KURABO

HANDBOOK

QuickGene-AutoS Plasmid Kit (AS-PL)

For extraction of plasmid DNA

Ver.1.1

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 plasmid 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, Plasmid DNA can be easily extracted from recombinant *E. coli.*
- DNA from *E. coli* samples can be simultaneously extracted in following time.
  QG-Auto12S: about 20 minutes for 12 sets of *E. coli* samples
  QG-Auto24S: about 20 minutes for 24 sets of *E. coli* samples
- High-purity plasmid DNA is obtained, which does not contain proteins or chaotropic salts. The resulting high-quality plasmid DNA is suitable for applications such as PCR and restriction endonuclease treatment.

2. Kit Content and Storage Conditions

2-1. Kit Components (48 Preps)

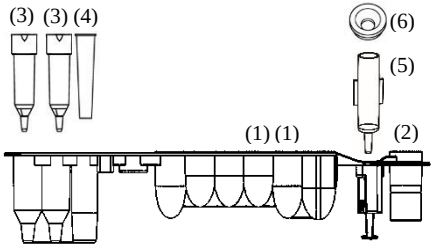
☐	RNase EDP	300 µl
☐	Resuspension Buffer RDP	10 ml
☐	Alkaline Solution ADP	10 ml
☐	Neutralization Buffer NDP	15 ml
☐	Lysis Buffer LDP	10 ml
☐	Reagent strip	48
☐	1 ml Long Tips	48
☐	Waste Tubes	48

2-2. Storage Conditions

All reagents are stable at room temperature (15-28°C) until expiring date indicated at outer box.
It is recommended that EDP be stored in a refrigerator (2°C to 8°C) after unpacking to maintain stability.

2-3. Reagent strip components

☐	Wash Buffer	WDP	750 µl	2 positions	(1)
☐	Elution Buffer	CDP	250 µl		(2)
☐	Short Tip		2		(3)
☐	Tip pack		1		(4)
☐	Cartridge		1		(5)
☐	Pressure Adapter		1		(6)



3. Other Required Materials, Not Supplied in This Kit

[1] Reagents

- High grade ethanol (>99%)
  Used for preparation of LDP

[2] Equipment

- QuickGene-Auto12S or QuickGene-Auto24S
- Micropipettes and tips
- 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.
- Tube stand
- Microcentrifuge (capable of centrifuging as much as 8,000×g (10,000 rpm)

4. Safety Warnings

Warning For research use only.
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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/)

◆ EDP (RNase)
- 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.

◆ RDP (Resuspension 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.

◆ ADP (Alkaline Buffer)
- The substance irritates the skin and eyes when touched.
- 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 appropriate protective gloves and goggles when handling this chemical.
- Since the pH of ADP is high, when disposing of the product without use, take appropriate measures such as neutralization.

◆ NDP (Neutralization Buffer)
- The substance irritates the skin and eyes when touched.
- Inhalation may be harmful.
- 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.
- Handle in well-ventilated area.
- Keep the container tightly closed.

◆ LDP (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.

◆ WDP (Wash Buffer)
- Flammable liquid is included. 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.

◆ CDP (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.

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

◆ **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
- This kit is based on the isolation of plasmid DNA from 1-2 ml of culture medium of transformed *E. coli* with high copy plasmid DNA.
- Yield is up to sample status. If the sample volume is large, cell lysis may not be sufficient or yield may be reduced.
- The use of endA+ *E. coli* may not result in the performance of the kits.
  Repeated freezing and thawing of frozen samples may reduce yield or decrease plasmid DNA size.

◆ Use of Reagent
- ADP may cause white deposits when room temperature is low. If any deposits occur, melt at 37°C and allow to warm to room temperature before use.

◆ Procedure of Extraction
- Do not take time during separation and perform the procedure quickly.
- This kit assumes dissolution with 50 µl of CDP. The amount of CDP may be changed, but the dissolution efficiency may change.
- 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 (Lysate 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°C 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 QG-Auto12S/QG-Auto24S for details.

6. Quality control

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

7. Product Description

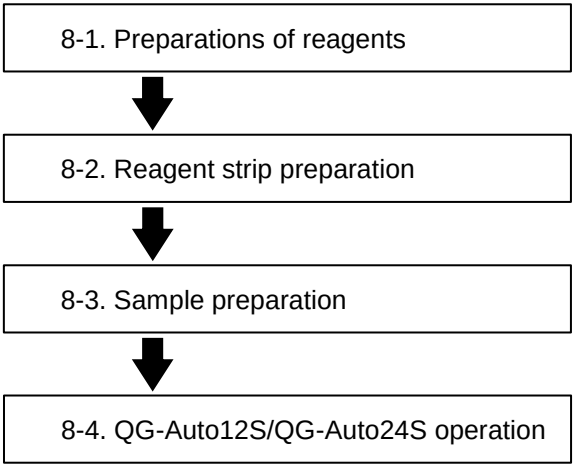
This kit corresponds to high copy plasmid DNA isolation from 1-2 ml of culture of transformed *E. coli* LB medium. The yield varies depending upon sample conditions. Table 1 shows the yield and purity of plasmid DNA obtained by transdorming DH5α with 1 kb recombinant plasmid DNA into pBlueScriptII vectors and isolating it from 1 ml of medium cultured overnight at 37℃ in LB medium.

Table 1 Examples of plasmid DNA yields

Sample	Yield of plasmid DNA (μg)	A260/280
pBlueScriptII/GAPDH/DH5α 1 x 10 ⁹ cells	23.9	2.09

8. Protocol

〔Overview Flow Chart〕



8-1. Preparation of Reagent

◆ EDP

It is recommended that EDP be stored in a refrigerator (2°C to 8°C) to keep it more stable.

◆ RDP

Before use, add all EDP to the RDP bottle and mix well. RDP with EDP should be stored in a refrigerator (2°C to 8°C) and used for about 6 months.

◆ ADP

Mix before use. Avoid vigorous shaking to foam. ADP may cause white deposits when room temperature is low. If any deposits occur, dissolve at 37°C and allow to warm to room temperature before use. Immediately after use, close the lid tightly. If left open, activity decreases.

◆ NDP

Mix thoroughly before use.

◆ LDP

It is delivered in a concentrated state. Before use, add exactly 22 ml of high-grade ethanol (>99%) to the bottle and mix well. Avoid vigorous shaking to foam. After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label. Close the cap firmly to prevent volatilizing.

8-2. Lysate Preparation Protocol

⚠ Note: Be sure to follow the sequence of steps <1> to <6>.
If the order is changed, the desired yield may not be obtained.

- Wear appropriate protective equipment to reduce the risk of chemical injury and infection.
- Wear gloves to avoid nuclease contamination when using reagent strips or tubes.

<1> Culture the transformed E. coli in 1-2 ml of LB medium for 12-16 hours. Transfer the 1 ml of the culture fluid into a 1.5 ml microtube, and centrifuge at 3,300 × g (6,000 rpm) for 10 min at room temperature to collect the bacterial cells. Then remove the medium. If pellet is prepared too hard, it will cause difficulty in resuspension thereof.

<2> Prepare RDP mix (Add total amounts of EDP-01 to RDP bottle, and mix well.) Add 100 µl of RDP mix to the pelleted bacterial cells. Stir it vigorously with vortex to surely suspend the bacterial cells. Thereafter, flash spin down for several seconds to remove drops from the inside of the lid.
If suspension is inadequate, bacteriolysis does not proceed well, resulting in deterioration in the yield of plasmid DNA. It is recommended to preserve RDP mix under refrigeration (2-8°C) and use within 6 months.

<3> Add 100 µl of ADP, and immediately mix by inverting the tube 5 times. Thereafter, flash spin down for several seconds to remove drops from the inside of the lid.
Mix slowly and surely without shaking to blend liquids. Addition of ADP renders the resulting mixture viscous. Vigorous mixing results in the copurification of much of genomic DNA. Too slow mixing causes inadequate blending of liquids, resulting in deterioration in the yield of plasmid DNA. Allowing the mixture to stand at room temperature for 5 min or more may cause denaturation of plasmid DNA. After use of ADP, close the cap tightly. If the precipitates are formed in ADP, when room temperature is low, dissolved them fully by incubation at 37°C. Cool down it to room temperature before use. Allowing the bottle to stand in an open state causes deterioration of the activity.

<4> Add 140 µl of NDP, and immediately mix by inverting the tube 5 times. Thereafter, flash spin down for several seconds to remove drops from the inside of the lid.
Mix slowly and surely without shaking to blend liquids. After addition of NDP, a white precipitate will be formed. Vigorous mixing may result in the copurification of much of genomic DNA. Too slow mixing causes inadequate blending of liquids, resulting in deterioration in the yield of a plasmid DNA.

<5> Centrifuge at 18,000 × g (14,100 rpm) for 10 min at room temperature to separate precipitate and supernatant. While centrifuging, dispense 320 µl of LDP (>99% ethanol added) into a new 1.5 ml microtube.
Be cautious in performing this operation, because much of genomic DNA would be copurified, if any of the precipitate is sucked in.

<6> Transfer the supernatant (about 330 µl) to the new 2 ml microtube (sample tube) with LDP prepared at step <5>, and vortex at the maximum speed for 30 sec (lysate).
Inadequate mixing results in the deterioration in the yield of the plasmid DNA.

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 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 “PLASMID DNA”.

<4> Choose the elution volume.

<5> Make sure all the accessories has been put 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.

- 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 plasmid DNA for a long time, it is recommended to preserve them at –20°C.

9. Trouble-shooting

Review the information below to troubleshoot the experiments with QuickGene-AutoS Plasmid Kit (AS-PL).

(1) Low yield or no DNA obtained

Causes	Measure
Insufficient lysis of bacteria	① Insufficient dispersion of bacteria in RDP. Disperse well. ② The mixing of ADP is insufficient. Gently and thoroughly mix the solution. ③ The amount of bacteria is too large. Approximately 1-2 ml of LB culture medium for 12-16 hours should be recommended.
Inadequate amount of reagent	Add the volume according to the protocol. Add all EDP to RDP for use.
Inadequate sample volume	Approximately 1-2 ml of LB culture medium for 12-16 hours should be used. Check that the bacteria have grown well. The culture medium incubated for too long contains lysed cells and degraded nucleic acid.
Vortex after addition of LDP is insufficient.	After adding LDP, vortex thoroughly at maximum speed (30 seconds).
Without adding a specified quantity of high grade ethanol to LDP	Before using LDP, a specified quantity of high grade ethanol (>99%) must be added. (Refer to 8-1, p.9)

(2) RNA was collected

Causes	Measure
Insufficient degradation of RNA	Add all EDP to the RDP bottle and mix well before use. If the sample volume is too large, reduce the sample volume to a predetermined volume (about 1-2 ml of culture medium for 12-16 hours in LB medium).

(3) Genomic DNA was collected

Causes	Measure
Incorrect lysis of cells	By inversion mixing in the process of adding or mixing ADP or NDP Mix the solution and do not agitate vigorously. Do not allow to stand for at least 5 minutes when adding or mixing ADP.
Inappropriate sample	If the incubation time is long, the number of lysed cells will increase. Therefore, culture should be maintained for about 12-16 hours.
Precipitates were contaminated during recovery of the supernatant after centrifugation.	Collect the supernatant to prevent precipitation after NDP addition.

(4) Clogging of Cartridge (CA) occurs

Causes	Measure
Too much samples.	Predetermined quantity (culture medium LB for 12-16 hours)
Inappropriate sample	If the incubation time is long, the number of lysed cells will increase. Therefore, culture should be maintained for about 12-16 hours.

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

Causes	Measure
Inappropriate amount of DNA is used	Determine the DNA concentration based on the absorbance at 260 nm.
DNA degradation	It is recommended that the plasmid DNA be stored at-20°C after elution. Plasmid DNA may be degraded when isolated from an old culture. If the pellet is not used immediately, it is recommended that it be stored frozen (-80°C). Allow to warm to room temperature before isolation.

(6) Precipitates occurred in the reagent.

Causes	Measure
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

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/

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