KURABO

HANDBOOK

QuickGene Plasmid kit S Ⅱ (PL-S2)

For extraction of plasmid DNA from *Escherichia coli*

Ver.2.0

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; moreover, with its patented thinmembrane, 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 using this kit with QuickGene, high quality and high yield plasmid DNA can be extracted and also purified from *E.coli*.
No hazardous organic solvents such as phenol and chloroform are used.
Plasmid DNA from 8 sets of *E. coli*. lysate samples can be simultaneously extracted in following time.
QuickGene-810/QuickGene-800 (QG-810/QG-800) : about 6 min
QuickGene-Mini80 (QG-Mini80) : about 4 min
The purified, high quality plasmid DNA is suitable for DNA sequencing, PCR, restriction enzyme digestion and other applications.

Please be sure to read the User's Guide of QuickGene carefully before using this kit.

2. Kit Components and Storage Conditions

2-1 Kit Components (96 Preps)

☐ RNase	EDP-01	600 µl
☐ Resuspension Buffer	RDP	20 ml
☐ Alkaline Solution	ADP	20 ml
☐ Neutralization Buffer	NDP	30 ml
☐ Lysis Buffer	LDP	20 ml
☐ Wash Buffer	WDP	64 ml
☐ Elution Buffer	CDP	100 ml
☐ Cartridges	CA	96
☐ Collection Tubes	CT	96
☐ Caps	CAP	96
☐ Waste Tubes	WT	96

2-2 Storage Conditions

All reagents are stable for 9 months after purchase at room temperature (15-28°C). We suggest keeping RNase (EDP-01) at 2-8°C to prolong the life.

3. Other Required Materials, Not Supplied in This Kit

[1] Reagents
- >99% Ethanol (for preparation of LDP and WDP working solution)

[2] Equipments
- QuickGene
- Centrifuge Tubes * (Large/Small sets)
- Micropipettes and tips
- 1.5 ml microtubes
- Tube stand
- Vortex mixer (maximum speed 2,500 rpm or more)
- Microcentrifuge (c.a. 18,000 × g (14,100 rpm))

*Centrifuge tubes are used with the QG-810/QG-800 as containers for WDP (>99% ethanol added) and CDP. They are unnecessary when QG-Mini80 is used.

Recommendation product of centrifuge tubes are following Table 1.
Use centrifuge tubes according to the number of Cartridges to use.

Table 1 Recommended centrifuge tubes (In case of QG-810/QG-800)

Size of Buffer Stand (Centrifuge Tube Holder)	The number of Cartridges	Type of centrifuge tube	Product name (Examples)
Standard	-16	Large centrifuge tube (for WDP)	BD Falcon™ 50 ml conical tube
		Small centrifuge tube (for CDP)	BD Falcon™ 15 ml conical tube
Large	-72	Large centrifuge tube (for WDP)	BD Falcon™ 175 ml conical tube
		Small centrifuge tube (for CDP)	BD Falcon™ 50 ml conical tube

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 Material Safety Data Sheet for specific recommendations, http://www.kurabo.co.jp/bio/English/)

◆ EDP-01 (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 Solution)
- **Irritating to skin and eyes.**
- 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 laboratory coat, gloves and safety goggles during experiments.
- As ADP has a high pH value, in the case of discarding unused ADP, perform suitable treatments such as neutralization, etc.

◆ NDP (Neutralization Buffer)
- **Irritating to skin and eyes.**
- **Harmful if ingested.**
- When it comes into contact with your skin or eyes, it irritates them.
- 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.
- It should be handled at a well-ventilated place.
- Keep the bottle tightly closed.

◆ LDP (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.

◆ WDP (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.

◆ 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.

◆ **In the case of using a 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

- The kit would be able to extract the high-copy plasmid DNA from 1-2 ml over-night culture of recombinant *E. coli* in LB medium.
- Yields vary depending upon sample conditions. In case there is a large amount of sample, it may be possible that cell lysis is not performed adequately, or yield may be decreased.
- In the case of using endA+ *E. coli*, there may be a possibility that the performance of the kit is not exerted.
- In the case of repeating thawing and freezing of a frozen sample, it may possibly cause decrease in yield or shortening of the plasmid DNA size.

◆ Use of Reagent

- If the precipitates are formed in ADP, dissolve them fully by incubation at 37°C. Cool down it to room temperature before use.

◆ Procedure of Extraction

- Use the QuickGene Plasmid kit S Ⅱ (PL-S2) at room temperature (15-30°C). In case of using at lower or higher temperature, it may affect the extraction performance.
- During the procedure, work quickly without interruption.
- It is assumed for this kit that elution is performed with 50 µl of CDP. The volume of CDP is changeable, but there is a possibility that the elution efficiency is changed.
- We recommend starting preparation of lysate after setup of QuickGene. Refer to the following pages.
  QG-810/QG-800 : 8-3 (p.14), Appendix 1 (p.24), Appendix 2 (p.25)
  QG-Mini80 : 8-4 (p.16)
- Refer to QuickGene User's Guide for details.

6. Quality Control

- As part of the stringent quality assurance program in KURABO INDUSTRIES LTD., the performance of QuickGene Plasmid kit S Ⅱ (PL-S2) 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

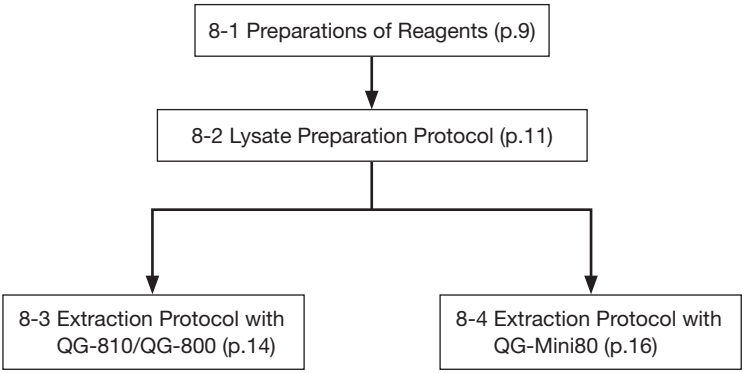
QuickGene Plasmid kit S Ⅱ (PL-S2) corresponds to the extraction/purification of a plasmid DNA from *E. coli*, which is transformed with a high-copy plasmid DNA, in 1-2 ml of the LB medium. For example, a plasmid DNA in which a 1 kb gene was recombined into pBlueScript Ⅱ Vector was transformed into DH5α followed by culture of the resulting *E. coli* in the LB medium at 37°C for overnight, and then the plasmid DNA was extracted from 1 ml of the culture fluid.

Table 2 Yield and purity of plasmid DNA (Data are the average of eight samples.)

Sample	Amount of plasmid DNA recovered (μg)	A260/280	A260/230
pBlueScript Ⅱ/GAPDH/DH5α 1×10^9	21.4	1.99	2.49

8. Protocol

[Overview Flow Chart]



8-1 Preparations of Reagents

◆ EDP-01 (600 µl)

We suggest keeping EDP-01 at 2-8°C to prolong its life.

◆ RDP (20 ml)

Before starting an extraction experiment, add total amounts of EDP-01 to RDP bottle, and mix well. In the case of storing RDP mix, it is recommended to preserve it under refrigeration (2-8°C) and use within 6 months.

◆ ADP (20 ml)

Mix thoroughly before use. Avoid vigorous shaking as it causes foaming.
If the precipitates are formed, dissolve them fully by incubation at 37°C. Cool down it to room temperature before use. Immediately after use, close the cap tightly. Allowing the bottle to stand in an open state causes deterioration of the activity.

◆ NDP (30 ml)

Mix thoroughly before use.

◆ LDP (20 ml)

LDP is supplied as a concentrate.
Add 44 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use. Avoid vigorous shaking as it causes foaming. After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label. Close the cap firmly to prevent volatilizing.

◆ WDP (64 ml)

WDP is supplied as a concentrate.
Add 256 ml of >99% ethanol into the bottle and mix by gently inverting the bottle before use. After adding ethanol, enter a check in the [ethanol added?] check box on bottle cap label. Close the cap firmly to prevent volatilizing.

◆ **CDP (100 ml)**

Use CDP for elution of plasmid DNA.

◆ **Required volume of WDP(>99% ethanol added) and CDP (In the case of using QG-810/ QG-800)**

Prepare the required volume of WDP and CDP into the tubes (see Table 1 p.4) : set them to Buffer Stand.

Table 3 Required volume of WDP and CDP

Number of Cartridges	WDP (QG-810/QG-800)	CDP (QG-810)	CDP (QG-800)
8	26 ml	9 ml	8 ml
16	44 ml	11 ml	11 ml
24	62 ml	13 ml	13 ml
32	80 ml	15 ml	15 ml
40	99 ml	17 ml	17 ml
48	117 ml	19 ml	19 ml
56	135 ml	21 ml	21 ml
64	154 ml	22 ml	22 ml
72	172 ml	24 ml	24 ml

*Required volume of discharge
QG-810 : WDP 8.0 ml, CDP 7.4 ml
QG-800 : WDP 8.0 ml, CDP 6.4 ml
Depending on the number of the Cartridges, add WDP and CDP.
Use WDP 1.5 ml and CDP 50 µl per 1 Cartridge.
For example, in case of using 2 Cartridges, 11 ml of WDP, 7.5 ml of CDP (QG-810) and 6.5 ml of CDP (QG-800) are required.
*Use appropriate tubes according to Table 1 (p.4).

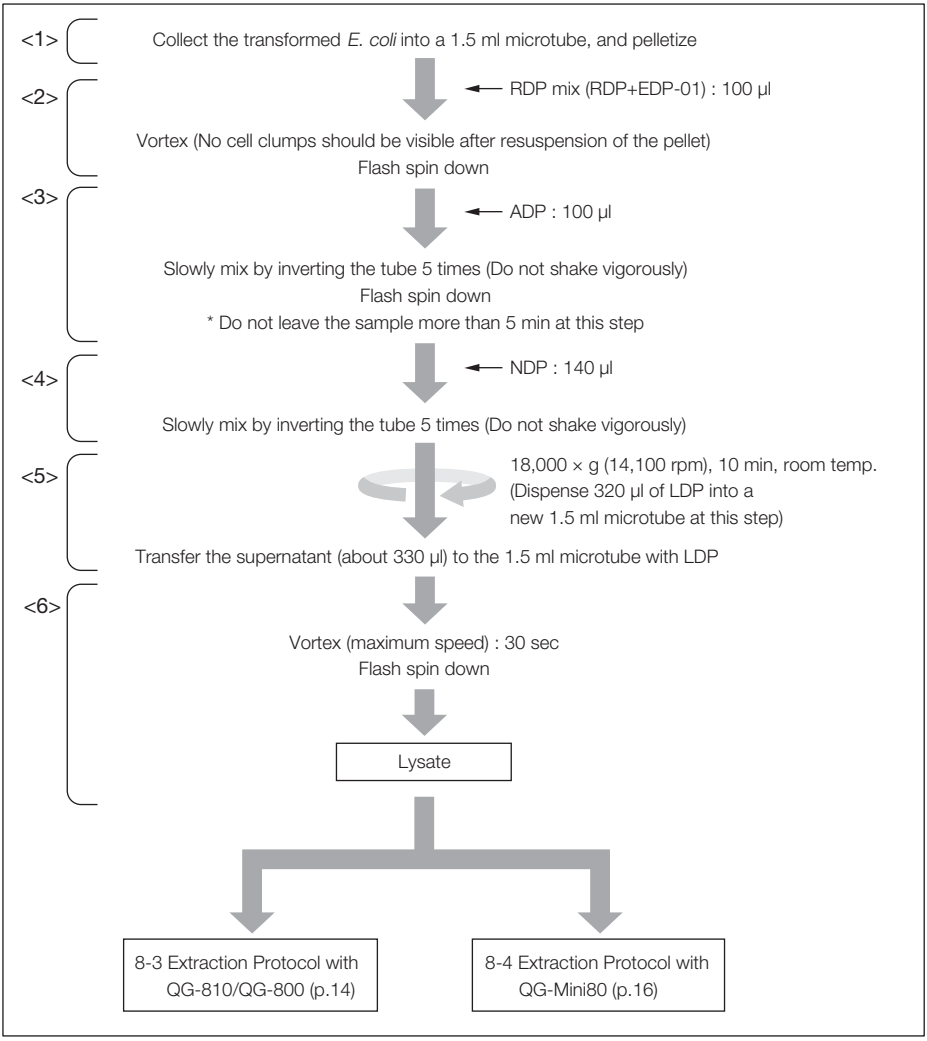
8-2 Lysate Preparation Protocol

QuickGene Plasmid kit S **II** (PL-S2) is corresponds for the extraction of high-copy plasmid DNA from 1-2 ml over-night culture of recombinant *E. coli* in LB medium.

[Important notes before starting]
- Cool down all reagents to room temperature before use.
- Follow the volume of buffers described in the workflow (p.12).
- To prevent cross-contamination, it is recommended to change pipette tips between all liquid transfers.
- During the procedure, work quickly without interruption.
- In case of disposing of ADP(pH is high), dispose after neutralizing it.
- When using potentially infectious samples for experiments, dispose of them according to the applicable regulations.

[Preparations for starting the experiment]
- Check that total amounts of EDP-01 are added to RDP before starting an experiment.
- LDP is supplied as a concentrate. Check that 44 ml of >99% ethanol is added to LDP before starting an experiment.
- WDP is supplied as a concentrate. Check that 256 ml of >99% ethanol is added to WDP before starting an experiment.

Lysate Preparation Workflow



Details of Lysate Preparation Workflow

<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 microtube 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.

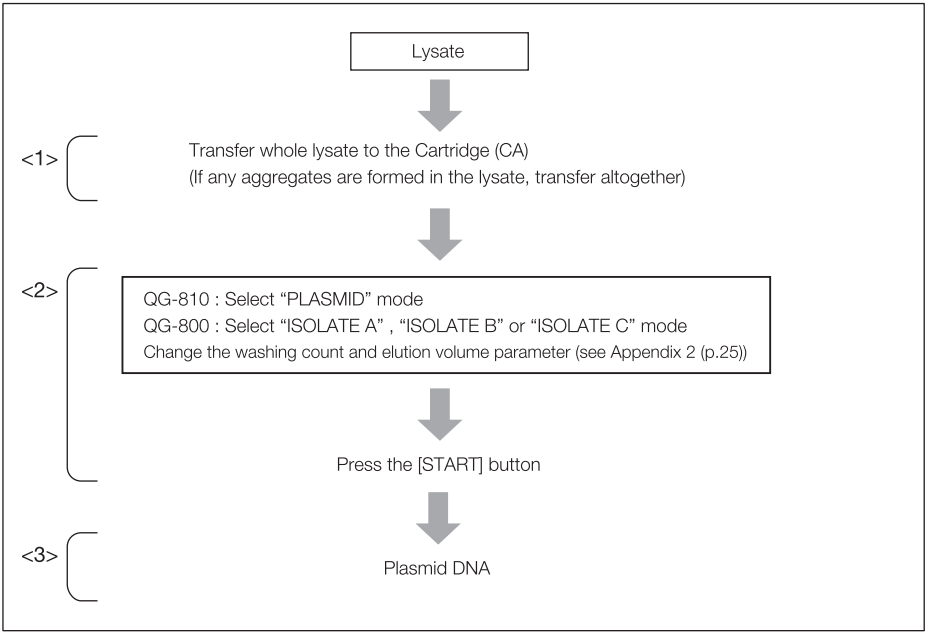
Perform the extraction operation quickly after completion of lysis.
QG-810/QG-800 (p.14)
QG-Mini80 (p.16)

8-3 Extraction Protocol with QG-810/QG-800

- Please read the User's Guide of QG-810/QG-800 for the details before using the system.
- Check that 256 ml of >99% ethanol is added to WDP before starting an experiment.
- Select "PLASMID" mode of QG-810 for plasmid DNA extraction. (see Appendix 2 (p.26))
- Select "ISOLATE A", "ISOLATE B" or "ISOLATE C" mode of QG-800 for plasmid DNA extraction, change the washing count and elution volume parameter.
- All reagent, Cartridges (CA) and tubes are manufactured in clean rooms. Wear gloves during the experiments to avoid nuclease contamination.
- Refer to the User's Guide of QG-810/QG-800 for the details of setting Cartridges (CA), tubes and each reagents.
- Open the front cover of QG-810/QG-800 and set the Collection Tubes (CT) and Waste Tubes (WT) in the Tube Holder (or Collection Tube Holder). Use the specified Cartridges (CA).
- Set WDP (>99% ethanol added) and CDP to QG-810/QG-800 referring to p.10.
- Incorrect Cartridge (CA) placement may result in the solution spilling or improper extraction.
- Press the [DISCHARGE] button after closed the front cover of QG-810/QG-800. Because of air in the lines, incorrect volume of reagents may occur without discharge operation.

- Avoid touching the filter in the Cartridge (CA) with the pipette tip.
- When using potentially infectious samples for experiments, dispose of them according to the applicable regulations.

QG-810/QG-800 Workflow



Details of QG-810/QG-800 Workflow

<1> <Applying lysate> Carefully transfer the whole lysate (see 8-2 (p.11)) to the each Cartridge (CA).

If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.

<2> <Extraction> Close the front cover of QG-810/QG-800. Confirm the appropriate mode on the operation panel and press the [START] button.

The operation panel shows “PROCESSING” (QG-810) or “EXECUTING” (QG-800) during extracting. In case of using QG-810, extraction progress can be checked by blinking of each lamp (BINDING, WASHING, ELUTION).

Warning Do not open the front cover during the extraction process (while “PROCESSING” or “EXECUTING” is shown on the operation panel). If the front cover is opened, the extraction process will be halted. Confirm it by Table 4.

Table 4 Movement when you opened a front cover during extraction

	QG-810	QG-800
Extraction process	Stop	Stop
Extraction continuation	possible*1	impossible*2

*1 QG-810 : See User’s Guide of QG-810, “3.5 Operations to Restart Program from Pause” (p.28).

*2 QG-800 : The sample that was on the way of the extraction cannot be used again. Discard according to the applicable regulations. Reter to “Disposal of waste fluid and consumables when using potentially infectious samples” of this handbook (p.6).

<3> <Extraction completion>
Operation panel displays the extraction results.

Table 5 Extraction result

	QG-810	QG-800	Remarks
Successfully extracted	v (Check)	○	
Extraction failure	- (Hyphen)	×	Cartridge is clogged
No Cartridge, or No sample	_ (Underscore)	▲	No Cartridge or occurrence of error before extraction

Open the front cover and remove the Collection Tubes (CT) from the Tube Holder after QG-810/QG-800 completely stopped.
The volume of the eluate from each Cartridge (CA) will be 50 µl.
Cover with the Caps (CAP) on the Collection Tubes containing the extracted plasmid DNA tightly.
In case of storing plasmid DNA for a long time, it is recommended to preserve at –20°C.

<4> Remove the Waste Tubes (WT) and dispose the waste fluid according to applicable regulations.
Remove the Cartridge Holder and dispose the Cartridges (CA).

8-4 Extraction Protocol with QG-Mini80

- Please read the User's Guide of QG-Mini80 for the details before using the system.
- Check that 256 ml of >99% ethanol is added to WDP before starting an experiment.
- Set Waste Tubes (WT) into Tube Holder.
- Set Tube Adapters to the Tube Holder, and set Collection Tubes (CT). In substitution for Collection Tubes, you can use 1.5ml microtubes. In this case Tube Adapters are unnecessary.
- Insert the Cartridge Holder into the Wash Position (W) of the Tube Holder. Then set Cartridges (CA) to the Cartridge Holder. Confirm the Release Lever is positioned at the left-hand end of the Cartridge Holder.

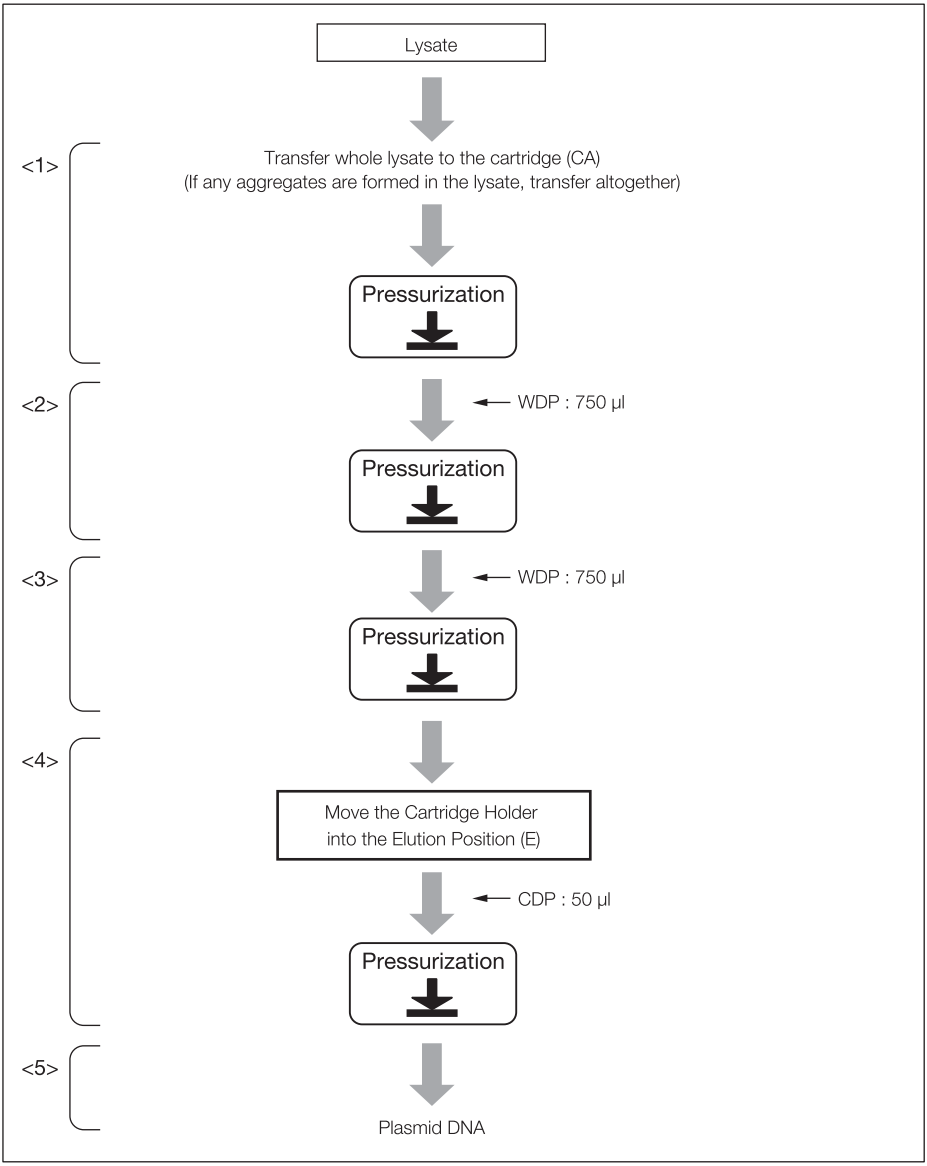
- When setting the Tube Holder and the Cartridge Holder to QG-Mini80, insert to the end.
- When pressuring lysates and WDP (>99% ethanol added), confirm that Wash Label on the Tray can be entirely seen.
- When pressuring CDP, confirm that Wash Label on the Tray can not be seen, being hidden below the Tube Holder.

- Avoid touching the filter in the Cartridge (CA) with the pipettle tip.
- When using potentially infectious samples for experiments, dispose of them according to the applicable regulations.

QG-Mini80 Workflow

The pressurization mark “Pressurization” in the workflow indicates the following operations.

1. Set the Cartridge Holder and Tube Holder in QG-Mini80.
2. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
3. Make sure that no liquid remains in the Cartridges and then return the Rotary Switch to the original position.
4. Pull out the Cartridge Holder and Tube Holder from QG-Mini80.



Details of QG-Mini80 Workflow

<1> <Applying Lysate> Carefully transfer the whole lysate prepared at 8-2 (p.11) to each Cartridge (CA).
Set the Tube Holder and Cartridge Holder into QG-Mini80. After setting it, check the Wash Label can be seen.
Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no lysate remains in the Cartridges and then return the Rotary Switch to the original position.

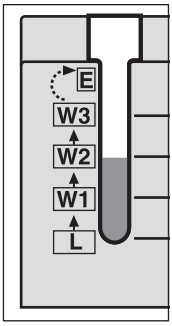
If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge.
Perform the extraction operation quickly after completion of lysis.
Pressure application automatically stops in about 1 min. If any lysate remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.

<2> <First wash> Pull out the Cartridge Holder and the Tube Holder and then apply 750 µl of WDP to the Cartridges (CA). Set the Tube Holder and the Cartridge Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no WDP remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any WDP remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.

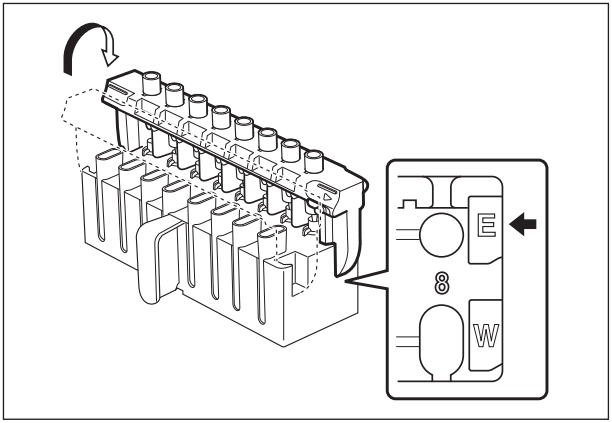
<3> <Second wash> Pull out the Cartridge Holder and the Tube Holder and then apply 750 µl of WDP to the Cartridges (CA). Set the Tube Holder and the Cartridge Holder into QG-Mini80. After setting it, check the Wash Label can be seen. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges.
Make sure that no WDP remains in the Cartridges and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any WDP remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.
After second wash, the waste fluid scale of the Tube Holder indicates [W2] position. (Refer the following illustration)
Do not add WDP three or more times. The contamination might be caused the waste fluid touching the Cartridges, or the waste fluid might overflow from the Waste Tubes.



<4> <Elution> Pull out the Cartridge Holder and the Tube Holder and then insert the Cartridge Holder into the Elution Position (E) of the Tube Holder. Do not touch the Release Lever. Apply 50 µl of CDP to the Cartridges (CA) and then set the Cartridge Holder and Tube Holder in QG-Mini80. After setting it, check the Wash Label cannot be seen by hiding under the Tube Holder. Rotate the Rotary Switch toward the front side to apply air pressure to the Cartridges. Make sure no CDP remains in the Cartridges, and then return the Rotary Switch to the original position.

Pressure application automatically stops in about 1 min. If any CDP remains in the Cartridges even after about 1 min has elapsed, return the Rotary Switch to the original position and then rotate it toward the front side to put pressurized air into the Cartridges again.



<5> Pull out the Tube Holder and the Cartridge Holder. Remove the Cartridge Holder from the Tube Holder and then dispose of the Cartridges (CA). When sliding the Release Lever to the right-hand end, all Cartridges will fall down. Remove the Collection Tubes and then insert the Collection Tubes into the Tube Rack (optional) and then put Caps (CAP).
When the Tube Rack is not used, remove the Collection Tubes after putting Caps on them.
When using commercially available 1.5 ml microtubes : Put caps on 1.5 ml microtubes and then remove them.
Dispose of the Waste Tubes and waste fluid according to appropriate laws and rules.

In case of storing plasmid DNAs recovered for a long term, it is recommended to preserve them at –20°C.

9. Troubleshooting

Review the information below to troubleshoot the experiments with QuickGene Plasmid kit S **II** (PL-S2).

(*) : For QG-810/QG-800
(**) : For QG-mini80

(1) Low yield or no Plasmid DNA obtained :

Cause	Action
Incompleately dissolved samples	1) Suspension of bacterial cells with RDP is inadequate. Suspend it well. 2) Mixing of ADP with stirring is inadequate. Mix well so that the mixture is well blended. 3) The amount of bacterial cells used is too much. Use 1-2 ml of the cultured LB medium for 12-16 hours.
Inappropriate addition order of reagents	Add each volume of liquids in accordance with the protocol. Use RDP to which EDP-01 is added. (See section 8-1 p.9)
Inappropriate amount of sample	Reduce sample volume. Use 1-2 ml of the cultured LB medium for 12-16 hours as a measure. Confirm the bacterial growth. In case culture period is too long, bacteriolysed cells and decomposed nucleic acids will contaminate in the resulting culture fluid.
Insufficient vortexing after addition of LDP	After adding of LDP, vortex thoroughly (for 30 sec) at the maximum speed.
No addition of the prescribed volume of ethanol to LDP	Before using LDP for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See section 8-1 p.9)
No addition of the prescribed volume of ethanol to WDP	Before using WDP for the first time, ensure that the prescribed volume of >99% ethanol has been added. (See section 8-1 p.9)
Incomplete addition of whole lysate to Cartridge (CA)	If any aggregates are formed in the lysate, transfer altogether with the aggregates to the Cartridge (CA).
Use of reagents other than CDP to elute plasmid DNA	Use CDP to elute plasmid DNA.
Rupture of filter	Be careful not to allow pipette tip to contact with a filter in the Cartridge (CA).
Insufficient amounts of reagents used (*)	Make sure that sufficient amount of reagent are in the reagent bottles.
Inappropriate mode or setting parameter (*)	Operate extracting under the mode set the applicable parameter. When using QG-800 set the parameter. (See Appendix 2 p.24)

(2) RNA is recovered :

Cause	Action
Insufficient RNA decomposition	Add total amounts of EDP-01 to RDP bottle, mix well, and then use. In case too much amount of sample is used, reduce sample volume to the prescribed one (1-2 ml of the cultured LB medium for 12-16 hours), and use.

(3) Genomic DNA is recovered :

Cause	Action
Inadequate cell lysis	In each of the addition and mixing processes of ADP or NDP, perform blending surely by upside-down mixing without stirring vigorously. In the addition and mixing processes of ADP, do not allow the resulting mixture to stand for 5 min or more.
Inappropriate sample	In case culture period is long, the amount of bacteriolysed cells increases. Therefore, culture for about 12-16 hours as a measure.
Contamination of supernatant with precipitate at the time of its recovery	Recover the supernatant without contamination with a precipitate after addition of NDP.

(4) Clogging of Cartridge (CA) occurs :

Cause	Action
Inappropriate amount of sample	Reduce sample volume to the prescribed one (1-2 ml of the cultured LB medium for 12-16 hours).
Not having been centrifuged	Centrifuge the precipitate after treatment with NDP.

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

Cause	Action
Inappropriate amount of plasmid DNA is used	Determine the plasmid DNA concentration based on the absorbance at 260 nm.
Degradation of plasmid DNA	It is recommended to preserve plasmid DNAs at –20°C. In the case where extraction is performed from an old culture fluid, decomposed plasmids are sometimes contained. When pellet is not used immediately, it is recommended to cryopreserve it as a pellet at –80°C. Before extraction, warm to room temperature and then perform operations.
Improper washing procedure (**)	Wash twice with 750 µl of WDP.

(6) A precipitate is formed in reagents :

Cause	Action
Stored at low temperature	Store buffers at the prescribed temperature (15-28°C). In case a precipitate is formed, dissolve the precipitate by incubation at 37°C. Use it after cooling back to room temperature.

(7) No sample is recovered in Collection Tube (CT) or 1.5 ml microtube (it is vacant) :

Cause	Action
Insufficient set of CDP or no operation of discharging (*)	Set the prescribed volume of CDP according to Table 3 (p.10). In addition, it is necessary to perform a discharging operation according to the User's Guide of QG-810/QG-800.
Not addition of CDP (**)	After insert the Cartridge Holder to the Elution Positon (E), add 100 µl of CDP to Cartridge.
No transfer of the Cartridge Holder to the Elution Position (E) when adding CDP (**)	When adding CDP, addition has to be started after the transfer of Cartridge Holder to the Elution Position (E).

(8) Cartridge (CA) can not be held on the Cartridge Holder :

Cause	Action
No return of the Release Lever to the left end (**)	Confirm that the Release Lever is positioned at the left-hand end of the Cartridge Holder, and then set Cartridges (CA).

10. Ordering Information

Product	Cat #
QuickGene DNA tissue kit S	DT-S
For extraction of genomic DNA from tissues	
QuickGene DNA whole blood kit S	DB-S
For extraction of genomic DNA from whole blood	
QuickGene RNA tissue kit S Ⅱ	RT-S2
For extraction of total RNA from tissues	
QuickGene RNA cultured cell kit S	RC-S
For extraction of total RNA from cultured cells	
QuickGene RNA cultured cell HC kit S	RC-S2
For extraction of total RNA from cultured cells	
QuickGene RNA blood cell kit S	RB-S
For extraction of total RNA from leukocytes	
QuickGene Plasmid kit S	PL-S
For extraction of plasmid DNA from *Escherichia coli*	
QuickGene Plasmid kit S Ⅱ	PL-S2
For extraction of plasmid DNA from *Escherichia coli*	

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Appendix 1 Setting of QG-810 Parameter

In the case of using QG-810 select “PLASMID” mode. The parameter of “PLASMID” is The follwing Table.

Display Sequence	LCD message	PARAMETER
1	BIND PEAK	120
2	WASH COUNT	2
3	WASH PEAK	110
4	WASH VOL1	750
5	WASH VOL2	750
6	WASH VOL3	750
7	WASH VOL4	750
8	WASH VOL5	750
9	WASH DIP TM	0
10	WAS2 WAIT T	0
11	WAS2 COUNT	0
12	WAS2 PEAK	110
13	WASH VOL1	750
14	WASH VOL2	750
15	WASH VOL3	750
16	WASH VOL4	750
17	WASH VOL5	750
18	ELUT VOL	50
19	ELUT PEAK	100
20	ELUT DIP TM	0

When changing the parameter, refer to QG-810 User’s Guide.

Appendix 2 Setting of QG-800 Parameter

In the case of using QG-800 the parameter of “ISOLATE A,B,C” is the following Table.
Extracting with this kit use any mode of “ISOLATE A,B,C” by changing parameter.
Refer to QG-800 User’s Guide.

*Gray shadow paramaters do not need to change the value.

Display Sequence	Operation menu	PARAMETER	Check	ISOLATE A, B, C default
1	SAMP PEAK	120		120
2	WASH COUNT	2		3
3	WASH PEAK	110		110
4	WASH VOL1	750		750
5	WASH VOL2	750		750
6	WASH VOL3	750		750
7	WASH VOL4	750		750
8	WASH VOL5	750		750
9	WAS2 COUNT	0		0
10	WAS2 PEAK	110		110
11	WAS2 VOL1	750		750
12	WAS2 VOL2	750		750
13	WAS2 VOL3	750		750
14	WAS2 VOL4	750		750
15	WAS2 VOL5	750		750
16	CLCT VOL	50		200
17	CLCT PEAK	120		120

<How to change parameters>

1. Select the extraction mode.
   Press the [MODE] button several times until “ISOLATE A,B,C” mode appears.

2. Press the [▲] [▼] button simultaneously.
   <Example of messages displayed in operation panel>

SETUP START
↓
ISOLATE A
The present mode is displayed.
↓
SAMP PEAK : 120*

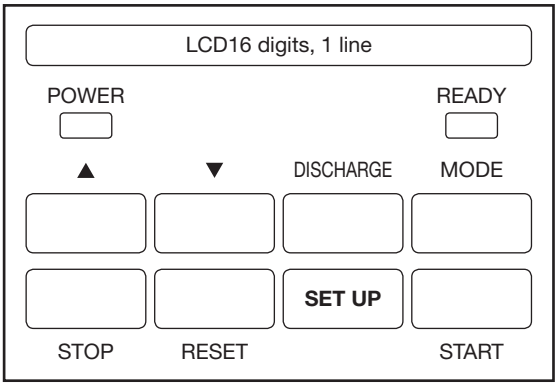
- The first item in operation menu and present set vaule are displayed.
- * is the present set value.

3. Display the item that you want to change.
   Press the [MODE] button continuously till the target item “WASH COUNT”, “CLCT VOL” are displayed.
   To return to the previous item, press the [DISCHARGE] button.

4. Change the setting.
   -[▲] button: raise the setting value.
   -[▼] button: lower the setting value.
   <Example of operating for changing parameter>
   “WASH COUNT” change to “2” → “WASH COUNT” appears by [MODE] button → change to “2”.

Operation items	Default	Changing Value
WASH COUNT	3	2
CLCT VOL	200	50

5. Save the settings.
   Press the [SET UP] button (No name button between [RESET] and [START])



<Display of operation panel>

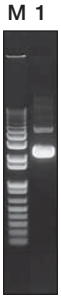
SETUP WRITING
Save setting content.
↓
SETUP FINISH
Returns to ready status.

Appendix 3 Examples of the Data with QuickGene Plasmid kit S Ⅱ (PL-S2)

● **Electrophoresis**

Figure 1 illustrates the results of electrophoresis of a plasmid DNA extracted with this kit.
For the yield and purity, see Table 2 (p.8).

Figure 1



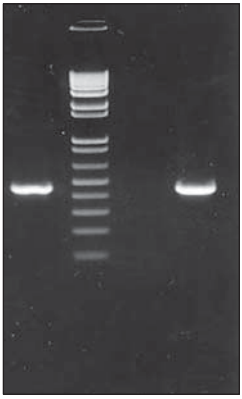
No.	Sample
1	pBlueScript Ⅱ/GAPDH/DH5α

M : 1 Kb Plus DNA Ladder (Invitrogen)

● **PCR Amplification of Recovered Plasmid DNA**

Figure 2 illustrates the results of PCR amplification of a plasmid DNA extracted with this kit.
PCR amplification is possible from 5 ng of template.

Figure 2



No.	Sample
1	pBlueScript Ⅱ/GAPDH/DH5α
2	Negative Control
3	Positive Control

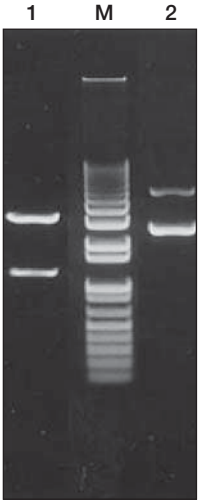
M : 100 bp DNA Ladder (Invitrogen)

● **Results of Restriction Endonuclease Cleavage of Recovered Plasmid DNA**

Figure 3 illustrates the results of restriction endonuclease cleavage of a plasmid DNA extracted with this kit.

Restriction endonucleases (0.5 µl each of Not Ⅰ and Xho Ⅰ) were added to 10 µl of a reaction solution (including 1 µl of the extracted plasmid), then it was incubated for 2 hours at 37°C. From these results, it is understood that restriction endonuclease cleavage is practicable.

Figure 3



No.	Restriction endonuclease added
1	Not Ⅰ+Xho Ⅰ
2	None

M : 1 Kb Plus DNA Ladder (Invitrogen)

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PL-S2_HB_E_V20