QuickClean II Plasmid Miniprep Kit

Cat. No.: L00420 Version 2018-07-16

Table of Contents

I Description 1
II Key Features 1
III Contents 2
IV Application 2
V Storage 2
VI Protocol 2
VII Examples 4
1 Absorbance analysis . . . 4
2 Agarose Analysis . . . 4
3 Enzymatic reactions analysis . . . 5
VIIITrouble Shooting 6
IX Ordering Information 7

I Description

QuickClean II Plasmid Miniprep Kit provides a fast, simple, and cost-effective method for purification of up to 20 µg high-copy plasmid from 1–5 mL of overnight cultures of *E. coli*. The DNA isolated by this Kit is ready for downstream applications such as restriction enzyme digestion, sequencing, PCR/Real-Time PCR and other downstream experiments.

II Key Features

The extracted DNA is immediately ready for routine molecular biology laboratory applications.

- **Fast Process**: Complete 24 preps within 24 min.
- **High Purity**: >90% removal of contaminants and inhibitors.
- **High Capacity**: up to 20 µg DNA.
- **High Reproducibility Yields**: Up to 99% recovery of plasmid DNA.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 Tel: 1-877-436-7274; 1-732-885-9188

III Contents

The components of QuickClean II Gel Extraction Kit are described in the table below.

Kit Contents	Volume (50 rxns)	Volume (100 rxns)
Resuspension Buffer	12.5 mL	25 mL
Lysis Buffer	12.5 mL	25 mL
Neutralization Buffer	17.5 mL	35 mL
Wash Buffer	15 mL	15 mL × 2
Elution Buffer	10 mL	20 mL
RNase A Solution	50 μL	100 μL
Spin columns	50(Column Volume:750μL)	100(Column Volume:750μL)

IV Application

The extracted DNA is immediately ready for further downstream applications such as:

- ◇ Sterile micro-centrifuge tubes (Volume: 1.5 mL)
- ◇ Sterile tips (Volume: 10 μL/100 μL/1000 μL)
- ◇ Absolute (100%) ethanol
- ◇ Microcentrifuge (capable of 14,000 xg)
- ◇ Vortex mixer

V Storage

This kit should be shipped and stored at room temperature (15-25°C), but the RNase A solution should be stored at 2-8°C. The kit can be stored for up to 18 months at room temperature (15-25°C). After addition of RNase A solution, Resuspension Buffer should be stored at 2-8 °C.

VI Protocol

Materials Supplied by the User

You will need to prepare the following reagents and equipments for PCR purification:

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 Tel: 1-877-436-7274; 1-732-885-9188

- ◇ Sterile microcentrifuge tubes (Volume: 1.5 mL)
- ◇ Sterile tips (Volume: 10 μL/100 μL/1000 μL)
- ◇ Absolute (100%) ethanol
- ◇ Microcentrifuge (capable of 14,000× g)

Important notes

The following steps may be performed ahead of time:

1. Transfer the RNase A solution to the Resuspension Buffer and mix well and store at 2-8 °C.
2. Add 100% ethanol (the volume of ethanol to be added is also shown on the bottle labels) to Wash Buffer and mix well.
3. Some precipitate may form in Lysis Buffer and Neutralization Buffer after long periods of storage. Dissolve the precipitate by warming in the 37 °C water bath. Please do not vortex Lysis Buffer acutely.
4. Close the lid immediately after using Lysis Buffer to avoid acidification.
5. The kit can extract high-quality plasmid DNA from 1-5 mL *E.coli* culture.
6. Adjust the Elution Buffer to the suitable volume if necessary.

Procedure

1. Transfer 1-1.5 mL E.coli culture to 1.5 mL microcentrifuge tube and centrifuge at 10, 000 rpm (8,000 - 10,000 ×g) for 30 s. Remove and discard the supernatant. Note: this step can be repeated for more than one time to collect enough cells.
2. Add 250 μL Resuspension Buffer to the pellet, cap the tube and resuspend the cells. No cell clumps should be visible after resuspension of the pellets.
3. Add 250 μL Lysis Buffer to the mixtureand mix gently by inverting the tube 4-6 times. To avoid contamination by genomic DNA. Do not vortex.
4. Add 350 μL Neutralization Buffer and mix gently by inverting the tube 4-6 times. The solution should become cloudy and no local precipitate should be visible.
5. Centrifuge at 13,000 rpm (>14, 000 ×g) for 10 min until a compact white pellet forms.
6. Transfer the supernatant to the Spin column and centrifuge for 30-60 s at 6,000 ×g. Discard all flow-through.
7. Add 650 μL Wash Buffer to the Spin column and Centrifuge for 30-60 s at 12,000 ×g. Discard the flow-through. Repeat this step once.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 Tel: 1-877-436-7274; 1-732-885-9188

8. Centrifuge for additional 1 min at 12,000 ×g to remove residual and transfer the Spin column to a sterile 1.5 mL microcentrifuge tube.
9. Add 50 µL Elution Buffer(ddH_2O or TE Buffer) to the Spin column and let the column stand for 1 min at room
10. temperature . The volume of Elution Buffer should be adjusted if necessary.
11. Centrifuge at 12,000 ×g for 1 min. The buffer in the microcentrifuge tube contains the plasmid DNA.
12. Store the microcentrifuge tube containing purified plasmid DNA at -20°C if not use immediately.

VII Examples

1 Absorbance analysis

Get some plasmid DNA, diluted in an advisable factor with elution buffer.
Survey the OD_{260} , OD_{280} and OD_{320}
Expressions: concentration (µg/m) =50×OD_{260}×dilution fact
Target: 2.0≥$OD_{260-320}$/ $OD_{280-320}$≥1.8
Notice: 1.0≥OD_{260}≥0.1, the result of ratio is much reliable.

2 Agarose Analysis

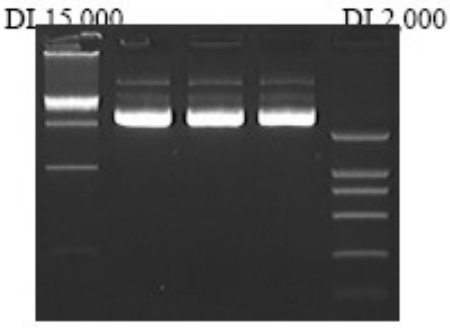

Figure 1: The following example shows the performance (1% Agarose gel) after purifying plasmid DNA from cultures of *E. coli* using this kit.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 Tel: 1-877-436-7274; 1-732-885-9188

The QuickClean II Plasmid Miniprep Kit is here compared to a commercially available kit (Competitor A) for rapid plasmid purification from *E. coli* bearing high copy number plasmid. Both kits are used in compliance with the protocols provided by the manufacturers. The results are shown in Figure 2.

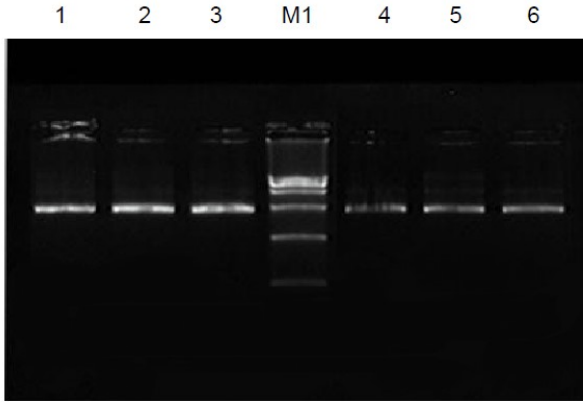

Figure 2: Plasmid DNA preparation from *E. coli* bearing high copy number plasmid.
M 1: Takara DL15,000 DNA Marker
Lane 1-3: Plasmid purified by GenScript kit
Lane 4-6: Plasmid purified by Competitor A

3 Enzymatic reactions analysis

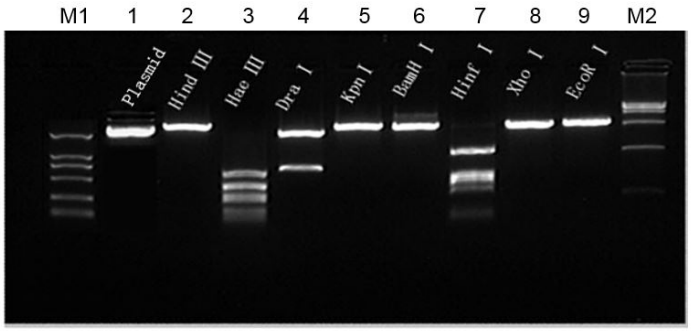

Figure 3: Enzymatic reactions analysis
M1: DL2,000™ DNA Marker (Takara)
M2: DL15,000 DNA Marker (Takara)
Lane1: Control
Lane 2 to 9: *Hind* III, *Hac* III, *Dra* I, *Kpn* I, *BamH* I, *Hinf* I,. *Xho* I, *EcoR* I

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-877-436-7274; 1-732-885-9188

VIII Trouble Shooting

Problem	Recommend
Get no plasmid DNA	If there is no the plasmid DNA in elution buffer, please check whether the ethanol had been added to Wash Buffer according to the volume be marked on bottle label.
Low plasmid DNA yields	1. Please check if the bacteria was cultured in the right way, 2. If the bacteria cells were resuspended completely. 3. Incubate the Elution Buffer in 30-60°C, it will increase the yields.
Absorbance problem	1. Absorbance is the difference from sample and criterion, please use the Elution Buffer to adjust to zero value and dilute the sample. 2. If the ratio of OD_{260}/ OD_{230} is too low, wash the spin column for one more time. 3. In the case of low ratio of $OD_{260-320}$/ $OD_{280-320}$, there is protein contamination, please add Neutralization Buffer, and then centrifuge buffer with sufficient rotating speed, thus to make precipitation compact; be careful to pipette supernatant to avoid pipette precipitation. 4. If the ratio of $OD_{260-320}$/ $OD_{280-320}$ is too high, add more RNase A to Resuspension Buffer to final concentration 100 μg/mL.
Electrophoresis problem	1. If there is genomic DNA in the result, invert the tube gently (step 3 and 4). 2. If there is RNA in the result, add more RNase A to Resuspension Buffer to final concentration 100 μg/mL.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-877-436-7274; 1-732-885-9188

IX Ordering Information

Product Name	Cat. No.
QuickClean II Plasmid Miniprep Kit	L00420
High-Stability PCR Kit	L00342
Green *Taq* DNA Polymerase, 100 µl (500 U)	E00043
10X *Taq* Buffer (with Mg2+), 1.5 ml	B0005
Stabilized dNTP Mix, 300 µl (10 mM each)	C01689

Contact us

Web: https://www.genscript.com
Email: product@genscript.com
Fax: 1-732-518-5150

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 Tel: 1-877-436-7274; 1-732-885-9188