Yeast Genomic DNA Purification Kit

CODE	DESCRIPTION	SIZE
1B1586-KIT-200RXN	Yeast Genomic DNA Pufication Kit	200 Reactions
1B1586-KIT-50RXN	Yeast Genomic DNA Pufication Kit	50 Reactions
1B1586-KIT-SAMPLE	Yeast Genomic DNA Pufication Kit	10 Reactions

General Information

AMRESCO's Yeast Genomic DNA Purification Kit yields high molecular weight DNA from yeast using a non-enzymatic lysis buffer and no glass beads, phenol, chloroform, or columns. The protocol is simple, requiring about 1 hour to complete, and may be scaled for large or small samples. The kit, which includes Yeast Lysis Solution, Protein Precipitation Solution, Ribonuclease A (RNase A), 10 mg/ml Solution and TE (Tris-EDTA) Buffer, pH 8.0, works with liquid cultures as well as colonies. The purified DNA yields vary for different yeast strains and is also dependent on cell density. The kit is optimized for *Saccharomyces cerevisiae*, with an average yield of 1.5 µg DNA per 5 x 10^7 cells, as measured by AMRESCO's Fluorescent DNA Quantitation Kit. High quality DNA obtained with the Yeast Genomic DNA Purification Kit is compatible with downstream applications, such as, PCR, restriction enzyme digestion and Southern blotting.

Storage/Stability

Store Ribonuclease A (RNase A) 10 mg/ml Solution at 2-8°C.

Store other kit components at 18-26°C.

Product Use Limitations

For Research Use Only. Not for Therapeutic or Diagnostic Use.

Hazard Precaution

Please see the Safety Data Sheet for information regarding hazards and safe handling practices.

Supplied Material

Reagent		200RXN	50RXN	Sample
1B1573	Yeast Lysis Solution	60 mL	15 mL	3 mL
E866	Ribonuclease A (RNase A), 10 mg/ml Solution	1 mL (x2)	1 mL	1 mL
N611	Protein Precipitation Solution	20 mL	5 mL	1 mL
E112	TE (Tris-EDTA) Buffer, pH 8.0	10 mL	1 mL (x2)	1 mL

Additional required materials not supplied

- Isopropanol
- 70% Ethanol

- Microcentrifuge tubes
- Heated water bath or heating block
- Ice
- Centrifuge

Protocol/Procedure

Notes: Volumes of reagents may be adjusted to accommodate variations in sample size and type. This kit is optimized for use with *S. cerevisiae*, but may also be used with other species of yeast.

Harvetsing Yeast Cells – Liquid Cultures

1. Centrifuge a 1.5 ml saturated yeast culture (8-10 A_{600} units) at ≥ 10,000 rpm for 2-5 minutes.
2. Discard supernatant and proceed to Cell Lysis and DNA Precipitation.

Harvesting Yeast Cells – Solid Media

1. Aliquot 300 µL of Yeast Lysis Solution to a microcentrifuge tube.
2. Transfer a single yeast colony (2 mm diameter) from solid medium into the Yeast Lysis Solution.
3. Resuspend the cells and proceed to Cell Lysis and DNA Precipitation, Step 2.

Cell Lysis and DNA Precipitation

1. To each tube of harvested cells, add 300 µL Yeast Lysis Solution and mix cells by vortexing or repeated pipetting.
2. Optional RNase A treatment: Add 10 µL Ribonuclease A (RNase A), 10 mg/ml Solution per 300 µL sample and mix thoroughly.
3. Incubate samples 15 minutes at 65°C.
4. Chill samples 5 minutes on ice.
5. Add 100 µL Protein Precipitation Solution and vortex samples for 10 seconds.
6. Centrifuge samples for 10 minutes at ≥ 10,000 rpm to pellet cellular debris.
7. Transfer supernatant to a clean microcentrifuge tube.
8. Add 500 µL isopropanol and mix by inversion.
9. Centrifuge samples for 10 minutes at ≥ 10,000 rpm to pellet the DNA. Discard the supernatant without disturbing the pellet.
10. Add 0.5 mL 70% ethanol to wash the pellet.
11. Centrifuge for 2 minutes at ≥ 10,000 rpm. Discard the supernatant without disturbing the pellet.
12. Air dry the pellet for approximately 5 minutes.
13. Resuspend the genomic DNA in 35 µL TE Buffer, pH 8.0.

Note: The average DNA yield for 5 x 10^7 *Saccharomyces cerevisiae* cells is approximately 1-2 µg

Frequently Asked Questions:*

Problem	Cause	Solution
Low DNA yield	Lost pellet	DNA pellet may be difficult to see during isopropanol precipitation. Be very careful when removing isopropanol.
	DNA degradation	Use nuclease-free tubes and tips.
	Insufficient cell lysis	Increase volume of Yeast Lysis Solution and mixing time/force.
	Old/overgrown culture	Lysis occurs readily in overgrown culture, leading to degradation of genomic DNA. Start over with fresh cultures.
Resuspension of DNA pellet is difficult	Overdried DNA	Try rehydrating DNA by incubation at 65°C for 1 hour in TE, pH 8.0 and/or allowing the DNA to sit overnight at room temperature or 4°C before use.
Sheared genomic DNA	Improper handling of samples	After step 5 of Cell Lysis and DNA Precipitation, mixing is to be done by gentle inversion.
	Old/overgrown culture	Lysis occurs readily in overgrown culture, leading to degradation of genomic DNA. Start over with fresh cultures.
Measured DNA concentration is inaccurate	Concentration determined by UV spectroscopy	A_{260} concentrations overestimate DNA yield, even for samples treated with RNase A. Quantitate DNA using a fluorometer and a fluorescent DNA quantitation assay.

For Technical Support

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