



HANDBOOK

QuickGene-AutoS DNA Blood Kit (AS-DB)

For extraction of genomic DNA from whole blood

Ver.1.1

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Warning

For research use only.

Not recommended or intended for diagnostic or clinical application for humans or animals.

1. Introduction

QuickGene porous membrane to immobilize nucleic acid has large specific surface area and uniform & fine porosity. So QuickGene successfully extracts genomic DNA with high yield. QuickGene also uses pressured filtration technology, which enables producing new, compact and automatic instruments for rapid nucleic acid purification.

This is a ready-to-use prepacked reagent kit for the extraction process of QuickGene-Auto12S (QG-Auto12S) or QuickGene-Auto24S (QG-Auto24S).

- When using this kit with QG-Auto12 or QG-Auto24S, high quality and high yield genomic DNA can be extracted and also purified from 200 µl of whole blood.
- DNA from whole blood samples can be simultaneously extracted in following time.
 - QG-Auto12S: about 23min for 12 sets of whole blood samples
 - QG-Auto24S: about 23min for 24 sets of whole blood samples
- The purified, high quality genomic DNA is suitable for PCR, restriction enzyme digestion, NGS analysis and other applications.

Please be sure to read the Operation Manual of QuickGene-Auto12S / QuickGene-Auto24S carefully before using this kit.

2. Kit Components and Storage Conditions

2-1. Kit Components (48 Preps)

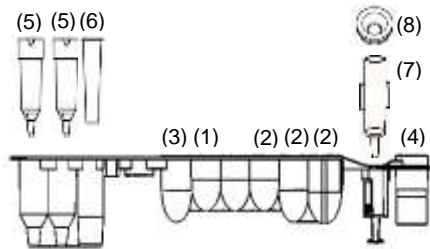
- | | | |
|--------------------------|---------------------------|--------|
| <input type="checkbox"/> | Protease(Lyophilized) EDB | 1 vial |
| <input type="checkbox"/> | Reagent strips | 48 pcs |
| <input type="checkbox"/> | 1 ml Long Tips | 48 pcs |
| <input type="checkbox"/> | Waste Tubes | 48 pcs |

2-2. Storage Conditions

All reagents are stable at room temperature (15-28°C) until expiring date indicated at outer box. Reconstituted EDB is stable for 2 months when stored at 4°C. Storage at -20°C will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at -20°C is recommended.

2-3. Reagent strip components

<input type="checkbox"/>	Lysis Buffer	LDB	270 µl	(1)
<input type="checkbox"/>	Wash Buffer	WDB	750 µl, 3 positions	(2)
<input type="checkbox"/>	(>99%) Ethanol		270 µl	(3)
<input type="checkbox"/>	Elution Buffer	CDB	250 µl	(4)
<input type="checkbox"/>	Short Tip		2	(5)
<input type="checkbox"/>	Tip pack		1	(6)
<input type="checkbox"/>	Cartridge		1	(7)
<input type="checkbox"/>	Pressure Adapter		1	(8)



3. Other Required Materials, Not Supplied in This Kit

[1] Reagents

- Nuclease-free water (for dissolving EDB)

[2] Equipment

- QuickGene-Auto12S or QuickGene-Auto24S
- Micropipettes and tips
- Tube stand
- 2 ml microtubes for samples

Recommendation product: BM EQUIPMENT Cat. BM4020

SARSTEDT Cat.72.695.700, Cat.72.695.500S

*When using a tube other than the recommended product, check the compatibility with the strip and equipment heater part beforehand.

- 1.5 ml or 2 ml microtubes for elution of DNA

Recommendation product: BM EQUIPMENT Cat. BM4015,

SARSTEDT Cat.72.706.700

*When using a tube other than the recommended product, check the compatibility with the Collection holder beforehand.

4. Safety Warnings

Warning

For research use only.

Not recommended or intended for diagnostic or clinical application for humans or animals.

- All reagents and items should be considered chemically and biologically hazardous. Wearing a laboratory coat, disposable gloves and safety goggles 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 Safety Data Sheet for specific recommendations, <http://www.kurabo.co.jp/bio/English/>)

◆ Protease EDB

- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ LDB (Lysis Buffer)

- Harmful if ingested.
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.
- Wear a laboratory coat, gloves and safety goggles during experiments.

◆ WDB (Wash Buffer)

- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ CDB (Elution Buffer)

- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

◆ EtOH (Ethanol)

- Highly flammable liquid. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources.
- Do not drink or ingest. Avoid contact with eyes.
- If contact with eyes, skin, or clothing occurs, rinse thoroughly with water. Consult a physician if necessary.

- ◆ Use or storage of Reagent strips at the specified temperature (15°C – 28°C).

- ◆ Any solution and waste fluid containing LDB should not be mixed with bleach.

◆ **In the case of using potentially infectious samples:**

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

◆ **Disposal of waste fluid and consumables when using potentially infectious samples:**

After use, dispose of potentially infectious samples and consumables by incineration, high-temperature decontamination, sterilization, or disinfection in accordance with applicable laws. When entrusting waste disposal to licensed hazardous waste disposal contractors, use

specially controlled waste management forms (manifest), if applicable.

5. Precautions

◆ Handling of Starting Material

- Small amount of samples should be adjusted to 200 µl with PBS (sterilized) before loading.
- Use a whole blood sample treated with EDTA-2Na, EDTA-2K, or heparin.
- Use a whole blood sample within 3 days after collection. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
- The yield of DNA might decrease when the number of leucocytes exceeds 2×10^6 cells/200 µl. In such cases, adjust the number of leucocytes by diluting the sample with PBS (sterilized) to below 2×10^6 cells/200 µl.
- The Cartridge (CA) might clog when the number of leucocytes exceeds 5×10^6 cells/200 µl. We recommend that you dilute the sample with PBS (sterilized) and then perform extraction.

◆ Use of Reagent

- After addition of nuclease-free water to EDB leave it for 30 min or more at room temperature with occasionally stirring. Use it after confirming the powder is completely dissolved. The yield of DNA might decrease or the Cartridge (CA) might clog when dissolution of EDB is insufficient.

◆ Procedure of Extraction

- Before starting operation, please make sure the following things:
 - Waste Tubes and 1.5 ml or 2 ml microtubes (for elution) are set in the Collection holder.
 - Reagent strips are set correctly in the Reagent holder.
 - 1 ml Long tips and 2 ml microtubes (Whole blood and EDB included) are set in the Reagent strip.
 - The lid of Reagent holder is completely closed.
 - Reagent holder and Collection holder are properly set in the holder guide.
- All operations should be performed at room temperature (15 to 28 °C). In case of using at lower or higher temperature, it may affect the extraction performance.
- Except for unavoidable circumstances, please do not turn off the QG-Auto12S or QG-Auto24S device during operation. You cannot resume operation from the same process.
- Refer to the Operation Manual of QuickGene-Auto12S / QuickGene-Auto24S for details.

6. Quality Control

- As part of the stringent quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene-AutoS DNA Blood Kit (AS-DB) is evaluated routinely on a lot-to-lot uniformity.
- Yield and quality of extracted genomic DNA are checked by measuring the absorbance at 260 nm, ratio of absorbance (260 nm/280 nm).

7. Product Description

Table 2 shows the average of yield and purity (A260/280) of genomic DNA extracted from 200 µl of whole blood samples.

The yield varies depending upon sample conditions.

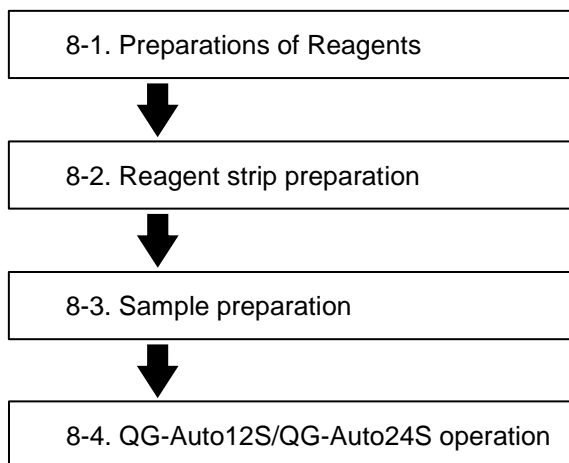
Table 2

Sample	Amount of genomic DNA (µg)	A260/280
Whole blood (200 µl)	5.8	1.95

*DNA and RNA are included in eluate extracted with this kit.

8. Protocol

[Overview Flow Chart]



8-1. Preparations of Reagents

◆ EDB (Lyophilized)

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

Notices

Dissolve EDB completely by the following method, and then use the solution.

Add 3.3 ml of nuclease-free water, close the cap and mix with inversion the bottle.

Leave it for 30 min or more at room temperature with occasionally stirring it. Use it after confirming the powder is completely dissolved. The yield of DNA might decrease or the Cartridge (CA) might clog when dissolution of EDB is insufficient.

8-2. Reagent strip preparation

- To avoid contamination of nuclease, wear disposable gloves during preparation of Reagent strips and microtubes.
 - Refer to the Operation Manual of QuickGene-Auto12S / QuickGene-Auto24S for details.
- <1> Prepare the Collection holder and Reagent holder on the workbench.
- <2> Load the waste tube and 1.5 ml or 2 ml microtube into the Collection holder.
- <3> Remove the Reagent strips from the kit box, place it in the Reagent holder, and insert 1 ml Long Tip in the specified position.

8-3. Sample preparation

Notices

Follow the protocol of <1> to <3> exactly.
In case the procedure is changed, the yield of DNA may not be obtained.

- Wear a suitable laboratory coat, disposable gloves and safety goggles during the experiments.
 - To avoid contamination of nuclease, wear disposable gloves during preparation of Reagent strips and microtubes.
 - Refer to the Operation Manual of QuickGene-Auto12S / QuickGene-Auto24S for details.
- <1> Add 30 μ l of EDB (previously dissolved in nuclease-free water) to the bottom of a 2 ml microtube.
Recommendation product of 2 ml microtube: M&S Instruments Cat. 4020
SARUSTEDT Cat.72.695.700
Cat.72.695.500S
- <2> Add 200 μ l of a whole blood sample.
- <3> Set the 2 ml microtube containing whole blood and EDB in the specified position on the Reagent strip.

8-4. QG-Auto12S/QG-Auto24S operation

- Please read the Operation Manual of QuickGene-Auto12S / QuickGene-Auto24S for the details before using the device.
- To avoid contamination of nuclease, wear disposable gloves during preparation of Reagent strips and microtubes.

<1> Open the front door and put the Collection holder and Reagent holder to the specified positions on the machine.

<2> Turn on the device.

The device proceeds through a self-check and moves to the home position about all moving parts.

<3> At the Home screen, select the "DNA WHOLE BLOOD".

<4> Chose the elution volume

<5> Make sure all the accessories has been putted in the system. Tick the check list then the "Next" button will show up.

<6> Press the "Next" button.

<7> Check the protocol information is correct, then press the "Start" button to proceed the isolation. Then processing will be started.

- During the running step, the touch panel show the processing and remaining time.
- Operation status can be confirmed by blinking process name (LYSIS, BINDING, WASH, ELUTE, FINISH).
- Do not open the front door of the device while running. If you open the front door, please read the Operation manual of QG-Auto12S / QG-Auto24S and resume operation.
- To pause, touch the "Pause" button on the operation panel. The end confirmation screen will be displayed, please press "Yes" to finish.

<8> After finishing the protocol, the beeper will call and the process name "FINISH" flashes on the operation panel.

After confirming that the device is completely stopped open the front door, take out the Reagent holder and the Collection holder.

Take out the elution tube from the Collection holder.

- The standard yield is 4 to 8 μg from 200 μl of whole blood samples.
- If you do not use DNA immediately, please close the tube lid tightly and store at 4°C or -20°C.
- In case of storing genomic DNA for a long time, it is recommended to preserve them at -20°C.

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene-AutoS DNA Blood Kit (AS-DB).

(1) Low yield or no DNA obtained

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
Inadequate dissolution of EDB	After addition of nuclease-free water to EDB leave it for 30 min or more at room temperature with occasionally stirring it. Use it after confirming the powder is completely dissolved.
Insufficient enzymatic activity of EDB	Reconstituted EDB is stable for 2 months when stored at 4°C. Do not use EDB preserved for a longer period than 2 months. Storage at -20°C will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at -20°C is recommended.
Inappropriate addition order of reagents and whole blood sample	When preparing lysates, perform the additions to a 2 ml microtube in the following order : EDB (previously dissolved in 3.3 ml of nuclease-free water) --> Whole blood sample
Inappropriate volume of whole blood sample	If the volume of a whole blood sample is too much, reduce it to the prescribed volume (200 µl). Small amount of sample should be adjusted to 200 µl with PBS (sterilized) before loading.
Use of too much amount of leucocytes	The yield of DNA might decrease when the number of leucocytes exceeds 2×10^6 cells/200 µl. In such cases, adjust the number of leucocytes by diluting the sample with PBS (sterilized) to below 2×10^6 cells/200 µl.
DNA degradation	Refer to (3) "DNA degradation".

(2) Clogging of Cartridge (CA) occurs

Cause	Action
Use of too much amount of a whole blood sample	Reduce it to the prescribed volume (200 µl).
Use of too much amount of leucocytes	The Cartridge (CA) might clog when the number of leucocytes exceeds 5×10^6 cells/200 µl. The yield of DNA might decrease when the number of leukocytes exceeds 2×10^6 cells/200 µl. In such case, we recommend that you dilute the sample with PBS (sterilized) to below 2×10^6 cells/200 µl, and then perform extraction.

(3) DNA degradation

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.

(4) Purity of DNA is low

Cause	Action
Inappropriate storage conditions for whole blood sample	Use a whole blood sample within 3 days after collection as much as possible. The yield of DNA might decrease, or degradation of DNA might be caused when a blood sample preserved for a long time is used.
Insufficient enzymatic activity of EDB	Reconstituted EDB is stable for 2 months when stored at 4°C. Do not use EDB preserved for a longer period than 2 months. Storage at -20°C will prolong the life of EDB, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at -20°C is recommended.

(5) Subsequent experiments such as PCR etc. do not proceed well

Cause	Action
Inappropriate amount of DNA is used	Determine the DNA concentration based on the absorbance at 260 nm.
Low purity of DNA	Refer to (4) "Purity of DNA is low".
DNA degradation	Refer to (3) "DNA degradation".

(6) A precipitate is formed in reagents

Cause	Action
Stored at low temperature	Store this kits at room temperature (15-28°C). If a precipitate is formed, dissolve the precipitate by incubation at 37°C. Cool down it to room temperature before use.



10. Ordering Information

Product	Content	Cat #
QuickGene-AutoS DNA Blood Kit	48 preps	AS-DB
QuickGene-AutoS DNA Tissue Kit	48 preps	AS-DT
QuickGene-AutoS Plasmid Kit	48 preps	AS-PL
QuickGene-AutoS RNA Blood Kit	48 preps	AS-RB
QuickGene-AutoS RNA Tissue Kit	48 preps	AS-RT
QuickGene-AutoS RNA Cultured Cell Kit	48 preps	AS-RC

* Trademark and exclusion item

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