INSTRUCTIONS

Yeast DNA Extraction Kit

78870

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Number	Description
78870	**Yeast DNA Extraction Kit,** contains DNA extraction and purification reagents for *Saccharomyces cerevisiae* **Kit Contents:** **Y-PER™ Reagent,** 25mL **DNA Releasing Reagent A,** 20mL **DNA Releasing Reagent B,** 20mL **Protein Removal Reagent,** 10mL **Storage:** Store at 4°C; if precipitant has formed, gently warm DNA Releasing Reagents A and B at 37°C for 1-5 minutes.

Introduction

The Thermo Scientific Yeast DNA Extraction Kit surpasses traditional methods of DNA isolation from yeast. Typically, extraction and purification of DNA from yeast are time-consuming and labor-intensive. The yeast proteinaceous cell wall is notoriously difficult to lyse and requires harsh treatments that are time-consuming and can damage the extracted DNA. The Yeast DNA Extraction Kit protocol requires less than one hour to complete, is effective without enzymatic treatment or glass beads, and yields little to no RNA contamination regardless of RNase presence. In studies with *Saccharomyces cerevisiae*, high yields of genomic and plasmid DNA are consistently obtained. DNA purified using this kit is suitable for PCR amplification, bacterial transformations, restriction digestions and hybridization applications.

Important Product Information

- **Optimized Conditions:** Scale the proportions outlined in the protocol as needed. The protocol is equally effective for fresh and frozen cells.
- **Species and Strain Variations:** This protocol was optimized with *S. cerevisiae*. For DNA extraction from organisms that are difficult to lyse, allow the samples to incubate for longer at Steps 2 and 3. Yield and RNA removal might be significantly affected when using organisms other than *S. cerevisiae*. In any case, no ribosomal RNA should be observed and small RNA species, if present, may be removed by adding DNase-free RNase A (100µg/mL) directly to the Thermo Scientific Y-PER Reagent before Step 2. When stored at 4°C, RNase A is stable in the Y-PER Reagent for over 6 months. To alter the DNA concentration, resuspend DNA in differing amounts of TE or sterile water at Step 7.
- **Single Colony:** When picking a single colony, use 20µL of the Y-PER Reagent, 16µL of DNA Releasing Reagent A, 16µL of DNA Releasing Reagent B, 8µL of the Protein Removal Reagent, and 24µL of isopropyl alcohol before suspending the DNA in 5µL of TE or sterile water. PCR amplification of plasmid DNA is reproducible in this format. Use electroporation when attempting to transform *E. coli* with DNA isolated from a single yeast colony, because a high transformation efficiency is required (> 10^8 cfu/µg supercoiled DNA). In general, it is best to use 5µL of the suspended DNA for these applications.
- **Yeast Double-stranded RNA Killer Virus:** The majority of *S. cerevisiae* laboratory strains contain a double-stranded RNA killer virus that produces a ~5000 bp band on an agarose gel. An extremely faint ~2000 bp band sometimes occurs that is also part of the killer virus. These bands do not interfere with most downstream applications, excluding exact quantitation of the sample. Decreasing the suggested amount of isopropyl alcohol addition by ½ (Step 5) will cure the DNA preparation of the double- stranded RNA virus; however, this will result in reduced genomic DNA yield.

- **Bacterial Transformations:** As with any yeast DNA purification, there will be a substantial amount of genomic DNA relative to plasmid DNA. Therefore, when using chemically competent *E. coli*, use only those cells that have ≥ 10^8cfu/µg of supercoiled DNA. Use 5µL of DNA sample for chemically competent *E. coli* transformations and 1-2µL for electrocompetent cells, when the suggested 50µL resuspension buffer (Step 7) is used. When using CEN-based plasmids, add 5µL of DNA sample to electrocompetent cells, having a transformation efficiency > 10^9 cfu/µg of supercoiled DNA for transformation into *E. coli.*
- **PCR:** DNA purified using this kit provides an excellent template for PCR. Use 0.5-1µL of the DNA suspended in 50µL of TE buffer (Step 7).

Extraction Protocol

1. Pellet a 10mL *S. cerevisiae* culture grown overnight, resuspend the cells and transfer entire suspension to a 1.5mL microcentrifuge tube. Pellet cells by centrifugation at 3000-5000 × *g* for 5 minutes at room temperature. Discard the supernatant. Typically this procedure will yield a 70-100mg pellet.
2. Suspend cells in an appropriate amount of the Y-PER Reagent. Scale the amount of Y-PER Reagent accordingly, maintaining a ratio of 8µL/1mg pellet. Mix by gently vortexing or inverting the tube or pipetting up and down until the mixture is homogenous. Once a homogenous mixture is established, incubate at 65°C for 10 minutes.
3. Centrifuge at 13,000 × *g* for 5 minutes, discard supernatant, add 400µL of DNA Releasing Reagent A, and 400µL of DNA Releasing Reagent B to the pellet for a total volume that should equal approximately 800µL. Mix to produce a homogenous mixture and incubate at 65°C for 10 minutes.
4. Add 200µL of Protein Removal Reagent to mixture and invert several times. Centrifuge at least 13,000 × *g* for 5 minutes and transfer supernatant to a new 1.5mL centrifuge tube.
5. Add 600µL isopropyl alcohol to fill tube. Mix gently by inversion. Precipitate genomic DNA by centrifuging the mixture at 13,000 × *g* for 10 minutes.
6. Remove supernatant, being careful not to discard any of the pellet, which is clear and hard to see. Add 1.5mL of 70% ethanol to the pellet, invert several times and centrifuge at 13,000 × *g* for 1 minute to wash off any residual salts or cellular debris clinging to the DNA or tube. Invert the tube to dry any residual ethanol before proceeding to Step 7. Alternatively, the dry sample in a vacuum centrifuge.
7. Resuspend in 50µL TE buffer or sterile water. Pellet should solubilize completely within 5 minutes. Flick the bottom of the tube carefully, or pipette solution up and down. Wash the sides of the tubes until all the genomic DNA is in solution.

Related Thermo Scientific Products

78990	**Y-PER Yeast Protein Extraction Reagent**
75768	**Yeast β-galactosidase Assay Kit**
78994	**Y-PER 6xHis Fusion Protein Purification Kit**
78997	**Y-PER GST Fusion Protein Purification Kit**