KURABO

HANDBOOK

QuickGene-AutoS DNA Tissue Kit (AS-DT)

For extraction of genomic DNA from tissues

Ver.1.2

Contents

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 porousness. 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 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 tissue samples.
- DNA from tissue lysate samples can be simultaneously extracted in following time.
  QG-Auto12S：about 30 min for 12 sets of whole blood samples
  QG-Auto24S：about 30 min 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 Auto-12S / QuickGene Auto-24S carefully before using this kit.

2. Kit Components and Storage Conditions

2-1 Kit Components (48 Preps)

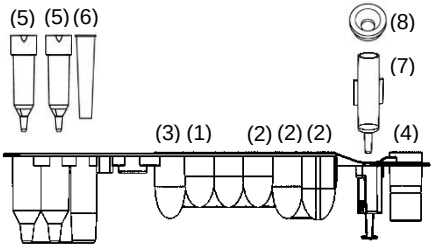
☐	Proteinase K EDT	1.25 ml
☐	Tissue Lysis Buffer MDT	12.5 ml
☐	Reagent strips	48
☐	1 ml Long Tips	48
☐	Waste Tubes	48

2-2 Storage Conditions

All reagents are stable at room temperature (15-28°C) until expiring date indicated at outer box. We suggest keeping EDT at 2-8°C to prolong its life.

2-3. Reagent strip components

☐	Lysis Buffer	LDT	270 µl		(1)
☐	Wash Buffer	WDT	750 µl	x 3potions	(2)
☐	(>99%)Ethanol	EtOH	240 µl		(3)
☐	Elution Buffer	CDT	250 µl		(4)
☐	Short Tip		2		(5)
☐	Tip pack		1		(6)
☐	Cartridge		1		(7)
☐	Pressure Adapter		1		(8)



3. Other Required Materials, Not Supplied in This Kit

[1] Reagents
* Prepare if necessary
- RNase A [Recommended products are listed as below.]
  - 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 : Life Technologies Cat. No. 12091

*1: Prepare 100 mg/ml solution with 10 mM Tris-HCl (pH 7.5) and 15 mM NaCl
*2: Incubate at 100°C for 15 min to deactivate DNase

[2] Equipment

- QuickGene-Auto12S or QuickGene-Auto24S
- Micropipettes and tips
- Tube stand
- 2 ml microtubes for samples
  Recommendation product：BM EQUIPMENT Cat. 4020
  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. 4015、Cat.4020
  SARSTEDT Cat.72.706.700

  *When using a tube other than the recommended product, check the compatibility with the Collection holder beforehand.
- Tube stand
- Microcentrifuge (c.a. 8,000 × g (10,000 rpm))
- Rotary shaker with heater (for tissue lysis at 55°C)

4. Safety Warnings

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- 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/)

◆ EDT (Proteinase K)
- 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.

◆ MDT (Tissue Lysis 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.
- Wear a laboratory coat, gloves and safety goggles during experiments.

◆ LDT (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.

◆ WDT (Wash Buffer)
- Include flammable liquids, so be careful with the fire
- 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.

◆ CDT (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 LDT 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

- QuickGene-AutoS DNA Tissue Kit (AS-DT) basically corresponds to genomic DNA extraction from 5 mg of animal tissue sample.

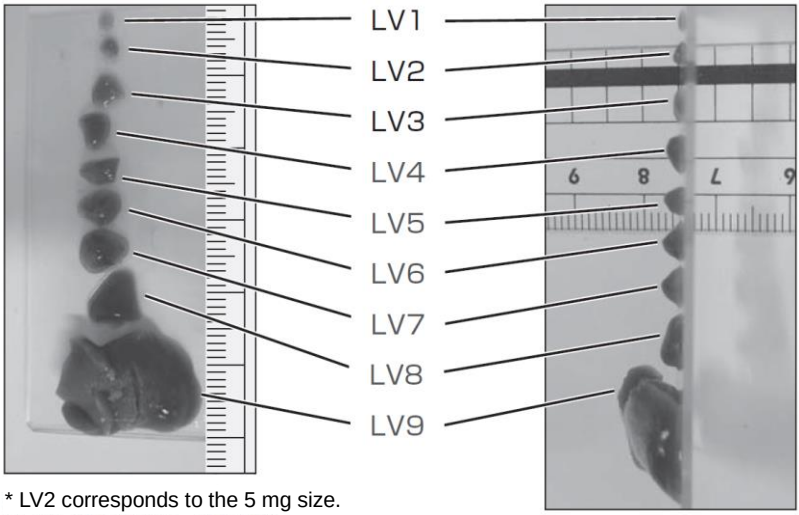
Table 1 : Maximum amount of starting material
This is an example of a normal tissue of Balb/c mouse (female, 7-week old).

Tissue	Maximum amount
Liver	10 mg
Lung	10 mg
Kidney	10 mg

- The maximum amount of tissue may vary depending on conditions and sites of tissue sample. The maximum amount of tissue may be decreased from the respective values shown in Table 1, depending upon the site, condition and digested state of a tissue sample.
- If you use QuickGene-AutoS DNA Tissue Kit (AS-DT) for the first time, start with 5 mg of tissue. Performing a preliminary test is recommended.
- Do not overload the Cartridge (CA), as this will significantly reduce genomic DNA yield and quality. In the worst case, the Cartridge may clog.
- RNA is purified together with genomic DNA. If contamination with RNA is not desired, perform RNase treatment.
- Keeping the tissues at room temperature for a long time and/or repeatedly freezing or thawing degrades the genomic DNA or lowers the yield.
- Figure 1 shows the relationship between the weight and the dimensions of samples of normal mouse tissue (liver). Please use this for reference. 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.

Figure 1 : Relationship between the weight and the dimensions of samples of normal mouse tissue(liver).

No.	Weight	Long axis	Short axis	Height	
LV1	2.3 mg	1.5 mm	1.5 mm	0.5 mm	Range within the capacity
LV2	5.0 mg	2.0 mm	2.0 mm	1.0 mm	
LV3	11.6 mg	4.0 mm	4.0 mm	1.0 mm	
LV4	16.2 mg	5.0 mm	4.0 mm	2.0 mm	Out of application
LV5	21.7 mg	5.0 mm	3.5 mm	2.5 mm	
LV6	25.6 mg	6.0 mm	5.0 mm	2.5 mm	
LV7	30.7 mg	7.0 mm	5.0 mm	2.5 mm	
LV8	56.7 mg	8.0 mm	7.0 mm	2.5 mm	
LV9	850.2 mg	20.0 mm	14.0 mm	8.0 mm	



* LV2 corresponds to the 5 mg size.

◆ Use of Reagent

- If the precipitates are formed in MDT during storage, dissolve them fully by incubating at 55°C. Cool down it to room temperature before use.

◆ 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 (containing tissue lysate) 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°C - 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 Tissue Kit (AS-DT) 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

QuickGene DNA tissue kit S (DT-S) corresponds to the extraction of genomic DNA from animal tissue, basically 5 mg of tissue.
Table 3 shows examples of genomic DNA yield and purity when this kit is used for extraction from normal mouse tissue (A260/280).

Table 3 : Examples of Yields and purities of genomic DNA obtained from normal tissues of Balb/c mouse (female, 7-week old), with RNase treatment.

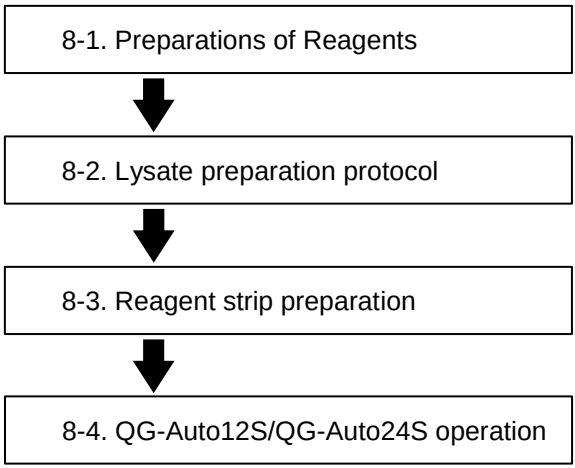
Sample	Amount of genomic DNA (µg)	A260/280
Liver	5.5	2.05

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

- Yields and purity may vary depending on the sample species, condition and tissue type.
- Repeatedly freezing or thawing degrades the genomic DNA or lowers the yield.
- RNA is purified together genomic DNA. If contamination with RNA is not desired, perform RNase treatment.
- When treating tissue rich in RNA such as a liver with RNase under standard protocol, RNA digestion may incomplete. The conditions for using RNase should be investigated.
- The default volume of CDT is 200 µl. The minimum elution volume is 50 µl, however the efficiency of elution may decrease when the volume collected is very small.

8. Protocol

[Overview Flow Chart]



8-1. Preparations of Reagents

◆ MDT

Mix thoroughly before use.
If the precipitates are formed, dissolve them fully by incubating at 55°C. Cool down it to room temperature before use.

◆ RNase A (When performing a RNase treatment)

RNase A is not supplied in this kit. Prepare according to 3-[1] (p.5).

8-2. Lysate preparation protocol

Notices Follow the protocol of <1> to <5> 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> Prepare a fresh or frozen tissue sample excised from animal.
Use the prescribed amount of tissue (in principle, 5 mg).
Excessive amounts of tissue sample results in clogging, low yield, and low purity. In case of clogging, reduce the sample amount.
Do not leave tissue at room temperature, as it might cause genomic DNA degradation.

<2> Cut tissue into small blocks 1.5 to 2 mm square using a pair of scissors, a hammer, and weigh the tissue into 2 ml microtube. Add 180 µl of MDT and subsequently 20 µl of EDT.
In case of using frozen tissue, add MDT immediately after thawing the tissue to room temperature.
In case of using fresh tissue, immediately add MDT to the tissue.

<3> Lyse the tissue completely with stirring at 55°C. If not stirring, imperfect lysing of some part may occur. If possible, stir with a rotary shaker with a heater. Or lyse tissue well by warming with occasionally vortexing.
The lysis time varies depending upon the types of tissue. For example, in the cases of brain, lung and kidney, take about 16 hours and in the case of liver, take about 3 hours. If tissue is lysed incompletely, extend the time.

<4> In order to remove unlysed portions, centrifuge at 8,000 × g (approximately, 10,000 rpm) at room temperature for 3 min. Transfer the supernatant to a new 2 ml microtube without sucking in the unlysed portion of tissue (unlysed residue, gelatinous substance, etc.).
Recommendation product of 2 ml microtube: M&S Instruments Cat. 4020
SARUSTEDT Cat.72.695.700
Cat.72.695.500S

<5> <u>RNase treatment</u>
RNA is copurified with genomic DNA. If contamination with RNA is not desired, perform a RNase treatment. Without RNase treatment, proceed to “8-3. Reagent strip preparation”. Add 20 µl of RNase A (in the case of Cat. No. 12091 (Life Technologies), 60 µl). Mix RNase A well with the sample fluid by tapping, or pipetting 5 times, vortexing for 5 sec. Flash spin down for several seconds to remove drops from the inside of the lid. Incubate at room temperature for 2 min.
Use a recommended RNase A. If using RNase A with DNase activity, perform the denaturation of DNase (100 C, 15 min) (3-[1] p.5).
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 A to be used.

8-3. 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 the 2 ml microtube containing tissue lysate and 1 ml Long Tip in the specified position.

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 TISSUE”.
<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 5.5 μg from 5 mg of mouse liver 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 Tissue Kit (AS-DT).

(1) Low yield or no DNA obtained

Cause	Action
Inappropriate storage conditions for tissue sample	Yield of genomic DNA varies depending upon the type, bulkiness, amount, storage period and storage conditions of a sample. Store sample under appropriate conditions. As soon as a tissue sample is excised from an animal, soak in MDT immediately or flash frozen with liquid nitrogen and store at -20°C or -80°C.
Imperfect lysing tissue	Soak tissue completely in MDT and EDT to lyse. When lysing, cut tissue into small pieces. Perform shaking with a rotary shaker with a heater. In case no shaker is used, incubate at 55°C with occasionally vortexing. Extend an incubation time for lysing as needed. In the case where a tissue amount exceeds 5 mg and the sample is to be extracted for the first time with QuickGene-AutoS DNA Tissue Kit (AS-DT), adjust the ratio of EDT to MDT for every 5 mg of tissue sample by proportional, so that it is 20 µl : 180 µl. Transfer 200 µl of the supernatant after centrifugation.
Use of too much amount of a tissue sample	Refer to Table 1 (p.9) to reduce tissue amount to the prescribed one.
DNA degradation	Refer to (3) “DNA degradation”.

(2) Clogging of Cartridge (CA) occurs

Cause	Action
Use of too much amount of a tissue sample	Refer to Table 1 (p.9) to reduce tissue amount to the prescribed one.
Imperfect lysing tissue	Soak tissue completely in MDT and EDT to lyse. When lysing, cut tissue into small pieces. Perform shaking with a rotary shaker with a heater. In case no shaker is used, incubate at 55°C with occasionally vortexing. Extend an incubation time for lysing as needed.
Clogging by the unlysed tissue portion	After tissue lysis with MDT and EDT, centrifuge at 8,000 × g (10,000 rpm) for 3 min to remove unlysed tissue portion.

(3) DNA degradation

Cause	Action
Allowing tissue to stand at room temperature	As soon as a tissue sample is excised from an animal, soak in MDT immediately or flash frozen with liquid nitrogen and store at -20°C or -80°C.

(4) Purity of DNA is low

Cause	Action
Imperfect lysing tissue	Soak tissue completely in MDT and EDT to lyse. When lysing, cut tissue into small pieces. Perform shaking with a rotary shaker with a heater. In case no shaker is used, incubate at 55°C with occasionally vortexing. Extend an incubation time for lysing as needed.

(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 kit at room temperature (15-28°C). If a precipitate is formed, dissolve the precipitate by incubation at 55°C for MDT and at 37°C for other solutions. 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

KURABO

KURABO INDUSTRIES LTD.

Bio-Medical Department, Advanced Technology Division

14-30, Shimokida-Cho, Neyagawa,
Osaka 572-0823, Japan
TEL +81-72-820-3079 FAX +81-72-820-3095
URL; http://www.kurabo.co.jp/bio/English/