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EZ-10 Spin Column Yeast Plasmid DNA Mini-Preps Kit

BS467 & BS468 & BS469

Version 5.2
ISO9001 Certified

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EZ-10 Spin Column Yeast Plasmid DNA Mini-Preps Kit

Product information for BS467/BS468/BS469:

Kit Contents

Component	BS467 10 Preps	BS468 50 Preps	BS469 250 Preps
Buffer P1 [(a)]	3 ml	15 ml	75 ml
Buffer P2 [(b)]	3 ml	15 ml	75 ml
Buffer P3 [(c)]	4 ml	20 ml	100 ml
Buffer DW1 [(d)]	5 ml	25 ml	125 ml
Universal Wash Buffer [(e)]	3 ml	12 ml	60 ml
Elution Buffer [(f)]	1 ml	5 ml	5 ml
RNase A Solution(10mg/ml)	25 µl	120 µl	600 µl
Snailase	60 mg	300 mg	1500 mg
Snailase Storage Buffer**	0.5 ml	2.5 ml	12.5 ml
Snailase Reaction Buffer	7 ml	35 ml	175 ml
EZ-10 Spin Column	10	50	250
2 ml Collection Tube	10	50	250
Protocol	1	1	1

Notes:

a) Before use, add entire contents of RNase A Solution to the bottle containing Buffer P1 and mix well. Solution I with RNase A should be stored at 4 ºC for frequent use and at -20 ºC for infrequent use.
b) Buffer P2 may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37 ºC.
c) Buffer P3 may form a precipitate upon low temperature storage. If necessary, dissolve the precipitate by warming the solution at 50 ºC.
d) Before use, add 1.25ml, 6.25ml or 31.25ml of isopropanol to Buffer DW1 in BS467, BS468 and BS469 respectively. For other volumes of Buffer DW1, simply add enough isopropanol to make a **1:4** ratio (volume of added isopropanol: volume of Buffer DW1 = 1:4).
e) Before use, add 12ml, 48ml or 240ml of 100% of ethanol to Universal Wash Buffer in BS467, BS468 and BS469 respectively. 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).
f) Elution Buffer is 2.0 mM Tris-HCl pH 8.0~8.5. The resulting yields may add up to 20% more recovery rates than TE buffer pH 8.0 or water.

** Please prepare snailase working stock: Transfer Snailase powder into 0.5 ml (BS467), 2.5 ml (BS468) or 12.5 ml (BS469) of enzymatic storage buffer and vortex thoroughly, Store at -20 ºC. If Lyticase was used, please use the same enzymatic storage buffer, and dissolve Lyticase to proper concentration.
Snail Storage Buffer contain: 20m M K_2HPO_4-KH_2PO_4 PH 7.4 and 50% glycerol

Storage

Snailase and snailase working stock should be stored at -20 ºC. RNase A can be stored at 4ºC.Other reagents can be kept at room temperature. The kit is stable for 12 months. For longer storage, keep all contents in cold place.

Introduction

This kit provides a simple and efficient method for Mini plasmid DNA purification from yeast cell. Yeast cell wall is first being degraded by enzymatic digestion (lyticase zymolyase, or snailase). Genomic DNA, protein and RNA are then precipitated through normal alkali lysis. Supernatant containing plasmids are transferred onto EZ-10 Spin Column. Up to 10 µg of DNA plasmid DNA can be selectively adsorbed on silica gel-based EZ-10 Spin Column while other impurities such as proteins, salts are washed away. Purified plasmid DNA can be used for any downstream application such as sequencing, restriction enzyme reactions, labelling, transformation, PCR and Southern-blot.

Features

√ Alkali lysis with enzymatic digestion.
√ Convenience and environment friendly. No phenol / chloroform extraction or ethanol precipitation needed.
√ High plasmid purity in small elution volume. Purified DNA can be used for many downstream applications including automatic fluorescence sequencing.

Procedures

1. Collect 1.0 ml yeast culture (~1×10^7 cell) in a 1.5 ml Eppendorf tube and centrifuge at 10,000 x g (12,000 rpm) for 30 seconds. Discard supernatant completely.
   Note: If the plasmid in yeast has low copy number, please increase to 3-5 ml yeast culture. Meanwhile, increase volume of Buffer P1, P2, and P3 and enzyme units proportionally.
2. Removal of yeast cell wall:
   a) Enzymatic Digestion: Add **600 µl** enzymatic reaction buffer, **1.2 µl** mercaptoethanol and **50 µl** Snailase Working Stock per 20 mg wet weight yeast in a 1.5 ml tube. Incubate at 37 ºC for 3 hours. Invert the tube periodically. If lyticase is used, add 50 µl lyticase enzymatic storage buffer containing 300U or more lyticase per 20 mg wet weight yeast. Centrifuge at 3,000 x g (5,000 rpm) for 10 minutes. Discard the supernatant.

3. Add 250 µl of Buffer P1 to the pellet, vortex vigorously.
4. Add 250 µl of Buffer P2 to the mixture, mix gently by inverting the tube 4-6 times and then keep the tube at room temperature for 1 minute.

Note: To prevent contamination from genomic DNA, do not vortex vigorously. In addition, the whole lysis should not exceed 5 minutes.

5. Add 350 µl of Buffer P3, and mix gently. Incubate at room temperature for 5 minutes.
6. Centrifuge at 10,000 x g (12,000 rpm) for 10 minutes.
7. Transfer the above supernatant (step 6) to an EZ-10 Spin Column. Centrifuge at 6,000 x g (8,000 rpm) for 2 minute
8. Discard the flow-through in the tube. Add 500 µl of buffer DW1 to the Spin Column, and centrifuge at 8,000 x g (10,000 rpm) for 1 minute.
9. Discard the flow-through in the tube. Add 500 µl of Universal Wash Solution to the Spin Column, and centrifuge at 8,000 x g (10,000 rpm) for 1 minute.
10. Repeat Step 9.
11. Discard the flow-through in the Collection Tube. Centrifuge at 8,000 x g (10,000 rpm) for an additional 2 minutes to remove any residual Wash Solution.
    Note: In order to gain high plasmid DNA elution efficiency, keep EZ-10 Spin Column at room temperature for 10 minutes or in dryer at 50 ºC for 5 minutes.
12. Transfer column to a clean 1.5 ml Eppendorf tube. Add 50 µl of Elution Buffer into the center part of the column and incubate at room temperature for 2 minutes. Centrifuge at 8,000 x g (10,000 rpm) for 2 minutes.
    Note: To improve plasmid DNA elution efficiency, the elution buffer or ddH_2O can be heated to 60 ºC prior.
13. Store purified DNA in freezer at -20 ºC.

Troubleshooting

Low plasmid yield:

1) Yeast aging: please spread new plate and select colony, and then prepare liquid culture.
2) Partial digestion: add the corresponding quantity of snailase, lyticase or zymolyase and prolong incubation time, according to different mass and different strains of yeast, so as to fully remove cell wall of yeast.

Note:
Buffer P3 and Buffer DW1 contain irritant compounds. Protection gloves must be worn. At the same time avoid contaminate clothes, eyes and skin.

Other Kits Available

EZ-10 Spin Column Plasmid DNA MiniPreps Kit

BS413 (50 Preps)
BS414 (100 Preps)
BS614 (250 Preps)

EZ-10 Spin Column PCR Products Purification Kit

BS363 (50 Preps)
BS364 (100 Preps)
BS664 (250 Preps)

EZ-10 Spin Column DNA Gel Extraction Kit

BS353 (50 Preps)
BS354 (100 Preps)
BS654 (250 Preps)

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

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