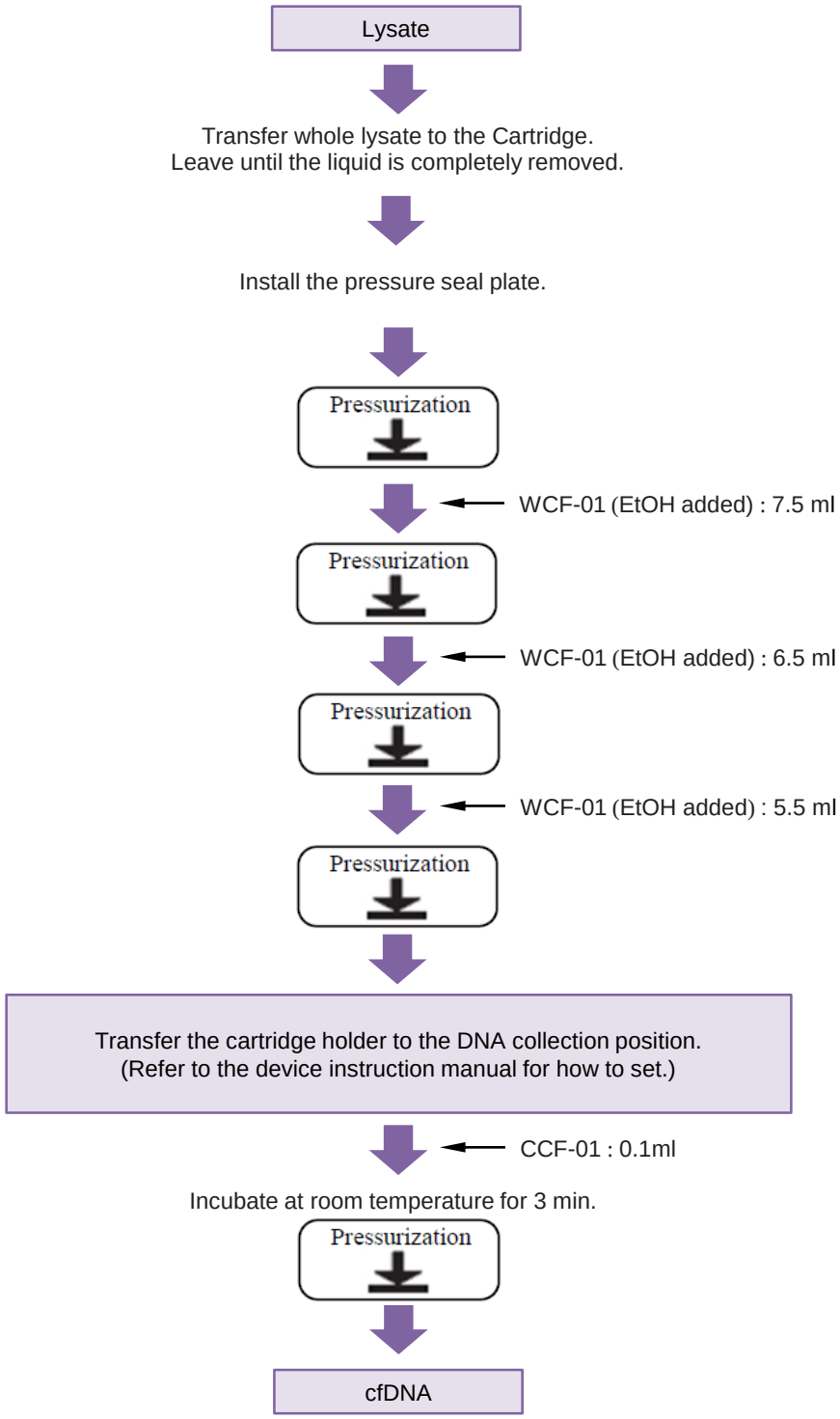
Perform the extraction operation within 30min after completion of lysis.

Next > > Step3A Extraction

Step3A Extraction (2 ml of plasma)

Isolate the target DNA using QuickGene-Mini8L.

QuickGene-Mini8L Workflow
The pressurization mark in the workflow indicates the following operations.
1. Set the Cartridge Holder and the Tube Holder in QG-Mini8L. Push until the first row of cartridges is directly under the pressure nozzle. (Refer to the device instruction manual for how to set.)
2. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
3. Make sure that no liquid remains in the Cartridges and then return the Rotary Switch to the original position.
4. Slide the holder, place the second row of cartridges directly under the pressure nozzle, and perform steps 2 and 3.
5. Pull out the Cartridge Holder and the Tube Holder from QG-Mini8L.



Step2B Protocol (3-5 ml of plasma)

Be sure to follow the steps below to obtain the desired yield.

1 Set the water bath to 56°C.

2 Set consumables in QuickGene-Mini8L.

Please refer to the QuickGene-Mini8L instruction manual for the set of consumables.

3 Preparation of lysate * (3) 3ml, (4) 4ml, (5) 5ml of plasma

1) Dispense (3) 0.45 ml, (4) 0.6 ml or (5) 0.75 ml of dissolved pretreatment enzyme (ECF-01) to the bottom of a 50 ml centrifuge tube.

2) Add (3) 3 ml, (4) 4 ml or (5) 5 ml of plasma. Perform 3) immediately after adding plasma.

Follow steps 3) and 4) one sample at a time.

3) Add (3) 3.75 ml, (4) 5 ml or (5) 6.25 ml of Lysis Buffer (LCF-01) and immediately shake vigorously up and down 10 times.

Make sure that the ECF-01, plasma, and LCF-01 are well mixed by shaking.
Vortex in the next step. If the maximum speed of your vortex is 2,500 rpm or less, mix thoroughly with shaking.

4) Vortex for 30 seconds at maximum speed (2,500 rpm or more recommended).

Insufficient mixing can result in reduced DNA yield and clogged cartridges.

5) Incubate for 5 minutes in a water bath set at 56 °C. Invert mix 3 times after 2 minutes.

Follow steps 6) and 7) one sample at a time.

6) Add (3) 1.8 ml, (4) 2.4 ml or (5) 3 ml of ethanol (> 99%) and immediately shake vigorously up and down 10 times.

7) Vortex for 30 seconds at maximum speed (2,500 rpm or more recommended).

Insufficient mixing can result in reduced DNA yield and clogged cartridges.

4 Completion of lysis

Perform the extraction operation within 30min after completion of lysis.

Next > > Step3B Extraction

Step3B Extraction (3-5 ml of plasma)

Isolate the target DNA using QuickGene-Mini8L.

QuickGene-Mini8L Workflow
The pressurization mark in the workflow indicates the following operations.
1. Set the Cartridge Holder and the Tube Holder in QG-Mini8L. Push until the first row of cartridges is directly under the pressure nozzle. (Refer to the device instruction manual for how to set.)
2. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
3. Make sure that no liquid remains in the Cartridges and then return the Rotary Switch to the original position.
4. Slide the holder, place the second row of cartridges directly under the pressure nozzle, and perform steps 2 and 3.
5. Pull out the Cartridge Holder and the Tube Holder from QG-Mini8L.