E.Z.N.A.® Yeast Plasmid Mini Kit

Table of Contents

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OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction and Overview

The E.Z.N.A.® family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is Omega Bio-tek's proprietary HiBind® matrix that avidly, but reversibly, binds DNA or RNA under optimized conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The E.Z.N.A.® Yeast Plasmid Mini Kit combines the power of HiBind® technology with the alkaline-SDS lysis of yeast cells to deliver high-quality DNA in less than 1 hour. Omega Bio-tek's HiBind® DNA Mini Columns facilitate the binding, washing, and elution steps, thus enabling multiple samples to be processed simultaneously. Yields will vary according to plasmid copy number, yeast strain, and growth conditions. Since yeast normally has very low plasmid copy number, the maximum yield from a 5 mL culture is ~ 1 µg.

This protocol has been successfully used to isolate autonomous plasmids from *S. cerevisiae*. Using a modified alkaline lysis procedure, genomic DNA is virtually eliminated from the preparation. Note that all centrifugation steps are to be performed at room temperature.

Binding Capacity:

Each HiBind® DNA Mini column can bind approximately 35 µg Plasmid DNA.

New in this Edition:

- This manual has been edited for content and redesigned to enhance user readability.
- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Product	D3376-00	D3376-01	D3376-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	5	50	200
YP I Buffer	5 mL	20 mL	60 mL
YP II Buffer	5 mL	20 mL	60 mL
YP III Buffer	5 mL	25 mL	80 mL
SE Buffer	3 mL	30 mL	110 mL
HBC Buffer	4 mL	25 mL	80 mL
DNA Wash Buffer	2 mL	15 mL	3 x 20 mL
Glass beads	270 mg	2.7 g	10 g
Lyticase (units)	250	2,500	4 x 2,500
RNase A	50 µL	100 µL	400 µL
Elution Buffer	5 mL	10 mL	40 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Yeast Plasmid Mini Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows. RNase A must be stored at 2-8°C. YP I Buffer (once RNase A is added) should be stored at 2-8˚C. Lyticase must be stored at -20°C. All remaining components should be stored at room temperature.

Preparing Reagents

1. Add vial of RNase A to bottle of YP I Buffer and Store at 4°C.

2. Add 10 µL 2-mercaptoethanol per 1 mL SE Buffer. Store at room temperature for up to one week.

3. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3376-00	6 mL
D3376-01	60 mL
D3376-02	80 mL per bottle

4. Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D3376-00	1.6 mL
D3376-01	10 mL
D3376-02	32 mL

5. Prepare a lyticase solution at 2500 Units/mL. Aliquot into adequate portions and store each aliquot at -20°C. Thaw before use. Each sample will require 20 µL lyticase solution.

Kit	SE Buffer to be Added
D3376-00	100 µL
D3376-01	1 mL
D3376-02	1 mL per tube

Determine the absorbance of an appropriate dilution (20- to 50- fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

DNA concentration = Absorbance 260 × 50 × (Dilution Factor) µg/mL

A value greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations.

Although the binding capacity of HiBind® DNA Mini Column is around 30 ug, the yield of the yeast plasmid depends on the yeast strain and plasmid type. High copy number plasmids generally yield up to 1 µg DNA from a 5 mL culture. The ratio of $(A_{260})/(A_{280})$ is an indication of nucleic acid purity. A value greater than 1.8 indicates >90% nucleic acid. Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatamers may also be present.

E.Z.N.A.® Yeast Plasmid Mini Kit - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000 x *g*
- Vortexer
- Water bath capable of 30°C
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol - Do not use other alcohols
- 2-mercaptoethanol (β-mercaptoethanol)
- Optional: 3M NaOH

Before Starting:

- Prepare DNA Wash Buffer, HBC Buffer, YP I Buffer, SE Buffer, and lyticase according to the instructions in the Preparing Reagents section on Page 4
- Set water bath to 30°C

1. Inoculate 5 mL YDP medium in a 10-20 mL culture tube with yeast carrying desired plasmid and grow at 30°C with agitation for 16-24 hours.

2. Centrifuge 1-3 mL yeast culture at 5,000 × *g* for 5 minutes. For best results use less than 2 x 10^7 cells.

3. Aspirate and discard medium.

4. Add 480 µL SE Buffer/2-mercaptoethanol and 20 µL lyticase solution. Vortex at maximum speed for 1 minute. Complete resuspension of the cell pellet is vital of obtaining good yield.

   Note: 2-mercaptoethanol must be added to SE Buffer before use. This mixture can be made and stored at room temperature for 1 week.

5. Incubate at 30°C for at least 30 minutes.

6. Centrifuge at 4,000 x *g* for 5 minutes at room temperature.

7. Aspirate and discard the supernatant.

8. Add 250 µL YP I Buffer.

   Note: RNase A must be added to YP I Buffer before use. Please see Page 4 for instructions.

9. Add 50 mg glass beads. Vortex at maximum speed for 5 minutes.

10. Let sit to allow the beads to settle. Transfer the supernatant to a new 1.5 mL microcentrifuge tube (not supplied).

11. Add 250 µL YP II Buffer. Invert and rotate the tube 4-6 times to obtain a cleared lysate.

    Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. A 5 minute incubation at room temperature may be necessary. Do not allow the lysis reaction to proceed more than 5 minutes. Store YP II Buffer tightly capped when not in use to avoid acidification from CO_2 in the air.

12. Add 350 µL YP III Buffer. Immediately invert several times until a flocculent white precipitate forms.

13. Centrifuge at ≥10,000 × *g* for 10 minutes at room temperature.

14. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Centrifuge at maximum speed for 30-60 seconds.
3. Discard the filtrate and reuse the collection tube.

15. Transfer 700 µL cleared lysate from Step 13 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

16. Centrifuge at maximum speed for 1 minute.

17. Discard the filtrate and reuse the collection tube.

18. Repeat Steps 15-17 until all cleared lysate has been transferred to the HiBind® DNA Mini Column.

19. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

20. Centrifuge at maximum speed for 1 minute.

21. Discard the filtrate and reuse collection tube.

22. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

23. Centrifuge at maximum speed for 1 minute.

24. Discard the filtrate and reuse the collection tube.

Optional: Repeat Steps 22-24 for a second DNA Wash Buffer wash step.

25. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

26. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

27. Add 50-100 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

    Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

28. Let sit at room temperature for 1 minute.

29. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

30. Store DNA at -20°C.

E.Z.N.A.® Yeast Plasmid Mini Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000 x *g*
- Vacuum Manifold
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath capable of 30°C
- 100% ethanol - Do not use other alcohols
- 2-mercaptoethanol (β-mercaptoethanol)
- Optional: 3M NaOH

Before Starting:

- Prepare DNA Wash Buffer, HBC Buffer, YP I Buffer, SE Buffer, and lyticase according to the instructions in the Preparing Reagents section on Page 4
- Set water bath to 30°C

1. Inoculate 5 mL YDP medium in a 10-20 mL culture tube with yeast carrying desired plasmid and grow at 30°C with agitation for 16-24 hours.

2. Centrifuge 1-3 mL yeast culture at 5,000 × *g* for 5 minutes. For best results use less than 2 x 10^7 cells.

3. Aspirate and discard medium.

4. Add 480 µL SE Buffer/2-mercaptoethanol and 20 µL lyticase solution. Vortex at maximum speed for 1 minute. Complete resuspension of the cell pellet is vital of obtaining good yield.

   Note: 2-mercaptoethanol must be added to SE Buffer before use. This mixture can be made and stored at room temperature for 1 week.

5. Incubate at 30°C for at least 30 minutes.

6. Centrifuge at 4,000 x *g* for 5 minutes at room temperature.

7. Aspirate and discard the supernatant.

8. Add 250 µL YP I Buffer.

   Note: RNase A must be added to YP I Buffer before use. Please see Page 4 for instructions.

9. Add 50 mg glass beads. Vortex at maximum speed for 5 minutes.

10. Let sit to allow the beads to settle. Transfer the supernatant to a new 1.5 mL microcentrifuge tube (not supplied).

11. Add 250 µL YP II Buffer. Invert and rotate the tube 4-6 times to obtain a cleared lysate.

    Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. A 5 minute incubation at room temperature may be necessary. Do not allow the lysis reaction to proceed more than 5 minutes. Store YP II Buffer tightly capped when not in use to avoid acidification from CO_2 in the air.

12. Add 350 µL YP III Buffer. Immediately invert several times until a flocculent white precipitate forms.

13. Centrifuge at ≥10,000 × *g* for 10 minutes at room temperature.

14. Prepare the vacuum manifold according to manufacturer's instructions.

15. Connect the HiBind® DNA Mini Column to the vacuum manifold.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Turn on the vacuum source to draw the NaOH through the column.
3. Turn off the vacuum.

16. Transfer 700 µL cleared lysate from Step 13 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

17. Transfer the cleared supernatant from Step 13 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

18. Turn on the vacuum source to draw the sample through the column.

19. Turn off the vacuum.

20. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

21. Turn on the vacuum source to draw the buffer through the column.

22. Turn off the vacuum.

23. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 6 for instructions.

24. Turn on the vacuum source to draw the buffer through the column.

25. Turn off the vacuum.

Optional: Repeat Steps 23-25 for a second DNA Wash Buffer wash step.

26. Transfer the HiBind® DNA Mini Column to a 2 mL Collection Tube.

27. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

28. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

29. Add 50-100 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

    Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

30. Let sit at room temperature for 1 minute.

31. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

32. Store DNA at -20°C

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Low DNA yields	Poor cell lysis	Do not use more than 5 mL culture with high copy plasmids or 10 mL culture with low copy plasmids with the basic protocol. Cells may not be dispersed adequately prior to addition of YP II Buffer. Vortex cell suspension to completely disperse. Increase incubation time with YP II Buffer to obtain a clear lysate. YP II Buffer, if not tightly closed, may need to be replaced.
	Yeast culture overgrown or not fresh	Do not incubate cultures for more than 24 hours at 30°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental.
	Low copy number plasmid used	Low copy number plasmids may yield as little as 0.5 µg DNA from a 5 mL overnight culture. Increase culture volume to 10 mL.
	Column matrix lost binding capacity during storage	Follow the **Optional Protocol for Column Equilibration** prior to transferring the cleared lysate to the HiBind® DNA Mini Column. Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x *g* for 30 seconds. Discard the filtrate.
Problem	Cause	Solution
No DNA eluted	DNA Wash Buffer not diluted with ethanol	Prepare DNA Wash Buffer according to instructions on Page 4.
	HBC Buffer not diluted with isopropanol	Prepare HBC Buffer according to instructions on Page 4.

Troubleshooting Guide

Problem	Cause	Solution
Optical densities do not agree with DNA yield on agarose gel	DNA Wash Buffer is diluted with ethanol containing impurities	Check the absorbance of the ethanol between 250 nm and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the binding column after washing and contribute to the absorbance in the final product.
	Background reading is high due to silica fine particulates	Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
	Purification is incomplete due to column overloading	Reduce the initial volume of culture.
Problem	Cause	Solution
Plasmid DNA floats out of well while loading agarose gel	Ethanol was not completely removed from column following wash steps	Centrifuge column as instructed to dry the column before elution.
High molecular weight DNA contamination of product	Over mixing of cell lysate upon addition of YP II Buffer	Do not vortex or mix aggressively after adding YP II Buffer.
	Culture overgrown	Overgrown cultures contain lysed cells and degraded DNA. Do not grow cell longer than 16 hours.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
DNase/RNase-free microcentrifuge tubes, 1.5 mL, 500/pk, 10 pk/cs	SSI-1210-00
DNase/RNase-free microcentrifuge tubes, 2.0 mL, 500/pk, 10 pk/cs	SSI-1310-00
Vacuum Manifold	VAC-08
HiBind® DNA Mini Columns (200)	DNACOL-02
Elution Buffer (100 mL)	PDR048
DNA Wash Buffer (100 mL)	PS010