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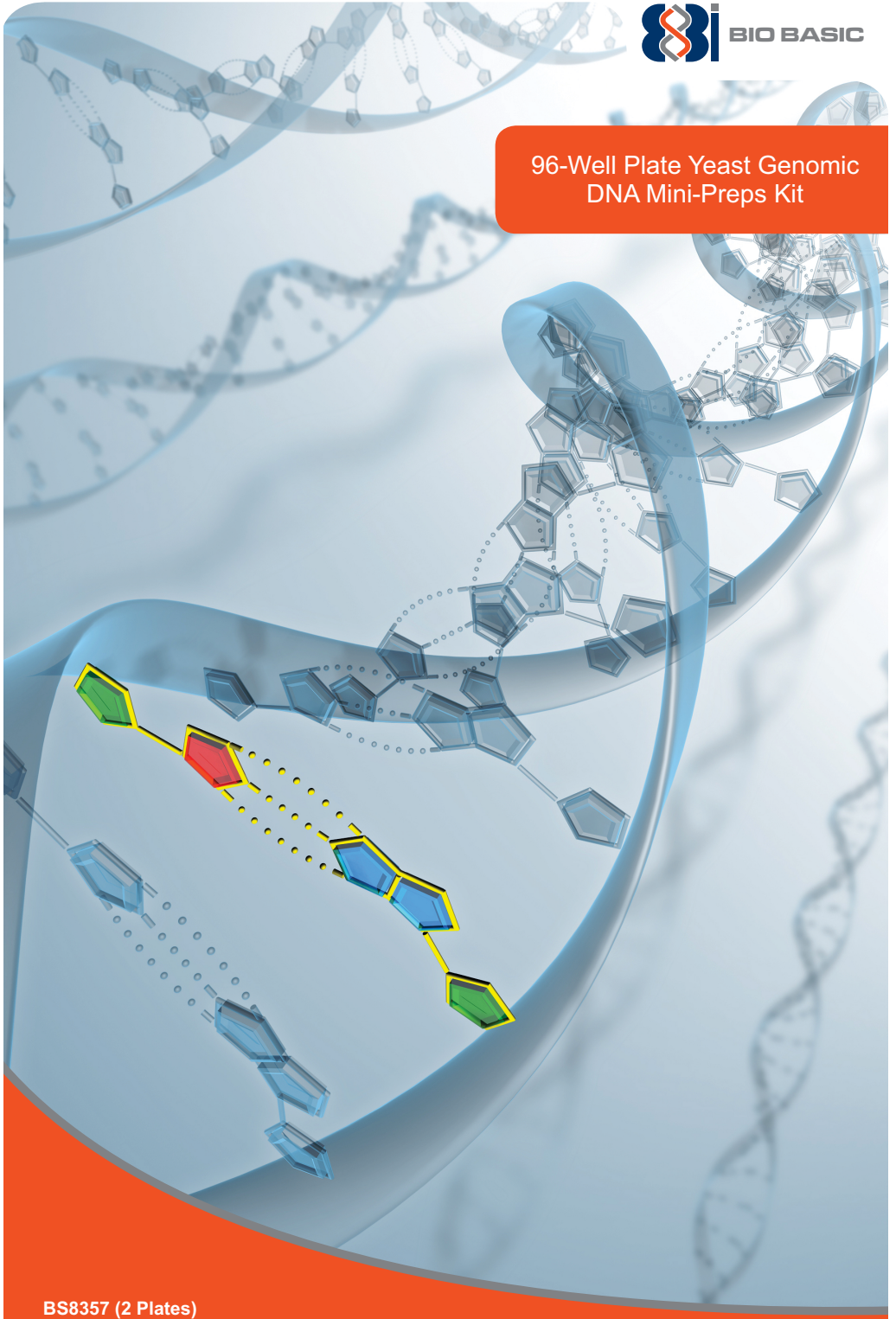
96-Well Plate Yeast Genomic DNA Mini-Preps Kit



BS8357 (2 Plates)
QF 24 TV4
CV1 2020

For Research Use Only

96-Well Plate Yeast Genomic DNA Mini-Preps Kit Code: BS8357 (2 Plates)

Description

The kit provides a simple and convenient high throughput approach to isolate high quality DNA from yeast. DNA of cell lysates is selectively adsorbed in a 96-Well plate, and impurities such as proteins, salts and nucleotides are washed away. No phenol/chloroform extraction and no ethanol precipitation are required. Purified genomic DNA can be up to 20 kb in length, and can be used for restriction endonuclease digestions and other downstream applications.

Features

- Fast & Simple: Using a rapid spin-column and high throughput format, the entire procedure takes approx 2 hours.
- High Quality of DNA: OD_{260}/OD_{280} of purified DNA is generally 1.7-1.9.
- Non-toxic: No phenol extraction or ethanol precipitation required.
- Economic.

12. Add 50 µl CE Buffer directly onto the center part of 96-Well Plate membrane. Incubate at room temperature for 2 minutes, and then centrifuge for 3 minutes at 5,000 x g to elute the DNA.

NOTE 1: Warming the CE Buffer to 60°C will increase the elution efficiency.

NOTE 2: Elution with more than 50 µl (e.g. 100 µl) increases the DNA yield, but the concentration will be lower.

NOTE 3: For maximum DNA yield, repeat elution once as described in this step.

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4. Add 100 µl Universal Buffer PY. Cover wells with Sealing film. Mix by inverting the plate a few times. Incubate at -20°C for 5 minutes.
5. Centrifuge at 5,700 x g for 5 minutes at room temperature. Transfer the supernatant to a new Deep Well Collection Plate.
6. Add 200 µl Universal Buffer BD, mix thoroughly.
7. Add 200 µl ethanol (96-100%). Mix thoroughly.

   NOTE: If a gelatinous material appears at this step, vigorous shaking or vortexing is recommended.
8. Transfer the mixture from step 7 (including any precipitate) into a 96-Well Plate placed on top of a Deep Well Collection Plate. Centrifuge at 5,700 x g for 10 minutes. Discard the flow-through.
9. Add 500 µl Universal PW Solution, and centrifuge for 5 minutes at 5,700 x g. Discard the flow-through.

   NOTE: Check the label to ensure PW Solution was diluted with isopropanol.
10. Add 500 µl Universal Wash Solution, and centrifuge for 3 minutes at 5,700 x g. Discard the flow-through.

    NOTE: Check the label to ensure Wash Solution was diluted with ethanol.
11. Place the empty 96-Well Plate in the Deep Well Collection Plate and centrifuge for an additional 2 minutes at 5,700 x g to dry the 96-Well Plate membrane. Discard flow-through and transfer the 96-Well Plate to a 96-Well Storage Plate.

    NOTE: It is important to dry the membrane of the 96-Well Plate, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

Kit Contents

Components	BS8357 (2 Plates)
Universal Buffer Digestion	48 ml
Universal Buffer PY	24 ml
Universal Buffer BD	48 ml
Universal PW Solution	72 ml
Universal Wash Solution	30 ml
CE Buffer (pH 9.0)	15 ml
Proteinase K	4.8 ml
Snailase Reaction Buffer	2 x 150 ml
Snailase Storage Buffer	20 ml
Snailase	2.4g
96-Well Plate	2
Deep Well Collection Plate	6
96-Well Storage Plate	2
Sealing film	8
Protocol	1

NOTE 1: Universal Buffer BD contains chaotropic salts; avoid contact with skin and eyes.

NOTE 2: Universal PW Solution and Universal Wash Solution are supplied as concentrates. Add 48 ml isopropanol to 72 ml Universal PW Solution and 90 ml ethanol (96-100%) to 30 ml Universal Wash Solution before use to obtain a working solution.

Storage

96 Well Plates and all buffers should be stored dry, at room temperature (15-25°C). Proteinase K is supplied as 10 mg/ml solution. The solution can be kept at room temperature for 6 months. For long-term storage please keep at -20°C.

Dilute 2.4g Snailase in 20 ml Snailase Storage Buffer before use. This is a Snailase Working Stock. Aliquot and store the Snailase Working Stock at -20°C.

Materials Supplied by User

- Microcentrifuge capable of at least 8,000 × g
- Pipettes and pipette tips
- Vortexer
- Isopropanol
- Ethanol (96-100%)
- RNase A (20 mg/ml, Optional for RNA-free DNA)
- β-mercaptoethanol
- Water bath for heating at 56°C

Before Starting

Snailase working stock: Add 2.4 g of Snailase into 20 ml of Snailase Storage Buffer. Mix well by gently inverting the tube. Then aliquot and store at -20ºC for further use.

Proteinase K is supplied as a ready-to-use solution.

RNase A is not provided in this kit. If RNA-free DNA is required, please prepare RNase solution and follow the protocol to add the RNA removal in step 3.

Check the Universal Buffer Digestion and Universal Buffer BD for salt precipitation before each use. If necessary, redissolve the precipitate by warming the solution at 56°C, then cool back down to room temperature before use.

CE Buffer is 10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0. Water can be used as elution in the final step if EDTA needs to be avoided for downstream applications; however this is not recommended if the pH of water is less than 7.0.

PW Solution and Wash Solution are supplied as concentrates. Before using them for the first time, add 48 ml isopropanol to 72 ml Universal PW Solution, and add 90 ml ethanol (96-100%) to 30 ml Universal Wash Solution.

Preheat the water bath or rocking platform to 56°C.

Protocol

1. Add 1.0 ml of yeast culture (1×10^7cells) to each well of a Deep Well Collection Plate. Centrifuge at 5,700 x g for 30 seconds to 1 minute. Discard the supernatant completely.

   NOTE: Using 1×10^8 or more cells may clog the wells in subsequent steps.

2. Add 600 µl of Snailase Reaction Buffer, 1.2 µl of β-mercaptoethanol (not supplied in the kit) and 50 µl of Snailase Working Stock per 20 mg wet weight yeast to the Deep Well Collection Plate. Cover plate with Sealing film and mix thoroughly by vortexing. Incubate at 37ºC for 3 hours. Invert the plate occasionally. If lyticase was used, please add 50 µl of Lyticase Enzymatic Storage Buffer (containing 300 U or more) per 20 mg wet weight yeast. Centrifuge at 4,500 x g for 10 minutes, discard the supernatant.

3. Add 200 µl Universal Buffer Digestion and 20 µl Proteinase K to the sample. Cover wells with Sealing film and mix thoroughly by vortexing. Incubate at 56°C for 30-60 minutes.

   NOTE: If RNA-free genomic DNA is required, add 20 µl RNase A (20 mg/ml) after step 3. Mix by vortexing. Incubate for 2 minutes at room temperature before continuing with step 4.