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Bacterial Genomic DNA Fast Kit

Product Number: DNK1101

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°C for at least 6 months, at 4°C for 12 months, and at -20°C for 2 years.
3. To avoid the volatilization, oxidation, and pH changes of reagents exposed to air for a long time, each solution should be covered tightly in a timely manner after use.

Components

Component	Storage	DNK1101 50 Preps	DNK1102 100 Preps	DNK1103 200 Preps
Balance Buffer	RT	5 ml	10 ml	20 ml
Buffer RB	RT	30 ml	60 ml	120 ml
Buffer CB	RT	11 ml	20 ml	40 ml
Buffer IR	RT	25 ml	50 ml	100 ml
Buffer WB	RT	13 ml	25 ml	50 ml
Buffer EB	RT	15 ml	15 ml	20 ml
Proteinase K	4°C	1 ml	1ml ×2	1ml ×4
Adsorption column AC	RT	50	100	200
Collection tube (2ml)	RT	50	100	200

Description

The unique Buffer CB/Proteinase K rapidly cleaves cells and inactivates intracellular nucleases, and then selectively adsorbs genomic DNA onto the silica matrix membrane in a highly dissociated salt state. Through a series of rapid rinsing centrifugation steps, Buffer IR and Buffer WB remove impurities such as cellular 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, single sample operations 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 and a length of up to 30kb-50kb. It can be directly used for PCR, Southern blot, and various enzyme digestion reactions.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm.
2. Preheat the water bath to 37℃ or 70℃ as needed before starting the experiment.
3. Self prepared isopropanol is required.
4. You need to provide 0.5M EDTA, Triton X-100, and Lysozyme (for Gram positive bacteria) by yourself.

5. 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.
6. 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.

Use of balance buffer

1. Description

During the long-term placement of nucleic acid adsorption silica gel membrane columns, they react with charges/dust in the air and affect their nucleic acid binding ability.After pre-treatment with equilibrium solution, the silica gel column can greatly reduce the hydrophobic groups of the silica gel membrane in the column and improve the binding ability of nucleic acids.Thus improving the recovery efficiency or yield of silicone columns. The equilibrium solution is a strong alkaline solution. If accidentally touched, please clean it with a large amount of tap water.After use, the bottle cap needs to be tightly closed to avoid contact with air. Store at room temperature. During storage, there may be precipitation formation. Please heat to 37℃ to completely eliminate the precipitation.

2. Protocol

Take a new silica gel membrane adsorption column and install it in a collection tube, and suck 100μl of equilibrium solution into the column.Centrifuge at 13000rpm for 1 minute, discard the waste liquid from the collection tube, and reposition the adsorption column in the collection tube. At this point, the balance liquid pre-treatment column is completed. Follow the subsequent operating steps.

Protocol(Please read the precautions before the experiment)

Tip: Before the first use, please add the specified amount of anhydrous ethanol to 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 adding it multiple times!

1. Take 0.5-2ml of culture medium (maximum of 2×10^9cells), centrifuge at 10000rpm for 30 seconds, and discard the supernatant as much as possible to collect the bacterial cells.

   The initial processing capacity can be adjusted based on bacterial density, cell type, and expected yield, but the maximum adsorption capacity of the centrifugal adsorption column is 30μg of genomic DNA. If the bacterial excess exceeds the maximum adsorption capacity, it will seriously reduce yield.
2. Add 200μl Buffer RB and resuspend, centrifuge at 10000rpm for 30 seconds, discard the supernatant. Shake or blow the cells thoroughly and resuspend them in 180μl Buffer RB.

   Note: For Gram positive bacteria that are difficult to break through, skip step 2 and add lysozyme for wall breaking treatment. The specific method is to add 180μL buffer (20 Tris, pH 8.0; 2mM Na2-EDTA; 1.2% Triton X-100; add lysozyme with a final concentration of 20mg/ml before use (lysozyme must be prepared by dissolving it in dry lysozyme powder in the buffer, otherwise it will cause no activity of lysozyme), and treat at 37 ℃ for more than 30 minutes.
3. Add 20μl Proteinase K (20mg/ml) solution, mix well, then add 200μl Buffer CB, immediately vortex and shake thoroughly, and let it stand at 70℃ for 10 minutes.

   Optional steps: If there is a large amount of RNA residue that needs to be removed, 5μl of RNase A (100 mg/ml) solution can be added before adding 200μl of Buffer CB, shaken well, and left at room temperature for 5-10 minutes.

   Balance liquid pretreatment adsorption column backup: Using balance liquid pretreatment silica gel membrane adsorption column is a necessary step. For specific methods, please refer to the previous section "Use of Balance Liquid"
4. After cooling, add 100μl of isopropanol and immediately vortex and shake thoroughly to mix well. At this time, flocculent precipitation may occur. It is very important to immediately vortex or blow thoroughly in the above steps. Insufficient mixing seriously reduces production. If necessary, if the sample is viscous and difficult to mix, vortex oscillation can be used for 15 seconds to mix.

5. 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.
6. Add 500μl Buffer IR, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
7. Add 600μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
8. Repeat step 7 once.
9. 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.
10. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 50-100μl of Buffer EB in the middle of the adsorption membrane (Buffer EB is better preheated in a 65-70℃ water bath beforehand). Leave it at room temperature for 2-4 minutes and centrifuge at 13000 rpm for 1 minute.

    The solution obtained from the first elution can be re added to 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 production.
11. DNA can be stored at -20℃, and if it needs to be stored for a long time, it can be stored at -70℃.