Ver: 2109

Yeast gDNA Isolation Kit

（BW-GD2415）

Contents

Kit Contents

Catalog#	BW-GD2415-00	BW-GD2415-01	BW-GD2415-02
Preps	4	50	250
DNA Mini Columns	4	50	250
2 mL Collection Tubes	4	50	250
Buffer YTL	1.0 mL	13 mL	65 mL
Buffer YBL	1.0 mL	13 mL	65 mL
Buffer KB	2.1 mL	26 mL	130 mL
DNA Wash Buffer*	2.0 mL	15 mL	3 x 24 mL
Glass Beads	210 mg	2.7 g	13 g
Elution Buffer	0.5 mL	6 mL	26 mL
Buffer SE	2.0 mL	25 mL	125 mL
Lyticase	200 µL	2200 µL	11,000 µL
Proteinase K	110 µL	1.3 mL	5 x 1.3 mL
RNase A	25 µL	270 µL	1.4 mL
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* Add 8 mL (BW-GD2415-00) or 60 mL (BW-GD2415-01) or 96 mL (BW-GD2415-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Introduction

The Yeast gDNA Isolation Kit allows rapid and reliable isolation of high-quality total cellular DNA from a wide variety of yeast species. Up to 3 mL of log-phase culture (OD_{600} of 1.0 in YPD medium) can be processed. The system combines the reversible nucleic acid-binding properties of ezBind matrix with the speed and versatility of spin column technology to yield approximately 15-30 µg of DNA. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. Yeast gDNA Kit will isolate all cellular DNA, including plasmid DNA.

Storage and Stability

Proteinase K is stable at room temperature (15-25℃) for one year. For long term, store aliquots at -20℃. Store aliquots of lyticase at -20℃. Store all other contents at room temperature (15-25℃). The guaranteed shelf life is 12 months from the date of production.

Before Starting

Please read the entire booklet to become familiar with the Yeast DNA Kit procedure. Carry out all centrifugation steps at room temperature.

Important Notes

Dilute DNA Wash Buffer with absolute ethanol as follows: Add 8 mL (BW-GD2415-00) or 60 mL (BW-GD2415-01) or 96 mL (BW-GD2415-02) of absolute ethanol to each bottle. The final concentration is 80%.

Materials not Supplied

✪ Tabletop microcentrifuge and nuclease-free 1.5 mL centrifuge tubes.

✪ Water bath set to 30℃ and 65℃.

✪ Shaking water bath set to 55℃.

✪ Absolute ethanol

Safety Information

Buffer YBL contains a chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the waste. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets.

Yeast DNA Kit Spin Protocol

1. Grow yeast culture to OD_{600} of 1.0. Harvest no more than 3 mL culture (< 2 $\times 10^7$) by centrifugation at 4,000 ×g for 10 minutes at room temperature.
2. Discard medium and resuspend cells in **480 µL Buffer SE** and **40 µL Lyticase solution**. Incubate at 30℃ for at least 30 minutes.
3. Pellet spheroblasts by centrifuging 10 minutes at 500 ×g at room temperature.
4. Resuspend cells in **200 µL Buffer YTL** and vortex for 5 minutes.

   Optional: If maximum DNA yield is desired, add around 30 mg of glass beads before vortexing.
5. Add **20 µL Proteinase K** solution and vortex for 10 senconds to mix well. Incubate at 55℃ for 60 minutes in a shaking water bath to complete lysis. If no shaking waterbath is available, incubate and shake or briefly vortex the samples every 20-30 minutes.
6. Add **5 µL RNase A** to samples and invert tube several times to mix. Incubate at room temperature for 5 minutes.
7. Centrifuge at 10,000 × g for 5 minutes to pellet insoluble debris. Carefully aspirate the supernatant and transfer to a sterile micro-centrifuge tube leaving behind any insoluble pellet.
8. Add **220 µL Buffer YBL** and vortex to mix at maximum speed for 15 seconds. Incubate at 65℃ for 10 minutes. A wispy precipitate may form upon addition of Buffer YBL (It does not interfere with DNA recovery).
9. Add 220 µL absolute ethanol and mix thoroughly by vortexing at maximum speed for 20 seconds. If any precipitation can be seen at this point, break the precipitation by pipetting up and down 10 times.
10. Insert a **DNA Mini Column** into the **2 mL Collection Tube**, and transfer the entire sample from step **9** into the **DNA Mini column**, including any precipitate that may have formed. Centrifuge at 10,000 ×g for 1 minute to bind DNA. Discard the flow-through and reuse the collection tube.
11. Add **500 µL Buffer KB**. Centrifuge at 10,000 ×g for 1 minute. Discard flow-through and reuse the **2 mL Collection Tube**.
12. Add **600 µL DNA Wash Buffer**. Centrifuge at 10,000 × g for 1 minute. Discard

flow-through and reuse the **2 mL Collection Tube**.

13. Centrifuge **DNA Mini Column**, with the lid open, into the collection tube and centrifuge for 2 minutes at 12,000 ×g for 2 minutes to dry the column. This step is critical for removal of trace ethanol that might otherwise interfere with downstream applications.
14. Place the column into a nuclease-free 1.5 mL microfuge tube and add **50-100 µL** of preheated (65℃) **Elution Buffer** to center of the ezBind matrix. Allow columns to incubate for 3 minutes at room temperature after addition of **Elution Buffer**.
15. To elute DNA from the column, centrifuge at 10,000 ×g for 1 minute.

    Optional: Reload the eluate into the center of the column for a second elution.

    Note: The first elution typically yields 60-70% of the DNA while the 2nd elution yields another 20-30% of the DNA bound to the column.
16. The total DNA yield can be determined by a spectrophotometer. DNA concentration is calculated as:

    [DNA] = (Absorbance260) x (0.05 µg /µL) x (Dilution factor)

    Note: The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio (A260/A280) of 1.7-1.9 corresponds to 85%-95% purity.

Yeast gDNA Isolation vacuum and spin protocol

Note: Please read through previous section of this manual before using this protocol.

1. Prepare samples and column by following the standard protocol in previous section (Step **1-9**).

   Prepare the vacuum manifold according to manufacturer's instructions and connect the V-Spin column to the manifold.
2. Load the sample/YBL/Ethanol mixture to the column. Switch on vacuum source to draw the sample through the column and turn off the vacuum.
3. Wash the column by adding **500 µL Buffer KB**, draw the Buffer KB through the column by turning on the vacuum source.
4. Wash the column by adding **600 µL DNA Wash Buffer** (add absolute ethanol before use), draw the wash buffer through the column by turning on the vacuum source.
5. Proceed to step **13-15** of Yeast DNA Spin Protocol on page **5**.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Clogged column	Incomplete lysis.	Add the correct volume of Buffer YTL and incubate at 55℃ to obtain complete lysis. It may be necessary to extend incubation time to 30 minutes.
	Sample too large.	Do not use greater than 3 mL culture at OD_{600} of 1.0 or $2x10^7$ cell per spin column. For larger volumes, divide sample into multiple tubes.
	Incomplete removal of cell wall.	Add more lyticase or extend the incubation time. It may be necessary to increase incubation by 60 minutes.
Low DNA yield	Clogged column.	See above.
	Poor elution.	Repeat elution or increase elution volume (see note on page 5). Incubation of column at 65℃ for 5 minutes after addition of Elution Buffer may increase yields.
	Improper washing.	DNA Wash Buffer concentrate must be diluted with absolute (96%-100%) ethanol.
Low A260/A280 ratio	Extended Centrifugation during elution step.	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation — it will not interfere with PCR or restriction digests.
	incomplete mixing with Buffer YBL.	Repeat the procedure, this time making sure to vortex the sample with Buffer YBL immediately and completely.
	Insufficient incubation.	Increase incubation time with Buffer YTL. Ensure that no visible cell clumps remain.
No DNA eluted	Poor cell lysis due to improper mixing with Buffer YBL.	Mix thoroughly with Buffer YBL and incubate at 70℃ prior to adding ethanol.
	Incomplete spheroblasting.	Add more lyticase or extend the incubation time. It maybe necessary to increase incubation by 60 minutes.
	No ethanol added to DNA Wash Buffer.	Before applying sample to column, an aliquot of ethanol must be added. See protocol above.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.

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