A world leader in serving science

BIO BASIC INC.

96-Well Plate Yeast Genomic DNA Mini-Preps Kit

BS8357

Version 4.3
ISO9001 Certified

20 Konrad Cres, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com

PRODUCT INFORMATION

Product information for BS8357:
Kit Content

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	2X150 ml
Snailase Storage Buffer	20 ml
Snailase	2.4 g
96-Well Plate	2
Deep Well Collection Plate	6
96-Well Storage Plate	2
Sealing film	8
Caps for Storage Plate	24
Protocol	1

Note 1: Universal Buffer BD contains chaotropic salt; 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%) for **30 ml** Universal Wash Solution before use to obtain a working solution.

Storage and Stability:

96 Well Plates and all buffers should be stored dry, at room temperature (15-25°C) and are stable for 1 year under these conditions. Proteinase K is supplied as 10 mg/ml solution, the solution can be keep at Room Temperature for 6 months, for long-term storage keep at -20°C.
Dilute 2.4 g Snailase in 20 mL Snailase Storage Buffer before use. This is a Snailase Working tock. Aliquot and store Snailase Working Stock at -20°C.

Introduction:

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

Features:

1. Fast & Simple: Using a rapid spin-column and high throughput format, the entire procedure takes approx 2 hours.
2. High Quality of DNA: OD_{260}/OD_{280} of purified DNA is generally1.7-1.9.
3. Non-toxic: No phenol extraction or ethanol precipitation is required.
4. Economic.

Materials Supplied by User:

Microcentrifuge capable of at least 8,000 × *g*
Pipets and pipet 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 2.4G of Snailase into 20ml of Snailase Storage Buffer, mix well by gently inverting, then aliquot and store at -20ºC for further use.
Proteinase K is supplied in a ready-to-use solution form, but RNase A is not provided in this kit, if RNA-free DNA is required, please prepare RNase solution and see protocol to add the RNA removal step.

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 should be avoided for the following applications, but it is not recommended if the pH of water is less than 7.0.

PW Solution and **Wash Solution** are supplied as concentrates. Before using for the first time, Add **48 ml** isopropanol (96-100%) to **72 ml** Universal PW Solution and **90 ml** ethanol for **30 ml** Universal Wash Solution, respectively.
Preheat the water bath or rocking platform to 56°C.

Protocol:

1. Collect 1.0 ml of yeast culture (1×10^7 cells) to a Deep Well Collection Plate, centrifuge at 5,700 x *g* for 30 seconds. Discard the supernatant completely.
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 tube occasionally. If lyticase was used, please use 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 with Sealing film and mix thoroughly by vortexing. Incubate at 56°C for 30-60 min.

   Note: If RNA-free genomic DNA is required, add 20 µl RNase A (20 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing with step 4, mix thoroughly by vortexing
4. Add 100 µl **Universal Buffer PY**, covered with Sealing film and mix by inverting, 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, vigorously 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 spin column 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.

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: Warm 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.

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR ANIMAL USE!

Please visit www.biobasic.com

BIO BASIC INC.

A world Leader in Serving Science