With QuickGene-Mini480

KURABO

Ver.16-08 E

Isolation of genomic DNA Quick Guide

Tissue DNA

ADS Biotec YOUR PATH TO DISCOVERY

QuickGene DNA tissue kit S (DT-S)

In this Quick Guide, the protocol for isolation of genomic DNA from whole blood is a digest from the Handbook of QuickGene tissue kit L (DT-S) and the Operation Manual of QuickGene-610L. * **Before using, please read the Operation 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 tissue kit S (DT-S)

High grade ethanol (>99%)

Benchtop microcentrifuge

Protective gloves

Safety goggles

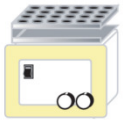
Microtubes (1.5 ml)

Heat block (use except for mouse tail)

Micropipettes (P-200, P-1000 or other types)

Tube mixer

or



Shaker with heater at 55°C (or water bath, mix occasionally)

RNase (refer to: step1-2) *Option

2 Preparations of Reagents

◆Proteinase K (EDT)

Store at 2-8℃.

◆ Tissue Lysis Buffer (MDT)

Mix thoroughly before use. If the precipitates are formed, dissolve fully by incubating at 55°C.

◆ Lysis Buffer (LDT)

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

◆ Wash Buffer (WDT)

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 (CDT)

Use CDT for elution of genomic DNA.

◆ RNaseWhen performing a RNase treatment, recommended products are listed as below (option)

- Ribonuclease A ; Sigma-Aldrich Cat. No. R5125 ✱1, ✱2 / R5500 ✱1, ✱2 / R6513 ✱1 / R4642
- Ribonuclease A ; MP Biomedicals Cat. No. 101076 ✱1, ✱2
- RNase A ; AMRESCO Cat. No. 0675 ✱1, ✱2
- RNase A ; QIAGEN Cat. No. 19101
- RNase A ; Thermo Fisher Scientific Cat. No. 12091

✱1 : Prepare 100 mg/ml solution with 10 mM Tris-HCl (pH7.5), 15 mM NaCl.

✱2 : When using R5125, R5500, 101076 or 0675, incubate at 100°C for 15 min to deactivate DNase.

Continue to Step.2

step2-1 protocol A

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

QuickGene DNA tissue kit S (DT-S) basically corresponds to genomic DNA isolation from 5 mg of animal tissue.
For example, from 5 mg liver tissue of Balb/c mouse (7 week, ♀), 4.0 µg genomic DNA can be gained.
The default volume of CDT is 200 µl. The volume of CDT can be reduced to 50 µl, but in that case, elution efficiency might be decreased.

* Following collection of tissue from animal, the described volume should be immediately immersed in MDT.
If the tissue sample is not used immediately, the tissue should be flash frozen with liquid nitrogen and stored at -20°C or -80°C.
Do not leave tissue at room temperature. Repeatedly freezing or thawing should be avoided. Genomic DNA may degrade.

1 Lyse the tissue (use 2 ml microtubes)

1) Set the temperature of shaker at 55°C.

2) To lyse the tissue well, cut tissue into small blocks 5 mm square or less and put the tissue into 2 ml microtubes.

3) Add 180 µl of MDT and subsequently 20 µl of EDT.

4) Lyse the tissue completely with stirring (use shaker) at 55°C for several hours till over night.

Remove unlysed portions by centrifugation or change the protocol referencing the trouble shooting in the handbook with this kit.

5) In order to remove unlysed portions, centrifuge at 8,000x g (10,000 rpm) at room temperature for 3 min.

6) Transfer the supernatant to a new 1.5 ml microtubes without sucking in the unlysed portion of tissue (unlysed residue, gelatinous substance, etc.).

2 Set the heat block at 70°C. (Except for mouse tail.)

3 RNase treatment ＊option

(Refer to step1. Preparations of Reagents)

1) Add 20 µl of 100 mg/ml RNase. (In the case of Cat. No. 12091, add 60 µl of 100 mg/ml RNase.)

Use DNase free RNase or do a DNase inactivation treatment.

*Depending upon the types of tissue, RNA contents vary. In the case of tissue with low contents of RNA, it is possible to reduce the amount of RNase to be used.
For example) You need 20 µl of 100 mg/ml RNase for breakdown RNA in 5 mg mouse liver.
You need 20 µl of 100 mg/ml RNase (Cat. No. R5125) for breakdown RNA in 5 mg Balb/c mouse (7 week, ♀) tail.

2) Mix RNase well with the sample fluid by tapping or pipetting 5 times.

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

4) Incubate at room temperature for 2 min.

Isolation of tissue genomic DNA

4 Add LDT, then voltex at the maximum rotation speed for 15 sec.

1) Add 180 µl of LDT and mix with the sample fluid by voltex at the maximum rotation speed for 15 sec .
2) When mixing is insufficiency, add tapping, pipetting or mixing upside-down.
3) Then flash spin down for several seconds to remove the drops from the inside of lip.

A white precipitate may form by addition of LDT, which in most cases will dissolve during the incubation at 70°C.

4) Incubation at 70℃ for 10 min.
5) Flash spin down for several seconds

5 Add ethanol (>99%), then voltex at the maximum rotation speed for 15 sec.

1) Add 240 µl of ethanol (>99%) and mix it with the sample fluid by voltex for 15 sec.
2) When mixing is insufficiency, add tapping, pipetting or mixing upside-down.
3) Then flash spin down for several seconds to remove the drops from the inside of lip.

If the room temperature is low, a white precipitate may form by addition of ethanol. After dissolving the precipitate during the incubation at 55°C, please use after returning to room temperature.

Isolation of mouse tail genomic DNA

4 Make the LDT/ethanol (>99%) mixture.

Mix LDT and ethanol (>99%).
(180 µl of LDT + 240 µl of ethanol / 1 sample)

5 Add LDT/ethanol mixture, then voltex at the maximum rotation speed for 15 sec.

1) Add 420 µl of LDT/ethanol mixture and mix with the sample fluid by voltex at the maximum rotation speed for 15 sec.
2) When mixing is insufficiency, add tapping, pipetting or mixing upside-down.
3) Then flash spin down for several seconds to remove the drops from the inside of lip.

If the room temperature is low, a white precipitate may form by addition of LDT/ethanol mixture. After dissolving the precipitate during the incubation at 55°C, please use after returning to room temperature.

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.

QuickGene DNA tissue kit S (DT-S) basically corresponds to genomic DNA isolation from 5 mg of animal tissue.
The default volume of CDT is 200 µl. The volume of CDT can be reduced to 50 µl, but in that case, elution efficiency might be decreased.

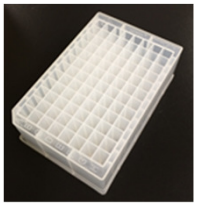
※ Following collection of tissue from animal, the described volume should be immediately immersed in MDT.
If the tissue sample is not used immediately, the tissue should be flash frozen with liquid nitrogen and stored at -20°C or -80°C.
Do not leave tissue at room temperature. Repeatedly freezing or thawing should be avoided. Genomic DNA may degrade.

1 Prerarations

Additional items are as follows in protocol B.

Multichannel pipettes *1

96 deep well plate *2



Pipetting reservoir

Water bath (70℃)

*1: Multichannel pipettes should be able to drew 180 µl, 240 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 Lyse the tissue (use 2 ml microtubes)

Unless otherwise noted, use single channel pipettes.

1) Set shaker at 55℃.

2) To lyse the tissue well, cut tissue into small blocks 5 mm square or less.

3) Add 180 µl of MDT and subsequently 20 µl of EDT.

4) Lyse the tissue completely with stirring (use shaker) at 55°C for several hours till over night.

Remove unlysed portions by centrifugation or change the protocol referencing the trouble shooting in the handbook with this kit.

5) In order to remove unlysed portions, centrifuge at 8,000x g (10,000 rpm) at room temperature for 3 min.

6) Transfer the supernatant to a new 96 well plate.

3 Set water bath at 70℃.

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

please turn over

4 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
LDT	4 ml	4.5 ml	5.5 ml	6 ml	7 ml	7.5 ml	8.5 ml	9 ml	10 ml
Ethanol (>99%)	5 ml	6 ml	7 ml	8 ml	9 ml	10 ml	11 ml	12 ml	13 ml
WDT with ethanol (>99%)	40 ml	50 ml	60 ml	70 ml	75 ml	85 ml	95 ml	105 ml	115 ml
CDT	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.

5 RNase treatment ＊option

(Refer to step1. Preparations of Reagents)

1) Add 20 µl of 100 mg/ml RNase. (In the case of Cat. No. 12091, add 60 µl of 100 mg/ml RNase)

Use DNase free RNase or do a DNase inactivation treatment.

*Depending upon the types of tissue, RNA contents vary. In the case of tissue with low contents of RNA, it is possible to reduce the amount of RNase to be used.
For example) You need 20 µl of 100 mg/ml RNase for breakdown RNA in 5 mg mouse liver.
You need 20 µl of 100 mg/ml RNase (Cat. No. R5125) for breakdown RNA in 5 mg Balb/c mouse (7 week,♀) tail.

2) Mix RNase and the sample fluid by pipetting 5 times.

3) Incubate at room temperature for 2 min.

6 Add LDT, then pipette 10 times.

Add 180 µl of LDT, then pipette 20 times by multichannel pipettes.

Pipette certainly to mix LDT and the sample fluid.
In case mixing is insufficient, the yield of DNA might decrease or the cartridge (CA) might clog.

7 Incubate at 70℃ for 15 min.

Cover the 96 deep well plate, then incubate in a water bath at 70℃ for 15 min.

Weight the 96 deep well plate down as necessary.

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

Add 240 µl of ethanol (>99%), then pipette 20 times by multichannel pipettes.

9 Complete the lysis.

Perform the isolation as soon as possible, after completing the lysis.
Leaving the lysis for a long time makes the yield low.
From next step, multichannel pipette can be used for applying lysate, WDT and CDT into the cartridge.

Continue to Step.3

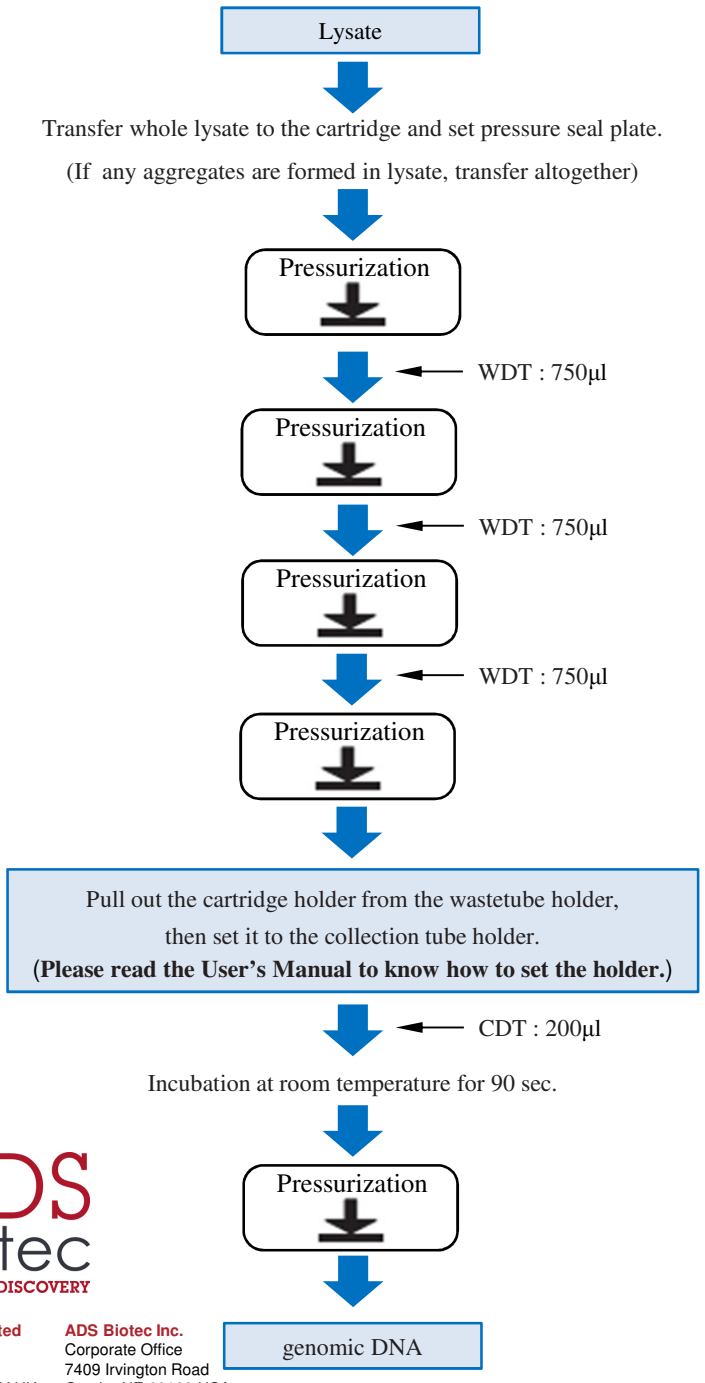
step3 Isolation protocol with QuickGene-mini480

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. ※**Please read the User's Manual to know how to set the holder.**
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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