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Yeast Genomic Fast Kit

Product Number: DNK2001

Shipping and Storage

1. Buffer CB or Buffer IR may precipitate and precipitate at low temperatures. It can be dissolved again in a water bath at 37℃ for a few minutes to restore clarity and transparency. After cooling to room temperature, it can be used.
2. Proteinase K is stored in a ready to use glycerol buffer and transported at room temperature. After receipt, store at room temperature not exceeding 25℃ for at least 6 months, at 4℃ for 12 months, and at -20℃ for 2 years.
3. Lytic Enzyme is a snail enzyme glycerol storage solution, which is relatively viscous. Please use it carefully and store at -20℃. Snail enzyme is a mixed enzyme prepared from the snail's pouch and digestive tract. It contains more than 20 enzymes, including cellulase, pectinase, amylase, protease, etc. Suitable for breaking and dissolving various yeast cell walls.
4. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly in a timely manner after use.

Components

Component	Storage	DNK2001 50 preps
Balance Buffer	RT	5 ml
Buffer YB	RT	20 ml
Buffer CB	RT	11 ml
Buffer IR	RT	25 ml
Buffer WB	RT	13 ml
Buffer EB	RT	15 ml
Lytic Enzyme	-20℃	2.5 ml
Proteinase K	4℃	1 ml
Adsorption column AC	RT	50
Collection tube (2ml)	RT	50

Description

This kit uses a DNA adsorption column and a unique solution system, suitable for rapid and simple extraction of genomic DNA from yeast cultures from various sources. Approximately 3ml of yeast culture medium in exponential growth stage can generally purify 10-15μg of high-quality genomic DNA in one extraction. The purified DNA product can be directly used for experiments such as PCR, enzyme digestion, and hybridization. After yeast cells are treated with Lytic Enzyme to remove the cell wall, the unique Buffer CB/Proteinase K rapidly lyses the cells and inactivates intracellular nucleases. Then, genomic DNA selectively adsorbs onto the silica matrix membrane in a high dissociation salt state. Through a series of rapid rinsing centrifugation steps, Buffer IR and Buffer WB remove impurities such as cell metabolites and proteins, Finally, low salt Buffer EB elutes pure genomic DNA from the silica matrix membrane.

Features

1. The silicon matrix membrane inside the centrifugal adsorption column is entirely made of specially designed adsorption membranes from world-renowned imported companies, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawback of unstable membrane quality in domestic reagent kits.
2. No toxic reagents such as phenol are required, and no steps such as ethanol precipitation are required.
3. Fast and simple, the operation after cracking a single sample can generally be completed within 30 minutes.
4. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of 1.7-1.9, which can be directly used for PCR,

Southern blot, and various enzyme digestion reactions.

Application

Suitable for rapid extraction of various yeast genomic DNA

Usage of Balance Buffer

1. Description

   During the long-term placement of the nucleic acid adsorption silica gel membrane column, it reacts with the charge/dust in the air, affecting its nucleic acid binding ability. After pre-treatment with Balance Buffer, the hydrophobic groups of the silica gel membrane in the column can be greatly reduced, improving the binding ability of nucleic acids. Thereby improving the recovery efficiency or yield of silicone columns. Balance Buffer is a highly alkaline solution. If accidentally touched, please clean with plenty of tap water. After use, it is necessary to tighten the bottle cap to avoid contact with air. Store at room temperature. During storage, precipitation may occur. Please heat to 37℃ to completely eliminate the precipitation.

2. Protocol

   Take a new silicone membrane adsorption column and place it in a collection tube. Take 100l of Balance Buffer and transfer it into the column. Centrifuge at 13000 rpm for 1 minute, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube. At this point, the Balance Buffer has completed preprocessing the columns. Follow the subsequent operating steps.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm.
2. We need to provide our own ethanol, isopropanol β- Mercaptoethanol.
3. Preheat the required water bath to 37℃ and 70℃ for later use before starting the experiment.
4. Buffer CB and Buffer IR contain irritating compounds, and latex gloves should be worn during operation to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, rinse with plenty of water or physiological saline.
5. Users need to bring their own Sorbitol Buffer (1M sorbitol, 0.1M Na2EDTA, 14 mM) β- Mercaptoethanol.

   Preparation method: Dissolve 182.2g of sorbitol in 600ml of deionized water, add 200ml of 0.5M Na2EDTA (pH 8.0), without adjusting the pH value, make up to 1L and store at 4℃. Add 0.2% before use β- Mercaptoethanol (commercialized) β- The molar concentration of mercaptoethanol is generally 14M.
6. When the OD600 value for bacterial concentration detection is generally 1, the brewing yeast cells are 1-2×10^7cells/ml, due to differences in bacterial strains and spectrophotometers, even with the same number of cells, the OD value varies greatly. The above is for reference only.
7. Buffer EB does not contain chelating agent EDTA and does not affect downstream enzyme cleavage, linking, and other reactions. Water can also be used for elution, but it should be ensured that the pH is greater than 7.5, as low pH can affect elution efficiency. Wash DNA with water and store it at -20℃. If DNA needs to be stored for a long time, it can be eluted with TE buffer (10mM Tris HCl, 1mM EDTA, pH 8.0). However, EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.

Protocol (please read the note before the experiment)

Tips:1)Before the first use, please add 60ml anhydrous ethanol to 15ml Buffer WB and mix well. After adding, please mark the box with a check mark indicating that ethanol has been added in a timely manner to avoid multiple additions!

2)Absorb the amount of Sorbitol buffer used and add 0.2% β- Mercaptoethanol, return to room temperature for later use.

1. Take 1-3ml of yeast culture (not exceeding 3×10^7 cells, preferably early logarithmic growth), centrifuge at 12000 rpm for 30 seconds, and discard the supernatant as much as possible to collect the bacterial cells.

   Collect more than 1.5ml of bacterial solution. After centrifuging and discarding the supernatant, add more bacterial solution to

the same 1.5ml tube and repeat step 1 until sufficient bacterial cells are collected.

2. Add 300μl Sorbitol Buffer and gently blow to resuspend cells; Add 50μl of Lytic Enzyme storage solution, thoroughly invert and mix well. Incubate at 37℃ for 1-3 hours to digest the cell wall, and invert several times to aid digestion.
   If the wall breaking effect is not good and leads to low yield, the amount of Lytic Enzyme can be increased to increase the enzyme working concentration, and the digestion time can be extended or the temperature can be raised to 45℃ to improve the effect. Yeasts that are not suitable for Lytic Enzyme digestion can choose other methods such as 0.5mm glass bead vortex beating, repeated freeze-thaw, etc. Glass bead method: Add 180μl Buffer YB to the bacterial body to completely suspend the bacterial body, add 0.1g of acid washed glass beads with a diameter of 0.45-0.55mm, vortex and shake for 10 minutes, let the glass beads settle for a few minutes, carefully suck the supernatant into a new tube, and then proceed to step 4.
3. Centrifuge at 13000rpm for 1 minute, discard the supernatant as much as possible, and add 180μl Buffer YB to resuspend the cell clusters.
4. Add 20μl of Proteinase K and immediately vortex and mix thoroughly.
5. Place the mixture in a 55℃ water bath for digestion until complete, gently shaking it a few times to aid in cracking.
   The required digestion time is related to the number, type, and growth status of yeast. Generally, 15 minutes is sufficient, but if convenient, overnight digestion will not have any adverse effects.
6. **Optional steps, generally not required:** If there is a lot of RNA residue and RNA needs to be removed, 20μl of RNase A (25mg/ml) solution can be added after completing step 5, shaken well, and left at room temperature for 5-10 minutes.
7. Add 200μl Buffer CB, immediately vortex and shake thoroughly, and let it stand at 70℃ for 10 minutes.
   Balance Buffer pre-treatment adsorption column backup: The use of Balance Buffer pre-treatment of silicone membrane adsorption column is a necessary step. For specific methods, please refer to the previous section "Usage of Balance Buffer"
8. After cooling, add 100μl of isopropanol and immediately vortex to mix thoroughly. At this time, flocculent precipitation may occur.
9. Add the previous mixture (including possible precipitates) to an adsorption column AC, centrifuge at 13000 rpm for 30-60 seconds (with the adsorption column placed in the collection tube), and discard the waste liquid in the collection tube.
10. Join 500μl Buffer IR, centrifuge at 12000rpm for 30 seconds, discard waste liquid.
11. Add 600μl of Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
12. Join 600μl Buffer WB, centrifuge at 12000rpm for 30 seconds, discard the waste liquid.
13. Put the adsorption column AC back into the empty collection tube, centrifuge at 13000rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.
14. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 100μl of Buffer EB to the middle of the adsorption membrane (Buffer EB is better preheated in a 65-70℃ water bath beforehand). Leave it at room temperature for 3-5 minutes and centrifuge at 12000 rpm for 1 minute. Add the obtained solution back into the centrifugal adsorption column, let it stand at room temperature for 2 minutes, and centrifuge at 12000 rpm for 1 minute.
    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 50μl. Small volume reduces DNA elution efficiency and reduces DNA production.
15. DNA can be stored at 2-8℃, and if it needs to be stored for a long time, it can be placed at -20℃.