FUJIFILM

HANDBOOK

QuickGene DNA whole blood kit S (DB-S)

For Isolation of Genomic DNA from whole blood

Contents

Warning: For research use only.
Not recommended and intended for diagnostic or clinical application for human and animals.

1. Introduction

Fuji Photo Film Co., LTD developed and patented an evolutionary, porous membrane to immobilize nucleic acid. Because of its large specific surface area and uniform & fine porousness, QuickGene successfully isolates genomic DNA with high yield; moreover, with its patented thin membrane, it eliminates most contaminants. QuickGene also uses pressured filtration technology, which cannot be successfully utilized with typical glass membranes; by using pressured filtration technology, new, compact and automatic instruments for rapid nucleic acid purification can be produced successfully.
When QuickGene DNA whole blood kit S is used with the QuickGene-series Automatic Nucleic Acid Isolation System, high quality and high yield genomic DNA can be isolated and also purified from whole blood. In addition, DNA from 8 sets of tissue lysate samples can be simultaneously extracted in only 6 minutes. The purified, high quality genomic DNA is suitable for PCR, restriction enzyme digestion, southern blotting and other applications.

Please be sure to read this handbook carefully before using the kit.

2. Components of the kit

The kit includes the reagents necessary for 96 sets of genomic DNA isolation.

- ☐ Protease (EDB)
- ☐ Lysis buffer (LDB)
- ☐ Wash buffer (WDB)
- ☐ Elution buffer (CDB)
- ☐ Cartridges (CA)
- ☐ Collection tubes (CT)
- ☐ Caps (CAP)
- ☐ Waste tubes (WT)

3. Storage conditions

Store all reagents at 15°C to 28°C.

4. Other required materials, not supplied in this kit

◆ **Reagents**
- >99% Ethanol
- Nuclease-free ultra pure water (for dissolving proteases)

◆ **Instruments and equipments**
- QuickGene-series Automatic Nucleic Acid Isolation System
- 1.5 ml Micro-centrifuge tubes
- Centrifuge tubes (see Table1)
- Micropipettes and tips
- Vortex mixer
- Micro-centrifuge (c.a. 5,000×g)
- Tube stands

Table1 Recommended centrifuge tubes

Size of QuickGene-series centrifuge-tube holder	Type of centrifuge tube	Product name (Examples)
Standard	Large centrifuge tube (for WDB)	BD Falcon™ 50 ml conical tube
	Small centrifuge tube (for CDB)	BD Falcon™ 15 ml conical tube
Large	Large centrifuge tube (for WDB)	BD Falcon™ 175 ml conical tube BD Falcon™ 225 ml conical tube
	Small centrifuge tube (for CDB)	BD Falcon™ 50 ml conical tube

Centrifuge tubes are used with the QuickGene-series Automatic Nucleic Acid Isolation System as containers for the wash buffer (WDB) with ethanol and collection buffer (CDB).

5. Safety warnings

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- All reagents and items should be considered chemically and biologically hazardous. Wearing a laboratory coat, gloves and safety glasses during the experiments are highly recommended. In case of contact between the reagents and the eyes, skin, or clothing, wash immediately with water. (See the Material Safety Data Sheet for specific recommendations, http://www.fujifilm.co.jp/msds)

  Protease (EDB)
  Don't put reagents in eyes and be careful of accidental ingestion.
  In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

  Lysis Buffer (LDB)
  Poisonous if swallowed
  Don't put reagents in eyes and be careful of accidental ingestion.
  In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.
  Wear laboratory coat, gloves and safety glasses during experiments.

  Wash Buffer (WDB)
  Don't put reagents in eyes and be careful of accidental ingestion.
  In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

  Elution Buffer (CDB)
  Don't put reagents in eyes and be careful of accidental ingestion.
  In case of contact between the reagents and eyes, skin or clothing, wash immediately with water.

- Keep away the Lysis Buffer (LDB) from heat. Do not mix with disinfectants such as bleach.
- For disposal of waste fluid and consumables: When using potentially infectious samples for experiments, dispose them according to applicable regulations.

6. Precautions

- Refer to the MSDS (Material Safety Data Sheet) for specific recommendations on properties and handling. The MSDS can be obtained from the World Wide Website (http://lifescience.fujifilm.com).
- Refer to the user's manual for the QuickGene-series Automatic Nucleic Acid Isolation System before using.

7. Quality controls

- The stability of the reagents is guaranteed for one year after purchase if stored at the specified temperature (15°C to 28°C).
- As part of the stringent of quality assurance program in Fuji Photo Film Co., LTD, the performance of QuickGene DNA whole blood kit S is evaluated routinely on a lot-to-lot uniformity.
- Quality and yield of isolated genomic DNAs are checked by measuring the absorbance at 260 nm, ratio of absorbance (260 nm/280 nm), and PCR amplification.

8. Protocols

8-1 Preparation of reagents

Protease (EDB)

Add 3.3 ml of nuclease-free ultra pure water to the vial containing the freeze-dried protease, and dissolve it carefully.
The dissolved protease (EDB) will be able to store for two months at 4°C.
The enzyme will be stable for a longer period at -20°C. Recommend to avoid repeated freezing and thawing.

Lysis Buffer (LDB)

Mix thoroughly before using.
If the precipitates are contained in Lysis Buffer, incubate the bottle in a water bath at 37°C and mix with inversion the bottle intermittently until the precipitates are dissolved. After dissolving the Lysis Buffer, cool down the bottle to room temperature before using.

Wash Buffer (WDB)

Provide the concentrated solution.
Add 160 ml of >99% ethanol into the bottle and mix with inversion the bottle gently at the beginning of use.

Requirements of Wash Buffer (WDB) with >99% ethanol and Elution Buffer (CDB)

Prepare the requirements of Wash Buffer (WDB) with >99% ethanol and Elution Buffer (CDB) according to the number of samples for isolation; refer to the following table.
Take some of the buffers into each tube and set the tubes in the QuickGene-series system tube holder. (See the user's manual of QuickGene-series Automatic Nucleic Acid Isolation System.)

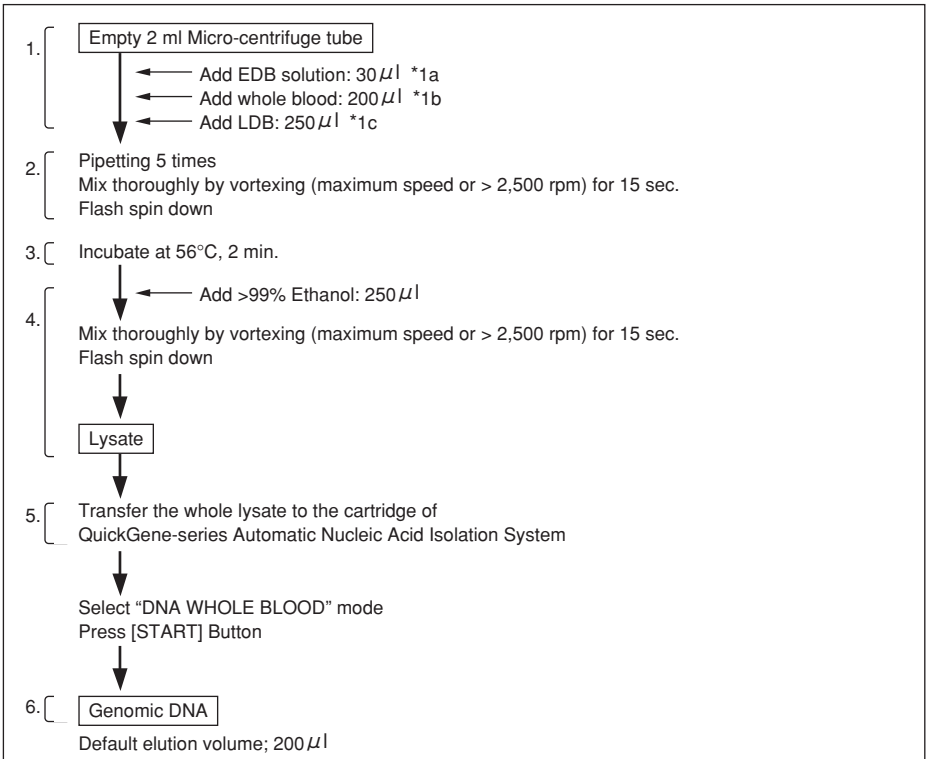
Table2 Buffer volume and the number of samples to set in the QuickGene System

Number of samples	WDB with Ethanol	CDB
8	26 ml	8 ml
16	44 ml	11 ml
24	62 ml	13 ml
32	80 ml	15 ml
40	99 ml	17 ml
48	117 ml	19 ml
56	135 ml	21 ml
64	154 ml	22 ml
72	172 ml	24 ml
80	190 ml	26 ml
88	209 ml	28 ml
96	227 ml	30 ml

8-2 Sample preparations

- The QuickGene DNA whole blood kit S is specifically designed for genomic DNA isolation from 200 µl of whole blood.
- Recommend to use the whole blood collected in EDTA·2Na, EDTA·2K or heparin.
- The yield will depend on the sample condition.
- Use the kit at room temperature (15°C to 30°C). When using the kit at lower or higher temperatures, the expected yield may not be obtained.
- Accurately measure the buffer volume during the experiments.

<Preparation workflow from whole blood>



Notice

1. Follow the protocol of 1a to 1c exactly. If you change the protocol, may be reduced the yield.
   1a; Add 3.3 ml of nuclease-free ultra pure water to the vial containing the freeze-dried protease, and dissolve it carefully.
   Put the 30 μl of EDB to bottom of 2 ml micro tube.
   1b; Add 200 μl of whole blood into the micro tube, and then add 250 μl of LDB immediately.
   (Leaving the samples long time before addition of LDB may be reduced the yield.)
   1c; Mix whole blood sample and LDB by pipetting 5 times.
   It is very important to mix thoroughly the sample with pipetting after addition of LDB.

2. Vortexing for 15 sec. with maximum speed and flash spin down to recover the lysate from cap and tube wall.
   Recommending vortex speed is 2,500 rpm and more. (If you do not have such a vortex mixer, pipetting completely at 1c.)
   Incomplete mixing at this time, the sample will be clogged the cartridge of QuickGeme-series Automatic Nucleic Acid Isolation System, or low yield.

3. Incubate at 56°C 2 min. The maximum incubation time is 5 min.

4. Add 250 μl >99% Ethanol and vortexing for 15 sec. with maximum speed.
   Flash spin down to recover the lysate from cap and tube wall.

5. Transfer the whole lysate to the cartridge of QuickGene-series Automatic Nucleic Acid Isolation System. Perform isolation within 30 min. after lysate preparation.
   If aggregates are present in the lysate, apply them along with the lysate to the cartridge.

6. Default elution volume is 200 μl but you may change the setting of elution volume less than default volume, minimum 50 μl. In case of setting to 50 μl, yield may decline.
   The standard yield of eluted genomic DNA is 4-8 μg from 200 μl whole blood.
   Store the eluted genomic DNA at -20°C for long storage.

8-3 Genomic DNA isolation using the QuickGene-series Automatic Nucleic Acid Isolation System

Notice: System set up and basic operations
Please read the user's manual of QuickGene-series Automatic Nucleic Acid Isolation System circumstantially for the details before using the system.

(1) Selection of isolation mode
Select "DNA WHOLE BLOOD" mode for genomic DNA isolation from whole blood with the kit. (See Appendix 1)

(2) Setting of cartridges and tubes
Open the front cover of the instrument and set the collection and waste tubes in the collection-tube holder.
- Use the specified Collection Tubes (CT) and Waste Tubes (WT) including the kit.

Attach the cartridge holder to the instrument and set 1-8 cartridges in the cartridge holder.
- Use the specified Cartridges (CA).

Notice: Refer to the user's manual for the QuickGene-series Automatic Nucleic Acid Isolation System for details of setting cartridges and tubes.
Incorrect cartridge placement may result in the solution spilling or improper isolation.
Wear gloves during the experiments to avoid nuclease contamination.

(3) Setting of reagents
Prepare the required volume (see 8-1 Preparation of reagents) of Wash Buffer (WDB) with >99% ethanol and Elution Buffer (CDB) into the tubes; set them to the holder; and put the holder to the designated positions of instrument.

Notice: Wear gloves during the handling of reagents to avoid nuclease contamination.
- Read the user's manual for the QuickGene-series Automatic Nucleic Acid Isolation System for details for setting reagents.

(4) Discharge
Set the "discharge tray" and check the collection holder and cartridge holder setting for the correct positions.
Press the "DISCHARGE" after closed the front cover of the instrument.

Notice: Because of air in the lines, incorrect volume of reagents may occur without discharge operation.

(5) Applying the prepared samples
Apply all contents of prepared lysate samples (see 8-2 Sample preparations) into the each Cartridge (CA) by using micropipettes (any aggregates in the lysate should be transfered into the cartridge).

(6) Isolation
Check if the materials—Wash Buffer (WDB) with >99% ethanol, Elution Buffer (CDB), Cartridges (CA) including samples, Waste Tubes (WT), and Collection Tubes (CT)—are well set.
Close the front cover of the instrument.
Confirm the appropriate mode on the operation panel and press the [START] button.

(7) Collection of genomic DNA

After completing the process, each sample result is indicated on the operation panel as follow;
[v (Check)]: Completed normally
[– (Hyphen)]: Not completed normally
[_ (Underscore)]: No cartridge or no sample

Open the front cover and remove the Collection Tube(s) (CT) from the collection-tube holder.

- As genomic DNA is eluted from the Cartridge(s) (CA) using 200 μl of Elution Buffer (CDB), the volume of recovered total DNA solution will be 200 μl.

Cover with the Caps (CAP) on the Collection Tubes (CT) containing the isolated genomic DNA.

(8) Clean up

Remove the Waste Tubes (WT) and dispose the waste fluid according to applicable regulations.
Remove the cartridge holder and dispose the Cartridges (CA).

Warning: Disposal of waste fluid and consumables
When using the potentially infectious samples for experiments, dispose them according to applicable regulations.

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene DNA whole blood kit S. For system-related problems (e.g., when an error message appears), see the QuickGene-series user's manual.

(1) Low yield or no DNA obtained

Cause	Possible Solution
Reagents and whole blood added in the wrong order	Add the reagents and samples to microtubes in the following order when preparing the lysate: Protease (EDB: dissolved in 3.3 ml of nuclease-free water) → whole blood → Lysis Buffer (LDB).
Excess amount of sample was used	Reduce the amount of whole blood to below the specified amount.
Insufficient homogenization following the addition of Lysis Buffer (LDB)	Vortex sufficiently (15 sec.) immediately after Lysis Buffer (LDB) addition.
Requirement volume of ethanol was not added to Wash Buffer (WDB)	Always confirm that the required volume of ethanol was added to the Wash Buffer (WDB) prior to use.
Old Wash Buffer (WDB: including ethanol) used	Flash remaining Wash Buffer (WRC: including ethanol) which may be one day old or more in the instrument prior to use.
Lysate is not fully applied to Cartridge(s) (CA)	If aggregates are present in the lysate, apply them along with the lysate to the cartridge.
Insufficient amounts of reagents used	Make sure that sufficient amount of reagent are in the reagent bottles.

(2) Clogging the cartridge

Cause	Possible Solution
Excess amount of sample was used	Reduce the amount of whole blood to below the specified amount.
Insufficient homogenization following the addition of Lysis Buffer (LDB)	Vortex sufficiently (15 sec.) immediately after Lysis Buffer (LDB) is added.

(3) Subsequent experiments (e.g., PCR) unsuccessful

Cause	Possible Solution
Improper amount of DNA used for subsequent experiments	Determine the concentration based on the absorbance at 260 nm.

(4) Supplying the precipitates in reagents

Cause	Possible Solution
Stored at low temperature	Store solutions at 15°C to 28°C. If the precipitates are contained, incubate the bottle in a water bath at 37°C and mix with inversion the bottle intermittently until the precipitates are dissolved.

(5) The collection tubes are empty after the elution.

Cause	Possible Solution
Missed the discharge	Set the "discharge tray" and check the collection holder and cartridge holder setting up into correct positions. Press the "DISCHARGE" after closed the front cover of the instrument. See the QuickGene-series user's manual.

10. Ordering Information

Product	Cat #
QuickGene-series Automatic Nucleic Acid Isolation Systems	
QuickGene DNA tissue kit S	DT-S
Dedicated reagent kit for QuickGene-series to isolate the Genomic DNA from the tissue	
QuickGene DNA whole blood kit S	DB-S
Dedicated reagent kit for QuickGene-series to isolate the Genomic DNA from whole blood	
QuickGene RNA tissue kit S	RT-S
Dedicated reagent kit for QuickGene-series to purify the total RNA from the tissue	
QuickGene RNA cultured cell kit S	RC-S
Dedicated reagent kit for QuickGene-series to purify the total RNA from cultured cell	
QuickGene Plasmid kit S	PL-S
Dedicated reagent kit for QuickGene-series to extract the Plasmid DNA	

11. Contact Information

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Appendix 1 “DNA WHOLE BLOOD” mode is set in the following parameter.

	DNA WHOLE BLOOD
PARAMETER	**SET VALUE**
BIND PEAK	120
WASH COUNT	3
WASH PEAK	110
WASH VOL1	750
WASH VOL2	750
WASH VOL3	750
WASH VOL4	750
WASH VOL5	750
WASH DIP TM	0
WAS2 WAIT T	0
WAS2 COUNT	0
WAS2 PEAK	110
WAS2 VOL1	750
WAS2 VOL2	750
WAS2 VOL3	750
WAS2 VOL4	750
WAS2 VOL5	750
ELUT VOL	200
ELUT PEAK	100
ELUT DIP TM	0