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Nuclefy
Plasmid DNA Isolation Mini Kit

Spin Column-Based DNA Extraction

For Research Use Only

Instruction Manual
Version 1.0

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1. INTRODUCTION

The Nuclefy Plasmid DNA Isolation Mini Kit is designed for the rapid, efficient, and reliable lysis, RNA removal and isolation of high-quality plasmid DNA (pDNA) from bacterial cells. The protocol utilizes specifically fromulated buffer systems, based on the well-established alkaline-SDS lysis of bacterial cells, for the fast and efficient processing of multiple samples.

2. KIT COMPONENTS

	Nuclefy Plasmid DNA Isolation Mini Kit 10 preps	Nuclefy Plasmid DNA Isolation Mini Kit 150 preps
Catalogue No.	**PCCSKU16092**	**PCCSKU16093**
Preps	10	150
Nuclefy Mini Kit P1 Buffer*	2.5 ml	40 ml
Nuclefy Mini Kit P2 Buffer	2.5 ml	40 ml
Nuclefy Mini Kit N3 Buffer	3.5 ml	55 ml
Nuclefy Mini Kit Binding Buffer	1.5 ml	24 ml
Nuclefy Mini Kit Wash Buffer	3 ml	45 ml
Nuclefy Mini Kit Elution Buffer	1 ml	15 ml
Nuclefy Mini Kit RNase A	20 µl	200 µl
Nuclefy Mini Kit Purification Columns	10	150
Nuclefy Mini Kit 2ml Collection Tubes	10	150

*P1 Buffer must be refrigerated (at +2 - +8 °C) after the addition of RNase A.

Table 1. Components of the Nuclefy Plasmid DNA Isolation Mini Kit.

3. STORAGE AND STABILITY

RNase A must be stored at -25 to -15 °C before added to P1 Buffer. P1 Buffer must be strored at +2 - +8 °C after the addition of RNase A. All other kit components can be stored at room temperature (+15 to +25 °C). Always keep bottles tightly closed, and columns, as well as collection tubes, sealed in the enclosed zip-lock bag. Some buffers may form precipitates when stored or transported under cool conditions. To redissolve them, warm the buffers at +37 °C and mix by shaking gently. Avoid shaking vigorously P2 Buffer and ensure that it is tightly closed after each use.

4. PRECAUTION

Proper laboratory safety practices should be employed, including the use of lab coat, gloves and protective goggles. For information regarding the composition of the buffers, please check the Safety Data Sheets (available upon request). N3 Buffer contains chaotropic salts, which are irritants, and they can form highly reactive compounds when combined with bleach. Each reagent is optimized for the specific use of this kit. Do not substitute reagents from this kit with reagents provided by any other manufacturer, and do not combine reagents with different Lot numbers.

5. PROCEDURE OVERVIEW

The procedure starts with the resuspension and lysis of bacterial cells, followed by the neutralization step that allows the cell debris to precipitate. In the next step, the supernatant is transferred into the purification column, and plasmid DNA (pDNA) binds to the silica membrane. A two-step washing procedure is then performed to remove detergents and other impurities. During the last step, pDNA is eluted using the Elution buffer.

6. ILLUSTRATED PROTOCOL

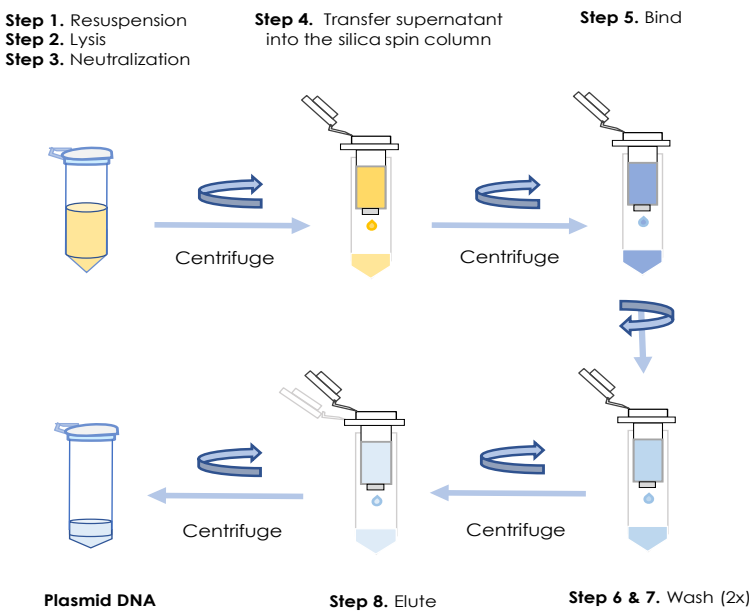

Figure 1. Illustrated protocol for plasmid DNA (pDNA) isolation.

7. REQUIRED MATERIALS NOT SUPPLIED

- Ethanol, absolute
- Isopropanol, ≥ 99.5 %
- Microcentrifuge capable of at least 13,000 x *g*
- Sample dispensing options
- Vortexer
- 1.5 ml or 2 ml microcentrifuge tubes (ideally low binding)
- Optional: Centrifuge for 15 ml tubes, capable of at least 5,000 x *g*
- Optional: 15 ml tubes

8. REAGENT PREPARATION

1. Add the provided RNase A to the bottle of P1 Buffer (as stated on the bottle label) and store P1 Buffer at +2 to +8 °C.
2. Add the appropriate amount of Ethanol (absolute) to the bottle of the Nuclefy Mini Kit Wash Buffer, as stated in Table 2 and on the bottle label.

Kit	Amount of Ethanol (absolute) required
Nuclefy Plasmid DNA Isolation Mini Kit - 10 preps	12 ml
Nuclefy Plasmid DNA Isolation Mini Kit - 150 preps	180 ml

Table 2. Amount of Ethanol (absolute) required for the preparation of the Nuclefy Mini Kit Wash Buffer.

3. Add the appropriate amount of Isopropanol (≥ 99.5 %) to the bottle of the Nuclefy Mini Kit Binding Buffer, as stated in Table 3 and on the bottle label.

Kit	Amount of Isopropanol (≥ 99.5 %) required
Nuclefy Plasmid DNA Isolation Mini Kit - 10 preps	3.5 ml
Nuclefy Plasmid DNA Isolation Mini Kit - 150 preps	56 ml

Table 3. Amount of Isopropanol (≥ 99.5 %) required for the preparation of the Nuclefy Mini Kit Binding Buffer.

9. SPECIFICATIONS

Typical yields from a 1-5 ml overnight culture in Luria Bertani (LB) medium can range from 3 to 15 µg of plasmid DNA. However, the yield and quality of the extracted plasmid DNA can vary according to the plasmid copy number per bacterial cell, the host strain, the input culture volume, and the size of insert. Depending on their origin of replication, the range of the plasmid copy number per bacterial cell might be from one to several hundred, while large plasmids usually occur in a very low copy number per cell. The isolated plasmid DNA can be directly used in a variety of applications, such as transformation and restriction enzyme digestion. Table 4 shows the expected plasmid DNA yields from 5 ml overnight LB cultures, using the Nuclefy Plasmid Isolation Mini Kit.

Plasmid	Origin of replication (*ori*)	Copy number	Expected yield
ColE14	ColE14	15-20	1-2 µg
pBR322 and its derivatives	pMB1	15-20	1-2 µg
pSC101 and its derivatives	pSC101	~5	0.5 µg
pACYC and its derivatives	p15A	~10	0.5-1 µg
pBluescript® vectors	ColE14	300-500	10-18 µg
pGEM® vectors	pMB1	300-400	10-20 µg
pUC vectors	pMB1	500-700	15-25 µg

Table 4. Expected plasmid DNA yields from 5 ml starting culture volume (LB medium).

10. RECOMMENDED BACTERIAL GROWTH AND CULTURE

a. Inoculation and culture media

It is strongly recommended that bacterial cultures, which are used for plasmid preparations, derive from a single colony picked from a freshly transformed or streaked plate. Subsequent inoculation of a suitable volume in a medium containing the appropriate antibiotic, followed by 12 to 16 hours incubation at 37 °C and shaking at ~300 rpm, are the recommended bacterial conditions for optimal results. The culture volume should not exceed ¼ the volume of the flask, as aeration is essential for bacterial growth. Variations in yield and plasmid loss can occur when bacterial cultures are subcultured directly from glycerol stocks or liquid cultures. It is recommended that Luria Bertani (LB) medium is used for the bacterial growth, as richer culture media (e.g., TB) might result in higher cell densities, overloading the purification column.

b. Cell density and input culture volume

In general, the input culture volumes should be dependent on the culture cell density. It is recommended that a bacterial density between 2.0 and 3.0 at OD_{600} is used. Input culture volumes might need to be adjusted, depending on the culture medium used or the plasmid copy number per bacterial cell (see also the Troubleshooting guide, Table 5).

11. ELUTION AND STORAGE

Buffer Composition
The Elution Buffer is 10 mM Tris-HCl, pH 8.0. Alternatively, nuclease-free water or other low ionic strength buffers can be used. Make sure that pH is adjusted from 8.0 to 9.0, if an alternative Elution buffer is used, as pH is critical for an efficient elution (see also the Troubleshooting guid, Table 5).

Volume
The recommended elution volume is 100 µl. A higher elution volume results in higher yield, but decreased pDNA concentration. A lower volume allows for higher concentration.

Storage
The eluted pDNA can be safely stored at +4 to +8 °C for some weeks to months. For long-term storage, store the eluted pDNA at -80 °C. Repeated freeze-thaw cycles should be avoided to maintain the integrity of pDNA.

12. PLASMID DNA ISOLATION PROTOCOL FROM 1-5 ml *E. coli* CULTURE

Preparation:

- For the harvest of bacterial cells, set a suitable centrifuge at 4 °C before starting.

1. Grow 1-5 ml culture overnight in a suitable culture tube or flask.

2. Harvest bacterial cells by centrifugation (e.g., in 15 ml tubes) at 4,500-5,000 x *g* for 10-12 minutes at 4 °C.

3. Decant or remove by pipetting the culture medium and discard it.

4. Add 250 µl P1 Buffer and vortex thoroughly until the pellet is completely resuspended.
Note: Make sure that RNase A has already been added to P1 Buffer.

5. Transfer the suspension into a new 2 ml microcentrifuge tube (not provided).

6. Add 250 µl P2 Buffer. Invert the tube gently until the lysate is clear. Do not vortex. Incubate the samples at room temperature (RT) for maximum 5 minutes.

7. Add 350 µl N3 Buffer. Immediately invert the tube gently several times until a white precipitate forms. Centrifuge at maximum speed (≥ 13,000 x *g*) for 10 minutes at RT.

8. Carefully transfer 700 µl of the clear supernatant into a Nuclefy Spin Column inserted into a 2 ml collection tube, without touching the pellet.

9. Centrifuge for 1 minute at maximum speed (≥ 13,000 x *g*) at RT. Discard the flow-through and reuse the collection tube.

10. Add 500 µl Binding Buffer. Centrifuge at maximum speed (≥ 13,000 x *g*) for 1 minute at RT. Discard the flow-through and reuse the collection tube.
Note: Make sure that the correct amount of Isopropanol, ≥ 99.5 % has already been added to the Binding Buffer.

11. Add 700 µl Wash Buffer. Centrifuge at maximum speed (≥ 13,000 x *g*) for 1 minute at RT. Discard the flow-through and reuse the collection tube.
Note: Make sure that the correct amount of Ethanol (absolute) has already been added to the Wash Buffer.

12. Repeat **step 11** for a second washing step.

13. Centrifuge the empty Nuclefy Spin column at maximum speed (≥ 13,000 x *g*) for 2 minutes.
Note: This step will allow the membrane to dry, removing traces of ethanol that might interfere with downstream applications.

14. Transfer the Nuclefy Spin Column into a clean 1.5 ml microcentrifuge tube (not provided).

15. Add 100 µl of Elution Buffer. Incubate for 1 minute at RT. Centrifuge at maximum speed (≥ 13,000 x *g*) for 1 minute at RT.

16. Store the eluted plasmid DNA (pDNA) at +4 °C to +8 °C for short-term or at -80 °C for long-term storage.

13. TROUBLESHOOTING GUIDE

Please use this guide to troubleshoot possible problems that may arise. For further assistance, please contact the technical support staff at support@procomcure.com.

Problem	Possible Cause	Solution
Low DNA Yield	Incomplete cell lysis.	Make sure that the cells are evenly suspended by vortexing thoroughly before the addition of P2 Buffer.
		Increase incubation time with P2 Buffer until the lysate is clear.
		Precipitates may have formed in P2 Buffer. Redissolve by warming at 37 °C. Do not shake P2 Buffer vigorously.
		Close P2 Buffer tightly after each use. If not tightly closed, a replacement might be necessary.
	Culture is overgrown	Culture incubation should not take place for more than 16 hours at 37 °C. Storage of the bacterial cultures for long periods before performing the plasmid DNA isolations can lead to low yields.
	Insufficient elution	The pH of the Elution Buffer or water is critical for an efficient elution, and it should range from 8.0 to 9.0. If other buffers are used for elution, make sure that the pH is within this range.
	Low copy number plasmid is used	Increase the input culture volume and double the amounts of P1, P2 and N3 Buffers. Do not increase or double the amounts of Binding Buffer, Wash Buffer, and Elution Buffer.
No DNA eluted	Ethanol was not correctly added to the Wash buffer	Add the correct amount of Ethanol (absolute) to the Wash buffer according to the instructions in Table 2, and as stated on the buffer label.
	Isopropanol was not correctly added to the Binding buffer	Add the correct amount of Isopropanol (≥ 99.5 %) to the Binding Buffer according to the instructions in Table 3, and as stated on the buffer label.
Contamination with genomic DNA	Incorrect handling after the addition of P2 Buffer	Handle gently by reverting the tube several times after the addition of P2 Buffer and do not vortex. If the lysate is too viscous and cannot be mixed gently, reduce the culture volume.
	Incorrect handling after the addition of N3 Buffer	Mix immediately but gently after the addition of N3 Buffer. Do not vortex.
	Culture is overgrown	Culture incubation should not take place for more than 12-16 hours at 37 °C.
	Too long lysis	Lysis, after the addition of P2 Buffer, must not take longer than 5 minutes.

Table 5. Troubleshooting guide.

Problem	Possible Cause	Solution
$A_{260/280}$ and $A_{260/230}$ ratios are out of the expected range	Ethanol used for the dilution of the Wash Buffer may contain impurities	Make sure that the Ethanol used for the dilution of the Wash Buffer is of high purity. Traces of impurities may remain on the silica column after the washing steps and disrupt the absorbance in the eluate.
	RNA in the eluate	Make sure that RNase A is added to P1 Buffer before the first use. Make also sure that RNase A is properly stored, as degradation may occur at high temperatures (> 65 °C).
	Incomplete purification due to overloading of the silica column	Reduce the initial culture amount.
RNA in the eluate	Incomplete RNase A digestion	Reduce the volume of the culture input, if necessary. Additional RNase A should be added, if P1 Buffer contains RNase A older than 6 months.
	RNase A digestion omitted	Make sure that RNase A is added to P1 Buffer before the first use.

Table 5 (cont.). Troubleshooting guide.

14. CONSUMABLES & RELATED PRODUCTS

Product	Content	Cat.No.
Nuclefy Plasmid DNA Isolation Mini Kit	150 preps	PCCSKU16093
Nuclefy Plasmid DNA Isolation Mini Kit (Test)	10 preps	PCCSKU16092
Nuclefy Genomic DNA Purification Kit	150 preps	PCCSKU16073
Nuclefy Genomic DNA Purification Kit (Test)	10 preps	PCCSKU16074
Aegis 96-well PCR Reaction Plate	10 plates	PCCSKU30001
Aegis 384-well PCR Reaction Plate	10 plates	PCCSKU30009
PhoenixDx® ABC qPCR Mastermixes	Test	PCCSKU12011
PhoenixDx® qPCR Mastermix Alpha	500 rxn	PCCSKU12012
PhoenixDx® qPCR Mastermix Bravo	500 rxn	PCCSKU12013
PhoenixDx® qPCR Mastermix Charlie	500 rxn	PCCSKU12014
PhoenixDx® RT-qPCR Mastermix Alpha	500 rxn	PCCSKU12015
PhoenixDx® RT-qPCR Mastermix Bravo	500 rxn	PCCSKU12016
PhoenixDx® RT-qPCR Mastermix Charlie	500 rxn	PCCSKU12017

Table 6. Related products and Consumables.

Ordering information

For ordering the Nuclefy Plasmid DNA Isolation Mini Kit and other products, visit us at

www.procomcure.com

or order via E-mail:

sales@procomcure.com

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