MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Yeast Genomic Fast Kit

Product Number: DNK2001

Shipping and Storage

1. Precipitation may occur in Binding Buffer CB or Protein Removal Buffer PE at low temperatures. Incubating in a 37°C water bath for several minutes can help redissolve the precipitates. Use after the solution becomes clear and transparent and cools to room temperature.
2. Proteinase K is supplied in a ready-to-use glycerol buffer and shipped at ambient temperature. Upon receipt, it can be stored for at least 6 months at ≤ 25°C, 12 months at 4°C, or 2 years at -20°C.
3. Lytic Enzyme is a snail enzyme glycerol stock solution and is therefore viscous. Handle with care. Store at -20°C. Snail enzyme is a mixture of enzymes prepared from the crop and digestive tract of snails, containing over 20 enzymes including cellulase, pectinase, amylase, protease, etc. It is suitable for breaking down the cell walls of various yeasts.
4. To prevent volatilization, oxidation, or pH changes due to prolonged exposure to air, cap all solutions tightly immediately after use.

Components

Component	Storage	DNK2001 50 preps
Balance Buffer	RT	5mL
Buffer SE	RT	15mL
Buffer YB	RT	10mL
Buffer CB	RT	11mL
Buffer PE	RT	16mL
Buffer WB	RT	13mL
Buffer EB	RT	15mL
Lytic Enzyme	-20°C	2.5mL
Proteinase K	4°C	1mL
Adsorption column AC	RT	50
Collection tube (2ml)	RT	50

Description

This kit utilizes centrifugal adsorption columns specifically designed for DNA binding and a unique buffer system for the extraction of genomic DNA from yeast cells. It eliminates the need for toxic reagents like phenol-chloroform and time-consuming alcohol precipitation steps, effectively removing proteins and other inhibitory impurities. The extracted DNA is ready for downstream applications such as restriction enzyme digestion, PCR, and Southern blotting.

Features

1. The silica membrane in the centrifugal adsorption columns is made from specially formulated adsorption material, ensuring minimal column-to-column variation in binding capacity and excellent reproducibility. This overcomes the instability issues often associated with membranes in domestic kits.
2. No need for toxic reagents like phenol or steps such as ethanol precipitation.
3. Fast and simple procedure. Processing after cell lysis can typically be completed within 30 minutes per sample.
4. Multiple washing steps ensure high purity, with typical OD260/OD280 ratios ranging from 1.7 to 1.9. The DNA is suitable for direct use in PCR, Southern blotting, and various enzymatic digestions.

Application

Suitable for yeast genomic DNA extraction.

Usage of Balance Buffer

1. Description

   Nucleic acid adsorption columns can react with airborne charges/dust during long-term storage, which may affect their nucleic acid binding capacity. Pretreating the silica column with Balance Buffer significantly reduces the inert groups on the silica membrane, enhancing nucleic acid binding. This improves the recovery efficiency or yield of the silica column.

2. Protocol

   Place a new silica membrane adsorption column into a collection tube. Pipette 100μL of Balance Buffer onto the column. Centrifuge at 13,000 rpm for 1 minute. Discard the flow-through from the collection tube and place the adsorption column back into the same tube. The Balance Buffer pretreatment is now complete. Proceed with subsequent steps.

Note

1. User must provide ethanol, isopropanol, and β-mercaptoethanol.
2. Binding Buffer CB and Protein Removal Buffer PE contain irritating compounds. Wear latex gloves during handling to avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with plenty of water or saline.
3. For cell concentration measurement, typically an OD600 value of 1 corresponds to approximately 1-2×10^7 cells/ml for Saccharomyces cerevisiae. Please note that OD values can vary significantly between different yeast strains and spectrophotometers, so this is for reference only.

Protocol (please read the note before the experiment)

Tips:1)Before first use, add the indicated volume of absolute ethanol to the bottles of Protein Removal Buffer PE and Wash Buffer WB as indicated on the labels. Mix thoroughly. Mark the bottles after ethanol addition to prevent multiple additions!

2)Add 0.2% β-mercaptoethanol to the required amount of Buffer SE and equilibrate to room temperature for use.

1. Harvest 1-3mL of yeast culture (not exceeding 3×10^7 cells, preferably from early-log phase) by centrifugation at 12,000 rpm for 30 seconds. Remove the supernatant as completely as possible and collect the pellet.

   For volumes exceeding 1.5mL, centrifuge, discard supernatant, add more culture to the same 1.5mL tube, and repeat step 1 until sufficient biomass is collected.

2. Resuspend the pellet thoroughly in 300μL Buffer SE by vortexing. Add 50μL Lytic Enzyme stock solution and mix well by inverting. Incubate at 37°C for 1-3 hours to digest the cell wall, inverting the tube several times during incubation to facilitate digestion.

   If cell wall disruption is inefficient, increase the amount of Lytic Enzyme to raise the working concentration, extend digestion time, or increase temperature to 45°C. For yeasts not suitable for Lytic Enzyme digestion, alternative methods such as glass bead beating (0.5mm beads), repeated freeze-thaw cycles, etc., can be used. Glass bead method: Resuspend the pellet thoroughly in 180μL Buffer YB. Add 0.1g of acid-washed glass beads (0.45-0.55mm diameter). Vortex vigorously for 10 minutes. Let stand for a few minutes for beads to settle. Carefully transfer the supernatant to a new tube and proceed to step 4.

3. Centrifuge at 13,000 rpm for 1 minute. Remove the supernatant as completely as possible. Add 180 μL Buffer YB and resuspend the pellet thoroughly.
4. Add 20μL Proteinase K and vortex immediately to mix thoroughly.
5. Incubate the mixture in a 55°C water bath until digestion is complete, gently vortexing several times during incubation to aid lysis.

   Digestion time depends on yeast quantity, species, and growth state. Typically 15 minutes is sufficient, but overnight digestion is also acceptable if convenient.

Optional step, usually not required: If significant RNA contamination is present, add 20μL RNase A (10 mg/mL) after completing step 5, vortex to mix, and incubate at room temperature for 5-10 minutes.

6. Add 200 μL Buffer CB, vortex immediately to mix thoroughly, and incubate at 70°C for 10 minutes.

Prepare Balance Buffer-treated adsorption column: Pretreating the silica membrane adsorption column with Balance Buffer is a mandatory step. Refer to the previous section "Regarding the Use of Balance Buffer" for the specific method.

7. After cooling, add 100μL isopropanol and vortex immediately to mix thoroughly. Flocculent precipitation may appear. Transfer the entire mixture (including any precipitate) to an Adsorption Column AC (placed in a collection tube). Centrifuge at 13,000 rpm for 30-60 seconds. Discard the flow-through.

Immediate and thorough vortexing/mixing in the above step is crucial. Inadequate mixing significantly reduces yield. If the sample is viscous and difficult to mix, vortex for 15 seconds if necessary.

8. Add 500μL Buffer PE (check if ethanol has been added). Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
9. Add 600μL Buffer WB (check if ethanol has been added). Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
10. Add another 600μL Buffer WB. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
11. Place the Adsorption Column AC back into the collection tube. Centrifuge at 13,000 rpm for 2 minutes to remove residual Wash Buffer WB as completely as possible, as residual ethanol may inhibit downstream reactions.
12. Remove the adsorption column AC and place it into a clean centrifuge tube. Add 100μL of Buffer EB to the central part of the adsorption membrane (preheating Buffer EB in a water bath at 80-100°C can enhance yield). Allow it to stand at room temperature for 3-5 minutes, then centrifuge at 12,000 rpm for 1 minute.

    The solution obtained from the first elution can be reintroduced into the centrifuge column, left at room temperature for 2 minutes, and centrifuged at 13000 rpm for 1 minute. It can increase the concentration by about 10%.

    The larger the elution volume, the higher the elution efficiency. If a higher DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 30μL. If the volume is too small, it will reduce the DNA elution efficiency and DNA yield.
13. DNA can be stored at -20°C. For long-term storage, use -70°C.