

# mi-Yeast Genomic DNA Isolation Kit

For highly pure and rapid purification of genomic DNA from yeast

Cat. No mi-YD100  
[100 Preparations]

This kit is for research purposes only.  
Not for use in diagnostic procedures.  
For in vitro use only.

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## Introduction

The mi-Yeast Genomic DNA Kit is used to quickly and efficiently isolate high molecular weight genomic DNA (50-100kb) from yeast. This kit is based on the efficient release of genomic DNA by using a special cell lysis buffer. A rapid separation of genomic DNA from proteins, polysaccharides and lipids by selective adsorption of DNA to the silica membrane becomes possible. The isolated DNA can be used for all molecular biology applications (RFLP, PCR, Southern Blotting, etc). This method is fast, safe and simple for DNA preparation from different sources of samples.

<b>Kit Contents</b>	<b>mi-Yeast Genomic DNA Kit</b>
Preparation	100 rxn/kit
Cell Lysis Buffer	35 ml
PPT Buffer	15 ml
Column Binding Buffer	65 ml (NOTE: Ethanol has been added)
Column Wash Buffer	25 ml (NOTE: Add 100 ml pure ethanol up to a final volume of 125 ml prior to use.)
RNAse	as powder (store at -20°C bevor addition to Lysis Buffer)
Spin column	100
Collection tube	100

## Required Equipment

Microcentrifuge (13,000 rpm or 12,000 x g)  
Vortexer  
Microcentrifuge tubes  
50 mM EDTA (pH 8.0)  
Lyticase / Zymolyase (20mg / ml)  
Proteinase K (10mg / ml)  
Distilled water (pH 7-8) or 10 mM TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)

## Kit Storage

Store dried RNAse at -20°C upon kit arrival.  
Store solved RNAse at -20°C  
At room temperature for 6 months.

## Precautions

See MSDS on our homepage ([www.mymetabion.com](http://www.mymetabion.com)).

## Protocol for the purification of DNA from yeast

**Note:** Before starting, please....

- Solve the RNase in 300µl of water. Store the stock solution (10 mg/ml) at -20°C.
- Make sure that you have completed the "Column Wash Buffer" by adding 100 ml of pure ethanol (99.9%) before first use.
- All centrifugation steps are at 13,000 rpm (or 12,000 x g). Remember to orient tubes with hinges pointing straight out from center of centrifuge rotor for all steps.

1. Add 1 ml cell resuspension (e.g. overnight culture containing approximately 1-2x10<sup>8</sup> cells) to a 1.5 ml tube on ice.  
Spin at 13,000 rpm (12,000 x g) for 1 min and then remove the supernatant.
2. Add 300 µl of 50 mM EDTA (pH 8.0) to the cell pellet and gently pipet up and down until cells are suspended.  
Add 7.5 µl of Lyticase / Zymolyase (not included) (stock solution 20 mg/ml) and invert the tube 25 times to mix.  
Incubate at 37°C for 30 min (optional: on shaker) to digest the cell walls. Invert the sample occasionally during the incubation.
3. Spin at 13,000 rpm (12,000 x g) for 1 min to pellet the cells. Remove the supernatant.
4. Add 400 µl of Cell Lysis Buffer and mix thoroughly.  
Add 10 µl of Proteinase K (stock solution 10 mg/ml) to the 1.5 ml tube and mix thoroughly.  
Incubate the sample at 65°C for 15 - 30 min.
5. Cool the sample to room temperature and add then 3 µl of RNase A (stock solution 10 mg/ml) to the 1.5 ml tube and incubate at 37°C for 15 - 30 min.
6. Add 100 µl of PPT Buffer and vortex for 20 sec.  
Incubate the sample on ice for 5 min.
7. Spin at 13,000 rpm (12,000 x g) for 1 min at room temperature.
8. Transfer the supernatant into a new 1.5 ml tube.  
Add 600 µl of Column Binding Buffer without wetting the rim and mix by vortexing.
9. Set one spin column into a collection tube.  
Transfer 650 µl of the sample (prepared step 8) to the spin column.

10. Spin at 13,000 rpm (12,000 x g) for 1 min and discard the flow through. The liquid will flow through the spin column membrane leaving the genomic DNA bound to the filter membrane. (Repeat steps 9 and 10 until all supernatant has passed through the column.)
11. Remove the spin column from the collection tube and discard the flow through. Place the spin column in the same collection tube. Add 650 µl of Column Wash Buffer without wetting the rim. Spin at 13,000 rpm (12,000 x g) for 1 min.  
**Note:** Please ensure you have prepared the “Column Wash Buffer” by adding 100 ml of pure ethanol before using.
12. Remove the spin column from the collection tube and discard the flow through. Replace the spin column in the same collection tube. Add 350 µl of Column Wash Buffer without wetting the rim. Spin at 13,000 rpm (12,000 x g) for 1 min.  
**Note:** Residual Column Wash Buffer in the silica membrane may cause problems in downstream applications. In these cases, spin again at full speed (more than 13,000 rpm) for 1 min.
13. Place the spin column in a clean 1.5 ml microcentrifuge tube (not included), and discard the collection tube containing the filtrate. Elute by adding 50 - 100 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or distilled water (pH 7-8). Incubate the spin column with TE buffer or distilled water at room temperature (15-25°C) for 1 min, and spin at 13,000 rpm (12,000 x g) for 1 min.  
[Incubating the spin column loaded with TE buffer or distilled water for 5 min at room temperature before centrifugation, generally increases DNA yield. The elution efficiency will decrease when using elution buffer with pH < 7.0]

The Yeast Genomic DNA is now ready to use!