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MasterPure™ Yeast DNA Purification Kit

Cat. No. MPY80200

1. Introduction

The MasterPure™ Yeast DNA Purification Kit provides all of the reagents necessary to consistently isolate highly purified DNA from a variety of yeast species including: *Candida, Saccharomyces, Pichia, and Schizosaccharomyces*. In addition, users can isolate DNA from filamentous fungal species such as *Aspergillus*[1] and *Penicillium*. This kit utilizes a nonenzymatic approach to lyse the yeast cell wall combined with a rapid precipitation step to remove contaminating macromolecules. The purified DNA can then be used in many applications including hybridization, restriction enzyme digestion, PCR amplification, and next-generation sequencing. We offer several products for PCR that incorporate the MasterAmp™ PCR Enhancement Technology+, which substantially improves product yield and decreases nonspecific product formation.

2. Product Designations and Kit Components

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
MasterPure™ Yeast DNA Purification Kit	200 Purifications	MPY80200	TE Buffer	SS000001-D2	8 mL
			RNase A (5 µg/µL)	SS000213-D3	200 µL
			MPC Protein Precipitation Reagent	SS000399-D3	60 mL
			Yeast Cell Lysis Solution	SS000405-D1	60 mL

3. Product Specifications

Storage: Store the MasterPure Yeast DNA Purification Kit at room temperature.

Storage Buffer: RNase A is supplied in a 50% glycerol solution containing 25 mM sodium acetate (pH 4.5).

Quality Control: The MasterPure Yeast DNA Purification Kit is function-tested by extracting DNA from a liquid culture of *S. cerevisiae*. DNA quality and yield are assayed by agarose gel electrophoresis, fluorimetry, and use as a template for PCR.

Reagents needed but not supplied: 0.1M Mg Cl_2

4. DNA Purification Protocols

The following protocol is provided for the purification of DNA from yeast and filamentous fungi. Lyse the tissue as outlined in either Part A or B; if desired, users may add the optional RNase A treatment (Part C step 1). Adjust the volumes of reagents proportionately for larger cell samples to scale purification reactions up or down.

A. Harvesting Cells From Liquid Cultures

Yeast Cells (e.g., *Saccharomyces, Candida, Pichia*)

1. Pellet the yeast cells from a saturated 1.5-mL culture (approximately 8-10 A_{600} units for each mL) by centrifugation in a microcentrifuge at ≥10,000 rpm for 2-5 minutes.

2. Remove all growth medium and continue with Cell Lysis and Precipitation of DNA in Part C.

Fungal Mycelium *(e.g., Aspergillus, Penicillium)*

1. Harvest the mycelium from a 10-mL overnight culture by transferring the culture to a vacuum filtration apparatus (such as a Buchner funnel with filter paper) and remove the culture medium by filtration.
2. Rinse the mycelium with several volumes (original culture volume) of 0.1 M $MgCl_2$ (not provided). Thoroughly dry the tissue onto the filter paper using vacuum.
3. Transfer the dried tissue (limit the amount of filter paper transferred) to a chilled mortar and grind the mycelium to a powder in the presence of liquid N_2.
4. Divide the powder equally between two microcentrifuge tubes and continue with Cell Lysis and Precipitation of DNA in Part C.

B. Harvesting Colonies From Solid Medium

1a. Scrape a single yeast colony (2 mm in diameter) from an agar plate (or similar medium) and transfer to a microcentrifuge tube containing 300 µL of Yeast Cell Lysis Solution. Suspend the cells as directed in Part C, Step 1. Continue with Cell Lysis and Precipitation of DNA in Part C, Step 2.

1b. For filamentous species, transfer a colony to a microcentrifuge tube and rinse the tissue with 1 mL of 0.1 M $MgCl_2$ (not provided); discard the wash solution. Briefly centrifuge the sample and remove any remaining wash solution. Continue with Cell Lysis and Precipitation of DNA in Part C.

C. Cell Lysis and Precipitation of DNA

Thoroughly mix the Yeast Cell Lysis Solution to ensure uniform composition before dispensing.

1. Add 300 µL of Yeast Cell Lysis Solution to each microcentrifuge tube of tissue collected in Parts A and B. Optional RNase A treatment: add 1 µL of 5 µg/µL RNase A to the tube. Suspend the cells by either vortex mixing or pipetting the cells repeatedly using a 1 mL capacity pipet tip.
2. Incubate the suspended cells at 65°C for 15 minutes.
3. Place the samples on ice for 5 minutes. Add 150 µL of MPC Protein Precipitation Reagent and vortex mix for 10 seconds.
4. Pellet cellular debris by centrifugation in a microcentrifuge for 10 minutes at ≥10,000 rpm.
5. Transfer the supernatant to a clean microcentrifuge tube and add 500 µL of isopropanol (not provided). Mix thoroughly by inversion.
6. Pellet the DNA by centrifugation in a microcentrifuge for 10 minutes at ≥10,000 rpm.
7. Remove the supernatant by pipeting and discard. Wash the pellet containing the DNA with 0.5 mL of 70% ethanol. Carefully remove the ethanol by pipetting and discard. Briefly centrifuge the DNA pellet and remove any remaining ethanol.

8. Suspend the DNA in 35 µL of TE Buffer. Store the DNA at 4°C.
9. Quantitate DNA yield by fluorimetry using Hoechst dye 33258.[2] (A_{260} estimates of DNA yield can lead to gross overestimation of nucleic acid content (up to 28 fold), even after ribonuclease treatment to degrade RNA.[1]

The average total yield of DNA from 1.5 mL of liquid culture is 3-4 µg for *Saccharomyces* species and 6-8 µg for *Candida* species. (The maximum theoretical yield of DNA from 1.5 mL of an early stationary phase culture of haploid yeast (e.g., *Saccharomyces*) is approximately 10 µg (assuming a cell density of 2 x 10^8 cells/mL of culture and a DNA concentration of 0.017 pg/cell).)[3] The purified DNA molecules average 40-50 kb in length.

5. References

1. Jin, J. *et al.*, (2004) *J. Clin. Microbiol.* **42**, 4293.
2. Ausubel, F. *et al.*, (eds.) (1995) *Current Protocols in Molecular Biology* (CD ROM ver. 3.7.5) John Wiley and Sons, New York, Appendix 3D.
3. Sherman, F. (1991) *Meth. Enzymol.* **194**, 3.