NIPPON Genetics EUROPE GmbH

FastGene® Plasmid Mini Kit

For isolation of high copy and low copy plasmid DNA

Cat.No.: FG-90402 for 100 preparations
Cat.No.: FG-90502 for 300 preparations

STORAGE
Store at room temperature (15-25°C)

FastGene® Plasmid Mini Kits are intended for research use only.

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CONTENTS

COMPONENTS

FastGene® Plasmid Mini Kit	100 preparations FG-90402	300 preparations FG-90502
FastGene® mP Column	100	300
2 ml Collection Tube	100	300
Resuspension Buffer mP1	25 ml	65 ml
Lysis Buffer mP2	25 ml	75 ml
Neutralization Buffer mP3	40 ml	100 ml
First Wash Buffer mP4	50 ml	130 ml
Second Wash Buffer mP5 concentrate	25 ml	40 ml
Elution Buffer mP6	10 ml	30 ml
RNase A (lyophilized)	10 mg	26 mg

Materials not supplied

Reagents:	96-100% EtOH
Consumables:	1.5 ml microcentrifuge tube, Disposable pipette tips
Equipment:	Manual pipettors, Centrifuge for microcentrifuge tubes, Heating block, Vortex mixer, Personal protection equipment (lab coat, gloves, goggles)

STORAGE AND PREPARATION

The FastGene® Plasmid Mini Kit should be stored dry at room temperature (15-25°C). Under these conditions, the kit is stable for up to 12 months without showing any reduction in performance and quality. Check buffers for precipitate before use and redissolve at 37°C if necessary.

Before starting any protocol prepare the following:

FastGene® Plasmid Mini Kit	100 preparations FG-90402	300 preparations FG-90502
Second Wash Buffer mP5 concentrate	add 100 ml ethanol (96-100%)	add 160 ml ethanol (96-100%)
Resuspension Buffer mP1 RNase A	Add 1 ml of Buffer mP1 to the RNase A vial and vortex. Transfer all of the resulting solution into the Buffer mP1 bottle and mix thoroughly. Store Buffer mP1 containing RNase A at 4 °C. The solution will be stable for at least six months.	

SAFETY INSTRUCTIONS – RISK AND SAFETY PHRASES

Warning: FastGene® Plasmid Mini Kits are intended for research use only. The kits are not recommended or intended for diagnosis of disease in humans or animals.
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. We strongly recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice.
The following components of the FastGene® Plasmid Mini kits contain hazardous contents. Follow the safety instructions given in this section.

Lysis Buffer mP2
Contains sodium dodecyl sulfate, sodium hydroxide: irritant. Risk and safety phrases: R36/38, S2-25-26-37-46-64

Neutralization Buffer mP3
Contains guanidine hydrochloride, acetic acid: harmful, irritant. Risk and safety phrases: R10-22-34, S1/2-13-25-26 -27/28-36/37/39-45-64

First Wash Buffer mP4
Contains guanidine hydrochloride: harmful. Risk and safety phrases: R22-36/38, S26-37

RNase A
Contains ribonuclease: Harmful. Risk and safety phrases: R42/43, S2-23-24-37-45-46-63

R10: Flammable; R22: Harmful if swallowed; R34: Causes burns; R36/38: Irritating to eyes and skin; R42/43: May cause sensitization by inhalation and skin contact; S1/2: Keep locked up and out of the reach of children; S2: Keep out of the reach of children; S13: Keep away from food, drink and animal feedingstuffs; S23: Do not breathe liquid; S24: Avoid contact with skin; S25: Avoid contact with eyes; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S27/28: After contact with skin, take off immediately all contaminated clothing, and wash immediately with plenty of water; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S37: Wear suitable gloves; S45: In case of accident or if you feel unwell, seek medical advice immediately S46: If swallowed, seek medical advice immediately and show container or label; S63: In case of accident by inhalation: remove casualty to fresh air and keep at rest; S64: If swallowed, rinse mouth with water (only if the person is conscious).

KIT SPECIFICATIONS

FastGene® Plasmid Mini Kits are designed for isolation and purification of high copy and low copy plasmid DNA.

Parameter	High copy plasmid	Low copy plasmid
Maximum Sample Volume	1-5 ml ON culture	5-10 ml ON culture
Typical yield	< 25µg	< 25µg
Elution volume	50 µl	50 µl
Binding Capacity	40 µg	40 µg
Vectors	< 15 kbp	< 15 kbp
Preparation time	26 min/12 preps	36 min/12 preps
Format	spin column	spin column

PROTOCOL - Fast High Copy Plasmid DNA preparation

Before starting the preparation please check if RNase A was redissolved in Resuspension Buffer mP1, and check if Second Wash Buffer mP5 was prepared according to section *Storage and Preparation.*

Unless otherwise noted the centrifugation steps are carried out at 13,000 rpm (~11,000 – 18,000 x g) in a conventional, table-top microcentrifuge.

Lysis and Neutralization Procedure

- Pellet 1-3 ml (typically 1,5 ml) of a bacterial overnight culture by centrifugation at >10,000 rpm for 1 minute at room temperature (15-25°C). Discard the supernatant and remove as much of the liquid as possible.
- Resuspend pelleted bacterial cells in 200 µl Resuspension Buffer mP1 (RNase A added) by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Lysis Buffer mP2.
- Add 200 µl of Lysis Buffer mP2 and mix gently by inverting the tube 10 times. **Do not vortex** in order to avoid shearing genomic DNA. Allow the mixture to stand for 2 minutes (not longer as 5 minutes!) at room temperature until the lysate clears.
- Add 300 µl of Neutralization Buffer mP3 and mix immediately by inverting the tube 10 times. **Do not vortex.** Centrifuge for 2 minutes at 13,000 rpm. Repeat this step if the supernatant is not clear!

DNA Binding

- Place a FastGene® mP Column into a 2 ml Collection Tube.
- Apply the clear lysate (supernatant) to the mP column by pipetting and centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FastGene® mP Column back into the Collection Tube.

Wash

- Add 150 µl First Wash Buffer mP4 to the FastGene® mP Column. Let this buffer adsorb to the membrane (10 seconds). **Do not centrifuge yet.**
- Overlay with 300 µl Second Wash Buffer mP5.
- Centrifuge at 13,000 rpm for 3 minutes.

DNA Elution

- Transfer FastGene® mP Column into a new microcentrifuge tube (not provided). Please avoid a carryover of the Wash Buffer.
- Add 50 µl Elution Buffer mP6 directly onto the centre of the membrane. Avoid residual buffer adhering to the wall of the column.
- Allow to stand for 2 minutes until Elution Buffer mP6 is absorbed by the membrane.
- Centrifuge at 13,000 rpm for 2 minutes to elute purified DNA.

PROTOCOL - Standard High Copy Plasmid DNA preparation

Before starting the preparation please check if RNase A was redissolved in Resuspension Buffer mP1, and check if Second Wash Buffer mP5 was prepared according to section *Storage and Preparation.*

Unless otherwise noted the centrifugation steps are carried out at 13,000 rpm (~11,000 – 18,000 x g) in a conventional, table-top microcentrifuge.

Lysis and Neutralization Procedure

- Pellet 1-5 ml (typically 1,5 ml) of a bacterial overnight culture by centrifugation at >10,000 rpm for 2 minutes at room temperature (15-25°C). Discard the supernatant and remove as much of the liquid as possible.
- Resuspend pelleted bacterial cells in 200 µl Resuspension Buffer mP1 (RNase A added) by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Lysis Buffer mP2.
- Add 200 µl of Lysis Buffer mP2 and mix gently by inverting the tube 10 times. **Do not vortex** in order to avoid shearing genomic DNA. Allow the mixture to stand for 2 minutes (not longer as 5 minutes!) at room temperature until the lysate clears.
- Add 300 µl of Neutralization Buffer mP3 and mix immediately by inverting the tube 10 times. **Do not vortex.** Centrifuge for 2 minutes at 13,000 rpm. Repeat this step if the supernatant is not clear!

DNA Binding

- Place a FastGene® mP Column into a 2 ml Collection Tube.
- Apply the clear lysate (supernatant) to the mP column by pipetting and centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FastGene® mP Column back into the Collection Tube.

Wash

- Add 400 µl First Wash Buffer mP4 to the FastGene® mP Column and centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FastGene® mP Column back into the Collection Tube.
- Add 600 µl Second Wash Buffer mP5 to the FastGene® mP Column and centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FastGene® mP Column back into the Collection Tube. Centrifuge again for 2 minutes to dry the membrane completely.

DNA Elution

- Transfer FastGene® mP Column into a new microcentrifuge tube (not provided). Please avoid a carryover of the wash buffer
- Add 50 µl Elution Buffer mP6 directly onto the centre of the membrane. Avoid residual buffer adhering to the wall of the column.
- Allow to stand for 2 minutes until Elution Buffer mP6 is absorbed by the membrane.
- Centrifuge at 13,000 rpm for 2 minutes to elute purified DNA.

PROTOCOL - Low Copy Plasmid DNA preparation

Before starting the preparation please check if RNase A was redissolved in Resuspension Buffer mP1, and check if Second Wash Buffer mP5 was prepared according to section *Storage and Preparation.*

Unless otherwise noted the centrifugation steps are carried out at 13,000 rpm (~11,000 – 18,000 x g) in a conventional, table-top microcentrifuge.

Lysis and Neutralization Procedure

- Pellet up to 10 ml (typically 5 ml) of a bacterial overnight culture by centrifugation at >10,000 rpm for 2 minutes at room temperature (15-25°C). Discard the supernatant and remove as much of the liquid as possible.
- Resuspend pelleted bacterial cells in 400 µl Resuspension Buffer mP1 (RNase A added) by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Lysis Buffer mP2.
- Add 400 µl of Lysis Buffer mP2 and mix gently by inverting the tube 10 times. **Do not vortex** in order to avoid shearing genomic DNA. Allow the mixture to stand for 2 minutes (not longer as 5 minutes!) at room temperature until the lysate clears.
- Add 600 µl of Neutralization buffer mP3 and mix immediately by inverting the tube 10 times. **Do not vortex.** Centrifuge for 3 minutes at 13,000 rpm. Repeat this step if the supernatant is not clear!

DNA Binding

- Place a FastGene® mP Column into a 2 ml Collection Tube.
- Apply 750 µl of the clear lysate (supernatant) to the mP column by pipetting.and centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FastGene® mP Column back into the Collection Tube.
- Apply the remaining clear lysate to the same mP column and centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FastGene® mP Column back into the Collection Tube.

Wash

- Add 400 µl First Wash Buffer mP4 to the FastGene® mP Column and centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FastGene® mP Column back into the Collection Tube.
- Add 600 µl Second Wash Buffer mP5 to the FastGene® mP Column and centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FastGene® mP Column back into the Collection Tube. Centrifuge again for 2 minutes to dry the membrane completely.

DNA Elution

- Transfer FastGene® mP Column into a new microcentrifuge tube (not provided).
- Add 50 µl Elution Buffer mP6 directly onto the centre of the membrane. Avoid residual buffer adhering to the wall of the column. If plasmid DNA is larger than 10kb, use preheated Elution Buffer mP6 (70°C).
- Allow to stand for 2 minutes until Elution Buffer mP6 is absorbed by the membrane.
- Centrifuge at 13,000 rpm for 2 minutes to elute purified DNA.

ORDERING INFORMATION

Product	Cat.No.
FastGene® Gel/PCR Extraction Kit (100 preps)	FG-91202
FastGene® Gel/PCR Extraction Kit (300 preps)	FG-91302
FastGene® Gel Band Cutter (50)	FG-830
FastGene® Plasmid Mini Kit (100 preps)	FG-90402
FastGene® Plasmid Mini Kit (300 preps)	FG-90502
FastGene® Dye Terminator Removal Kit (50 preps)	FG-9411

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