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IsoPure™

DNA Purification Kits

PCR Purification Prep Kit
Cat. CM0100-50 & CM0100-100

Gel Extraction Prep Kit
Cat. CM0200-50 & CM0200-100

Plasmid Mini Prep Kit
Cat. CM0300-50 & CM0300-100

Plasmid Midi Plus Prep Kit
Cat. CM0310-10 & CM0310-20

Instruction Manual

Version 4.0
Rev. 02/28/11

For research use only

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3. Troubleshooting for Gel Extraction and PCR Purification Protocols

Low yield

There is a number of variables that can cause low yield.

- a. Each of the steps has to be performed thoroughly.
- b. Make sure that the column binding capacity (20µg) is not exceeded.

Sample floats upon loading into the agarose gel

The sample contains ethanol from the washing step. Discard the liquid waste from the collection tube after the washing step and spin again for additional two minutes (alternatively open up the column and let air dry for a few minutes) before the final elution step.

The salt concentration is too high in the eluted DNA

Use distilled water (molecular biology grade) for the final elution. DNA is an acid and it will hydrolyze in pure water fairly quickly therefore when plasmid DNA is eluted with water it needs to always be stored frozen (-20°C).

DNA purified from the gel is not functional in downstream application.

Limit the time of gel exposure to UV light. If possible use lower intensity setting on UV lamp when cutting the gel slice. Prolonged gel exposure to UV rays may significantly damage the DNA and lead to low or no efficiency of downstream applications such as ligation.

4. Kit Components Available Separately:

Catalog number	Product description
CM0600-01	Binding Solution I - [25ml]
CM0600-02	Binding Solution II - [25ml]
CM0600-05	Wash Buffer - [25ml]
CM0600-11	Solution I - [25ml]
CM0600-12	Solution II - [25ml]
CM0600-13	Solution III - [25ml]
CM0600-06	Elution Buffer - [25ml]
CM0600-10A	RNase A [1ml - 10mg/ml]

- Place the column in a clean 1.5ml microfuge tube. Add 30-50ul of Elution Buffer to the center of the column and incubate at room temperature for 2 minutes. Centrifuge at 16,000 x g for 1 minute to elute DNA.

Note: It is extremely important to add the Elution Buffer into the center part of the column.

Incubating the column with the Elution Buffer at higher temperatures (5 min at 50°C) may slightly increase the yield especially of large (>10,000bp) DNA fragments. Alternatively the Elution Buffer can be prewarmed to 75°C before it is applied to the column.

For higher concentration of DNA, two elution steps may be carried out with 20µl of Elution Buffer each, rather than one 50µl step.

- Store the purified DNA at -20°C.

The Gel Extraction Kit can be used to purify DNA from enzymatic reactions. Follow the protocol for PCR purification below using Binding Solution II instead of Binding Solution I.

2. Protocol for Purification of DNA from PCR Reaction:

- Transfer PCR reaction mixture to a 1.5ml microfuge tube and add 3 volumes of Binding Solution I.
- Transfer the above mixture solution to the IsoPure-10 column and let stand at room temperature for 2 minutes. Centrifuge at 8,000 x g for 1 minute.
- Remove the flow-through in the tube. Add 500ul of Wash Solution to the column and centrifuge at 16,000 x g for 1 minute.
- Repeat washing procedure in step 3. Spin at 16,000 x g for an additional minute to remove any residual Wash Solution.
- Transfer the column into a clean 1.5ml microfuge tube and add 30-50µl of Elution Buffer. Incubate at room temperature for 2 minutes. Centrifuge at 16,000 x g for an additional 2 minutes to elute the DNA.

Note: It is extremely important to add the Elution Buffer into the center part of the column.

Incubating the column with the Elution Buffer at higher temperatures (5 min at 50°C) may slightly increase the yield especially of large (>10,000bp) DNA fragments. Alternatively the Elution Buffer can be prewarmed to 75°C before it is applied to the column.

For higher concentration of DNA, two elution steps may be carried out with 20µl of Elution Buffer each, rather than one 50µl step.

- Store the purified DNA at -20°C.

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I. Introduction

The IsoPure DNA Purification Kits provide a simple and efficient method for purification of Plasmid DNA, extraction of DNA from agarose gels, and purification of DNA from enzymatic reactions such as PCR or restriction enzyme digestions.

The DNA is selectively adsorbed in the silica gel-based IsoPure column and other components are washed away. The DNA is then eluted off the column and can be used for any downstream application.

The purification method used in these protocols does not require use of phenol, chloroform or CsCl. The DNA is purified without an additional step of ethanol precipitation.

1. Features

- ❑ Fast and efficient
- ❑ Preparation of high quality DNA which can be used in any downstream applications such as sequencing (automatic and manual), PCR, transformation, restriction enzymatic digestion, or ligation
- ❑ High purification capacity. Up to 20µg of DNA per column on the Mini Prep, Gel Extraction, PCR Purification Kits; and up to 200µg of DNA per column on the Midi Prep Kit
- ❑ No phenol / chloroform extraction or ethanol precipitation is required

2. Limitations of Use

These kits are designed for research only. The purified plasmid DNA should not be used for live animal transfection. It is also not to be used for human diagnostic or drug production purposes.

3. Applications

Plasmid Mini Prep Kit (Cat. CM0300-50 & CM0300-100)

Plasmid Midi Plus Prep Kit (Cat. CM0310-10 & CM0310-20)

- ❑ Plasmid DNA purification from bacterial cells. The silica-gel based column can be used to purify plasmid DNA from 60bp up to 40,000bp.
- ❑ The IsoPure Mini Prep and Midi Plus Prep Kits are suitable for purifying low copy plasmid DNA. When working with low copy plasmid it is recommended to increase the starting culture volume to obtain the maximum yield. To insure complete cell lysis and protein precipitation when double culture volume is used it is necessary to double the volume of Solution I, II and III. The kit is supplied with 25% more buffer than required for single culture volume use. If all the preps are processed with double culture volume it is necessary to purchase additional Solution I, II and III. All kit components (except spin columns) can be purchased separately (see buffers and solutions listed at the end of this manual).

PCR Purification Prep Kit (Cat. CM0100-50 & CM0100-100)

- ❑ Recovery of 60bp to 40,000bp DNA fragments from reaction solutions.

Gel Extraction Prep Kit (Cat. CM0200-50 & CM0200-100)

- ❑ Recovery of 60bp to 40,000bp DNA fragments from all types of agarose gels. Using low melt agarose will slightly accelerate the purification process. This kit can also be used for purification of DNA fragments from various enzymatic reactions.

III. *IsoPure* PCR Purification and Gel Extraction Kits

Components	CM-0100-50	CM-0100-100	CM-0200-50	CM-0200-100
	50 Preps	100 Preps	50 Preps	100 Preps
Binding Solution I	20ml	2x20ml	-	-
Binding Solution II	-	-	2x30ml	4x30ml
Wash Solution	12ml	24ml	12ml	24ml
Elution Buffer	5ml	10ml	5ml	10ml
IsoPure-10 Column	50	100	50	100
Collection Tube	50	100	50	100
Instruction manual	1	1	1	1

- A) Before use, the following volume of ethanol has to be added to the Wash Solution:
CM-0100-50 add 48ml of 96-100% ethanol to 12ml of Wash Solution
CM-0100-100 add 96ml of 96-100% ethanol to 24ml of Wash Solution
CM-0200-50 add 48ml of 96-100% ethanol to 12ml of Wash Solution
CM-0200-100 add 96ml of 96-100% ethanol to 24ml of Wash Solution
For other volumes of wash solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol : volume of Wash Solution = 4:1).
- D) Elution Buffer is 2mM Tris-HCl pH 8.5. Although TE buffer pH 8.0 or water may be substituted, the resulting yields may be up to 20% lower. Storage: The kit is stable for 12 months at room temperature. For longer storage, keep all contents at 4 °C.

1. Protocol for DNA Purification from Agarose Gel:

1. Excise the DNA fragment from the gel with a clean, sharp scalpel. Weigh the gel slice and transfer to a 1.5mL microfuge tube.
2. Add 400µl of Binding Solution II for each 100mg of gel weight (example – a gel slice weighing 125mg would require 500µl of Binding Solution II). Incubate at 50-60°C for 10 minutes and shake occasionally until agarose is completely dissolved. For high concentration gels (1.5% and up), 700µl of Binding Solution II per 100mg of gel is required.
3. Add the above mixture to the IsoPure column and let stand for 2 minutes. Centrifuge at 8,000 x g for 1 minute and discard the flow-through.
4. Add 500µl of Binding Solution II to the column, and centrifuge at 16,000 x g for one minute. Discard the the flow-through. (This step is optional. To order more buffer, use part # CM0600-02 Binding Solution II - [25ml].)
5. Add 500µl of Wash Solution, and centrifuge at 16,000 x g for one minute. Discard the flow-through.
6. Repeat step 5. Spin at 16,000 x g for an additional minute to remove any residual Wash Solution.

3. Troubleshooting for Mini and Midi Plus Prep Protocols

□ Low yield

There are a number of variables that can cause low yield.

- Each of the steps has to be performed thoroughly.
- Make sure that there is no precipitant in buffer I, II or III - if precipitant in the buffer can be seen, heat up the solution to 37°C and shake well.
- Low culture density - make sure that the temperature in the incubator is stable and the shaking speed provides sufficient aeration of the culture
- Very high cell density, therefore incomplete cell lyses - double the volume of solution I, II and III.
- When proper culture density is verified and the yield of plasmid is still low, it is recommended to verify that the antibiotic is fully functional and that its concentration is sufficient. For difficult to grow bacteria strains chose richer media such as *Terrific Broth*TM. It is critical not to over grow the culture. Cultures should not be allowed to grow for more than 16 hours. Extended culture times will result in the growth of bacteria that have no plasmid and will have a negative impact on plasmid yield and purity. The maximum time depends on the type of antibiotic used as well as a type of bacteria and size of the vector. All of these affect the propagation of the plasmid. Be sure to follow the recommendations for antibiotic concentration.

□ Contamination of chromosomal DNA

Do not vortex (or shake vigorously) the sample after adding Solution II and III. Vigorous shaking will cause shearing of chromosomal DNA. Smaller pieces of chromosomal DNA will be captured on the silica gel and carried over with the purified plasmid DNA.

□ Sample contains RNA

RNase activity is weakened or lost. Add additional dose of RNase A (50µg/ml) to buffer I and store the buffer I at 4°C

□ Sample floats upon loading into the agarose gel

The sample contains ethanol from the washing step. Discard the liquid waste from the collection tube after the washing step and spin again for additional two minutes (alternatively open up the column and let air dry for a few minutes) before the final elution step.

□ The O.D. ratio outside of 1.9 to 2.2 range

If the O.D. ratio between 260nm and 280nm is above 2.2 there may be traces of ethanol present (see point 4. for recommended solution).

If the O.D. ratio is lower than 1.9 there is a chance of protein contamination. Make sure that the sample is mixed well after Solution III is added and that after spinning down there are no precipitated particles transferred into the column.

□ The salt concentration is too high in the eluted Plasmid DNA

Use distilled water (molecular biology grade) for the final elution. DNA is an acid and it will hydrolyze in pure water fairly quickly. Therefore when plasmid DNA is eluted with water it needs to be stored frozen (-20°C)

II. IsoPure Plasmid Mini and Midi Plus Prep Kits

Components	CM-0300-50	CM-0300-100	CM-0310-10	CM-0310-20
50 Preps		100 Preps	10 Preps	20 Preps
RNase A (10mg/ml)	0.12ml	0.24ml	1.2ml	2x1.2ml
Solution I	6ml	12ml	22ml	44ml
Solution II	12ml	24ml	2x25ml	4x25ml
Solution III	25ml	2x25ml	3x30ml	6x30ml
Wash Solution	12ml	24ml	24ml	48ml
Elution Buffer	5ml	10ml	15ml	30ml
IsoPure-10 Column	50	100	10	20
Collection Tube	50	100	20	40
Instruction manual	1	1	1	1

- Before use, add the RNase A Solution to the bottle containing Solution I and mix well. Solution I with RNase should be stored at 4°C for frequent use and at -20°C for infrequent use.
- Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37°C.
- Before use, the following volume of ethanol must be added to the Wash Solution:

CM-0300-50 add **48ml** of 96-100% ethanol to **12ml** of Wash Solution
CM-0300-100 add **96ml** of 96-100% ethanol to **24ml** of Wash Solution
CM-0310-10 add **96ml** of 96-100% of ethanol to **24ml** of Wash Solution
CM-0310-20 add **192ml** of 96-100% ethanol to **48ml** of Wash Solution

For other volumes of wash solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol : volume of Wash Solution = 4:1).
- Elution Buffer is 2mM Tris-HCl pH 8.5. Although TE buffer pH 8.0 or water may be substituted, the resulting yields may be up to 20% lower.

Storage: With the exception of the RNase A, the kit may be stored at room temperature. The RNase A should be stored at 4°C. The kit is stable for 12 months at room temperature. For longer storage, keep all contents at 4°C.

2. Protocol for Mini Prep

1. Add 1.5ml overnight culture to a 1.5ml microfuge tube and centrifuge at 10,000 x g for 15 seconds. Drain the liquid completely. For low copy plasmid, see the next protocol on page 6.
2. Add 100µl of Solution I to the pellet and mix well. Be sure that there are no visible clumps of cells before proceeding to the next step.
3. Add 200µl of Solution II to the mixture, mix gently by inverting the tube 4-6 times and incubate at room temperature for 1 minute. **Do not vortex.** (Vortexing will cause genomic DNA contamination). The solution should become viscous and clear.
4. Add 350µl of Solution III, and mix gently by inverting the tube immediately after the solution is added. Incubate at room temperature for 1 minute. **Do not vortex.**
5. Centrifuge at 16,000 x g for 5 minutes.
6. Transfer the above supernatant (step 5) to the IsoPure-10 column. Centrifuge at 10,000 x g for 30 seconds.
7. Discard the liquid from the tube. Add 500µl of Wash Solution to the column, and centrifuge at 16,000 x g for 30 seconds.
8. Repeat wash procedure in step 7.
9. Discard the flow-through in the collection tube. Centrifuge at 16,000 x g for an additional 1 minute to remove any residual Wash Solution.
10. Transfer the column to a clean 1.5ml microfuge tube. Add 50µl of Elution Buffer into the center part of the column and incubate at room temperature for 2 minutes. Centrifuge at 16,000 x g for 2 minutes. It is recommended to repeat the elution with another 50µl of Elution Buffer.
11. Store the purified DNA at -20°C.

Note: It is extremely important to add the Elution Buffer into the center part of the column.

Incubating the column with the Elution Buffer at higher temperatures (5 min at 50°C) may slightly increase the yield especially of large (>10,000bp) DNA Plasmids. Alternatively the Elution Buffer can be prewarmed to 75°C before it is applied to the column.

For higher concentration of DNA, two elution steps may be carried out with 25µl of Elution Buffer each, rather than two 50µl steps.

When working with **low copy plasmid** it is recommended to start with a large culture volume of 3-6ml. The volume of the Solution I, II, and III needs to be doubled in order to achieve complete cell lyses and obtain maximum efficiency.

**NOTE: MIDI PLUS PROTOCOL HAS BEEN UPDATED.
PLEASE FOLLOW NEW INSTRUCTIONS CAREFULLY!**

3. Protocol for Midi Plus Prep (New!)

1. Add 100ml overnight culture to an appropriate centrifuge tube and centrifuge at 5,000 x g for 10 minutes to pellet the culture. Drain the liquid completely.
2. Add 4ml of Solution I to the pellet, mix gently and incubate for 2 minutes.
3. Add 8ml of Solution II to the mixture, mix gently by inverting the tube 4-6 times and incubate at room temperature for 2 minutes. **Do not vortex.** (Vortexing will cause genomic DNA contamination).
4. Add 14ml of Solution III, and mix gently by inverting the tube immediately after the solution is added. Incubate at room temperature for 2 minutes. **Do not vortex.**
5. Centrifuge at 10,000 x g for 10 minutes.
6. Transfer the above supernatant (step 5) to the IsoPure-200 column and let stand at room temperature for 5 minutes. Centrifuge at 6,000 x g for 3 to 5 minutes (it is recommended to use swing out rotor).
7. Discard the flow-through in the tube. Add 5ml of Wash Solution to the column, and centrifuge at 6,000 x g for 3 to 5 minutes (it is recommended to use swing out rotor).
8. Repeat wash procedure in step 7.
9. Discard the flow-through in the collection tube. Centrifuge at 6,000 x g for an additional 5 minutes to remove any residual Wash Solution.
10. Transfer the column to a clean 50ml collection tube. Add 500µl of Elution Buffer into the center part of the column and incubate at room temperature for 2 minutes. Centrifuge at 6,000 x g for 2 minutes. It is recommended to repeat the elution with another 500µl of buffer.
11. Store the purified DNA at -20 °C.

Note: It is extremely important to add the Elution Buffer into the center part of the column.

Incubating the column with the Elution Buffer at higher temperatures (5 min at 50°C) may slightly increase the yield especially of large (>10,000bp) DNA Plasmids. Alternatively the Elution Buffer can be prewarmed to 75°C before it is applied to the column.

For higher concentration of DNA, two elution steps may be carried out with 25µl of Elution Buffer each, rather than one 50µl step.

When working with **low copy plasmid** it is recommended to start with a large culture volume of 200-300ml.