With QuickGene-Mini480

KURABO

Ver.16-08 E

Isolation of genomic DNA Quick Guide

Whole Blood DNA

ADS Biotec YOUR PATH TO DISCOVERY

QuickGene DNA whole blood kit S (DB-S)

In this Quick Guide, the protocol for isolation of genomic DNA from whole blood is a digest from the Handbook of QuickGene whole blood kit S (DB-S) and the User's Manual of QuickGene-mini480. ***Before using, please read the User's Manual.**

Wear protective gloves and safety goggles during the experiments.

step1 Preparations

In order to isolate the target genomic DNA, please prepare the following items.

1 Preparations

QuickGene-Mini480

DNA Whole blood kit S (DB-S)

High grade ethanol (>99%)

Nuclease-free water

Heat block (at 56℃)

Benchtop microcentrifuge

Protective gloves

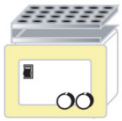
Safety goggles

Microtubes (1.5 ml)

Micropipettes (P-200, P-100 or other types)

Tube mixer

or



2 Preparations of Reagents

◆ Protease (EDB)

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

(Reconstituted EDB 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 (LDB)

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

◆ Wash Buffer (WDB)

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

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

◆ Elution Buffer (CDB)

Use CDB for elution of genomic DNA.

Continue to Step.2

step2-1 protocol A

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

The standard yield is 4 to 8µg from 200µl of whole blood samples. The volume of the eluate from each Cartridge is 200µl.
The volume of CDB can be reduced to 50µl, but in that case, elution efficiency might be decreased.

Using a whole blood sample treated with EDTA-2Na, EDTA-2K, or heparin, within 3 days after blood collection.

1 Set heat block at 56℃.

2 Using 1.5ml microtubes, follow the protocol of 1) to 3). (Do not change the procedure)

1) Add 30µl of EDB (previous dissolved in nuclease-free water) into bottom of a 1.5ml microtube.

2) Add 200µl of a whole blood sample. (After adding the whole blood, immediately process to step3))

3) Add 250µl LDB, then pipette 5 times (or mix upside-down).

Pipette (or mix upside-down) thoroughly to mix EDB, Whole Blood sample and LDB well.
Next step is mixing the solutions by vortex mixer. If you don't have a vortex mixer at the speed of 2500rpm or more,
please pipette (or mix upside-down) completely in this step.

3 Vortex at the maximum speed (2500 rpm or more) for 15 sec.

Surely Vortex for 15 sec at maximum speed.

Flash spin down for several seconds to remove drops from the inside of the lid.

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

4 Incubate at 56℃ for 2 min.

Incubation time up to 5 min does not affect the yield of DNA.

5 Adding 250 µl ethanol（>99%）, and vortex at the maximum speed for 15 sec.

After adding 250 µl of ethanol (>99%), vortex at maximum speed for 15 sec, flash spin down for several seconds.
Then flash spin down for several seconds to remove the drops from the inside of lip.

6 Complete the lysis

Perform the isolation operation within 30 min, after completing the lysis

Continue to Step.3

step2-2 protocol B

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

The Standard yield is 4 to 8 µg from 200 µl of whole blood samples. The volume of the eluate from each Cartridge is 200 µl.
The volume of CDB can be reduced to 50 µl, but in that case, elution efficiency might be decreased.

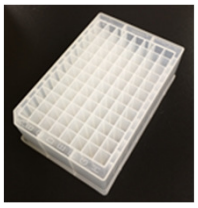
Using a whole blood sample treated with EDTA-2Na, EDTA-2K, or heparin, within 3 days after blood collection.

1 Preparations

Additional items are as follows in protocol B.

Multichannel pipettes *1

96 deep well plate *2



Pipetting reservoir

Water bath (56℃)

*1: Multichannel pipettes should be able to drew 200 µl, 250 µl, 750 µl regents and have 8 or more channel.

*2: Recommended product：Corning® 96 Well Clear V-Bottom 2 mL Polypropylene Deep Well Plate, Sterile (Product #3960)

2 Set water bath at 56℃.

Aqueous surface in the water bath should be the half level of 96 deep well plate.

3 Transfer the minimum required regents to the reservoir depending on the number of sample.

Number of samples	16	20	24	28	32	36	40	44	48
LDB	5 ml	6 ml	7 ml	8 ml	9 ml	10 ml	11 ml	12 ml	13 ml
Ethanol (>99%)	5 ml	6 ml	7 ml	8 ml	9 ml	10 ml	11 ml	12 ml	13 ml
WDB with Ethanol(>99%)	40 ml	50 ml	60 ml	70 ml	75 ml	85 ml	95 ml	105 ml	115 ml
CDB	4.5 ml	5 ml	6 ml	7 ml	7.5 ml	8.5 ml	9 ml	10 ml	11 ml

If the amount beyond the minimum necessary regents are transferred, they might be insufficiency.

4 Using 96 deep well plate, follow the protocol of 1) to 4). (Do not change the procedure)

Unless otherwise noted, use single channel pipettes.

1) Add 30 µl of EDB (previous dissolved in nuclease-free water) into bottom of 96 deep well plate.

2) Add 200 µl of a whole blood sample. (After adding the whole blood, immediately process to step3))

3) Add 250 µl LDB, then pipette 20 times by multichannel pipettes.

Pipette certainly to mix EDB, whole blood and LDB.
In case mixing is insufficient, the yield of DNA might decrease or the cartridge (CA) might clog.

please turn over

5 Incubate at 56℃ for 15 min.

Cover the 96 deep well plate, then incubate in a water bath at 56℃ for 15 min.

Weight the 96 deep well plate to make sure it does not move.

6 Add ethanol (>99%), then pipette 10 times.

Add 250 µl of ethanol (>99%), then pipette 20 times by multichannel pipettes.

7 Incubate at room temperature for 10 min.

Incubation time up to 5 min does not affect the yield of DNA.

8 Complete the lysis

Perform the isolation as soon as possible, after completing the lysis.
From next step, multichannel pipette can be used for applying lysate, WDB and CDB into the cartridge.

Continue to Step.3

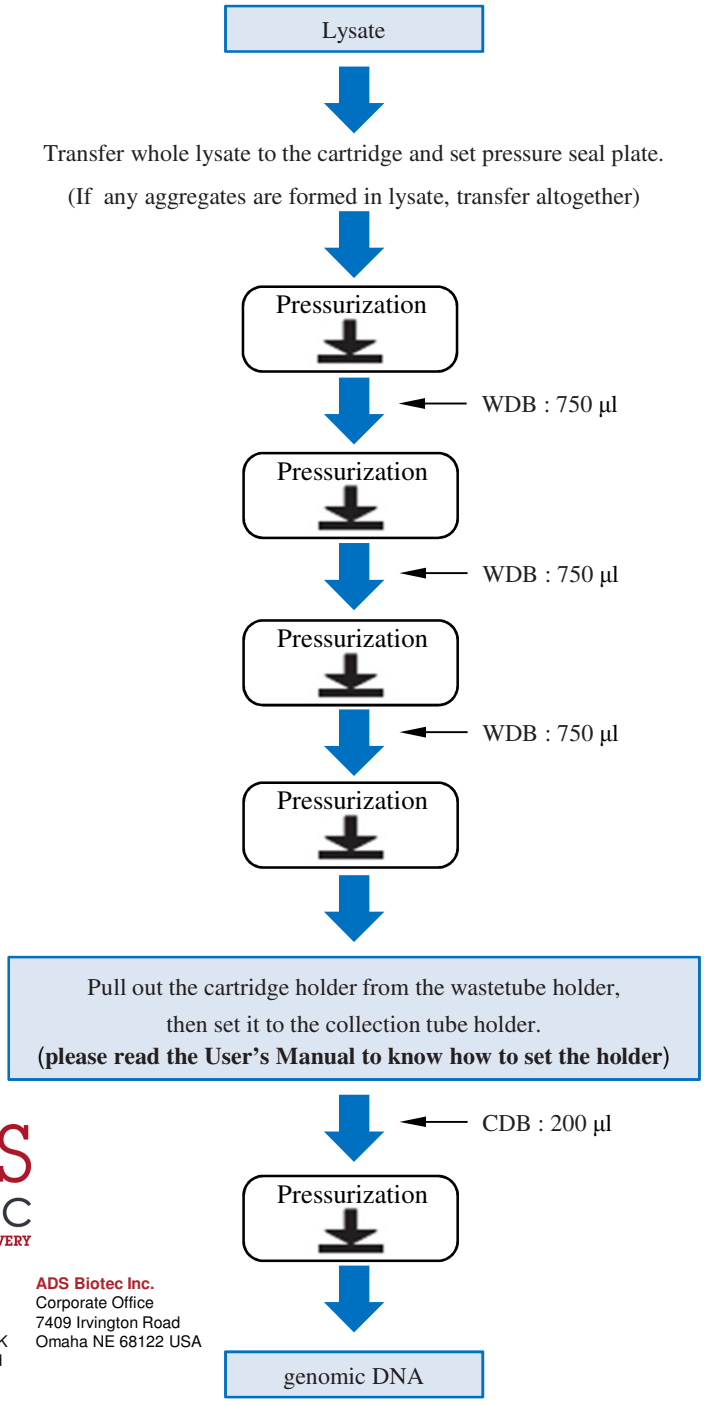
step3 Isolation protocol with QuickGene-mini480

Use QuickGene-Mini480 to isolate genomic DNA from whole blood sample.

1 QuickGene-Mini480 Workflow

The Pressurization mark in the workflow indicates the following operations.

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



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