RPM Yeast Plasmid Isolation Kit

Revision No. 2069-999-8C03W

Overview

* Purification of Plasmid DNA from Yeast for PCR and Bacterial Transformation
* No Phenol/Chloroform Extractions
* No Alcohol Precipitation

Shipping & Storage

The RPM Yeast Plasmid Isolation Kit is shipped and stored at ambient temperature

Kit Components

Catalog No. 2069-000 (12 preps)

CAT. NO.	DESCRIPTION	SIZE/VOL.
2069-001	Yeast Lysis Matrix	0.4 g x 12 tubes
2069-002	Alkaline Lysis Solution	4 ml
2069-003	Neutralizing Solution	4 ml
2069-004	GLASSMILK®Spin Buffer	5 ml
2069-005	Wash Solution Concentrate*	7 ml
2069-006	SPIN Filters	12
2069-007	Catch Tubes	12

*Add 7 ml of 100% Ethanol to Wash Solution Concentrate and mix before initial use

Catalog No. 2069-200 (25 preps)

CAT. NO.	DESCRIPTION	SIZE/VOL.
2069-201	Yeast Lysis Matrix	0.4 g x 25 tubes
2069-202	Alkaline Lysis Solution	8 ml
2069-203	Neutralizing Solution	8 ml
2069-204	GLASSMILK® Spin Buffer	8 ml
2069-205	Wash Solution Concentrate*	14 ml
2069-206	SPIN Filters	25

2069-207 Catch Tubes 25
*Add 14 ml of 100% Ethanol to Wash Solution Concentrate and mix before initial use

Catalog No. 2069-400 (100 preps)

CAT. NO.	DESCRIPTION	SIZE/VOL.
2069-401	Yeast Lysis Matrix	0.4 g x 100 tubes
2069-402	Alkaline Lysis Solution	32 ml
2069-403	Neutralizing Solution	32 ml
2069-404	GLASSMILK®Spin Buffer	32 ml
2069-405	Wash Solution Concentrate	56 ml
2069-406	SPIN Filters	100
2069-407	Catch Tubes	100

*Add 56 ml of 100% Ethanol to Wash Solution Concentrate and mix before initial use

Introduction

This is a dual purpose kit for the isolation of plasmid DNA from yeast and bacteria and is especially useful during screening studies of two-hybrid transformants.

The Yeast Plasmid Isolation Kit is a Rapid Pure Miniprep Kit designed for yeast cells. The plasmid isolated from yeast is suitable for PCR and bacterial transformation. The same Kit can be used to isolate plasmid from bacterial transformants for use in any downstream application (PCR, sequencing, restriction enzyme digestion, labeling, etc.) This dual purpose kit is ideal for screening yeast transformants obtained from two-hybrid screens.

Identical solutions are used for plasmid isolation from yeast and bacteria. However a larger amound of some of the solutions is included in the kit for working with yeast cells.

Protocol

1. Temporarily remove **Yeast Lysis Matrix** from screw cap tube by pouring into another clean tube or container. Add 1.5 ml of yeast culture to the empty tube and spin for 30 seconds to pellet the cells. Discard the supernatant.

2. Pour the **Yeast Lysis Matrix** back into the tube, add **250 µl Alkaline Lysis Solution** and vortex continuously for 5 minutes or shake for 10 seconds in the FastPrep Instrument.

Note: The presence of detergents in the **Lysis Solution** will cause the sample to foam. To facilitate the processing of multiple samples, use a multi-tube holder attached to the vortex machine. FastPrep is a controlled high-speed shaking device available through BIO 101 to extract nucleic acids from virtually any source: cells, tissues, organs, and organisms; it can process up to 12 samples simultaneously.

3. Add **250 µl Neutralizing Solution**; mix by brief vortexing and spin 2 minutes at room temperature. Transfer the supernate to a **Spin Filter**, avoiding the precipitated debris and Lysis Matrix.

4. Add **250 µl Glassmilk Spin Buffer**; invert to mix; spin 1 minute and decant the **Catch Tube**.

5. Add **500 µl Wash Solution**; spin 1 minute and decant the wash. Repeat the wash step; decant the **Catch Tube** and spin for 1 minute to drive the last of the liquid out of the **Spin Filter**. Transfer the **Filter** to a new **Catch Tube**.

6. Add 100 µl sterile H2O; vortex briefly (at no more than half speed) to resuspend and spin 30 seconds to collect the DNA in the bottom of the **Catch Tube**; discard the **Filter** containing used **Matrix**. Use 5 µl for transformation and PCR analysis.

Note: Electroporation is preferred over CaCl2-heat-shock treatment when transforming bacterial cells.

When using the kit to isolate plasmid DNA from bacteria, **do not use** the yeast lysis matrix in step 2. Use 50, 100, 100 µl of the lysis reagents in steps 2, 3, and 4, respectively, and elute with 50 µl of sterile water in step 6.