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Bacterial Genomic DNA Fast Kit

Product Number: DNK1101

Scope of application

Suitable for genomic DNA extraction from Gram-positive and Gram-negative bacteria.

Shipping and Storage

1. At low temperatures, CB may precipitate and precipitate. It can be dissolved again in a 37°C water bath for a few minutes to restore clarity and transparency. **After cooling to room temperature**, it can be used.
2. Protease K is stored in ready to use glycerol buffer and transported at room temperature. After receipt, store at room temperature not exceeding 25℃ for at least 6 months, 4℃ for 12 months, and -20℃ for 2 years.
3. To avoid the volatilization, oxidation, and pH changes of reagents exposed to air for a long time, the lid of each solution should be promptly closed after use.

Components

Component	Storage	DNK1101	DNK1102	DNK1103
		50 Preps	100 Preps	200 Preps
Balance Buffer	RT	5mL	10mL	20mL
Buffer GY	RT	10mL	20mL	40mL
Buffer CB	RT	11mL	20mL	40mL
Buffer PE	RT	16mL	32mL	64mL
		Add absolute ethanol as indicated on the bottle label before first use.		
Buffer WB	RT	13mL	25mL	50mL
		Add absolute ethanol as indicated on the bottle label before first use.		
Buffer EB	RT	10mL	10mL	20mL
Proteinase K	4°C	1mL	1ml ×2	1ml ×4
Adsorption column AC	RT	50	100	200
Collection tube (2ml)	RT	50	100	200

This reagent kit can be stored at room temperature for 12 months without affecting its effectiveness.

Description

The unique binding buffer and Proteinase K rapidly lyse cells. The silica-membrane spin column specifically adsorbs DNA. Toxic reagents such as phenol-chloroform and time-consuming alcohol precipitation are not required, which maximally removes proteins and other inhibitory impurities. It is suitable for efficient extraction of genomic DNA from a variety of Gram-positive and Gram-negative bacteria. The extracted DNA can be directly used for restriction digestion, PCR, Southern Blot and other experiments.

Features

1. No need to use toxic phenol or other reagents, and no need for time-consuming ethanol precipitation.
2. It delivers high extraction efficiency and high DNA concentration. It is compatible with various Gram-positive and Gram-negative bacteria, and can successfully extract DNA from strains that fail to be extracted or yield poor results with kits from other suppliers.
3. Fast, simple, and the operation of a single sample can generally be completed within 30 minutes.
4. Multiple column rinses ensure high purity, with a typical OD260/OD280 ratio of 1.7-1.9 and a length of up to 30 kb-50 kb. It can be directly used for PCR, Southern blot, and various enzyme digestion reactions.

Note

1. All centrifugation is completed at room temperature, and the operating speed can reach 13, A traditional desktop centrifuge with a speed of 000 rpm.
2. You need to bring your own isopropanol (for the steps of adding isopropanol, it is recommended to use isopropanol first, and ethanol can also be used as a substitute without isopropanol). RNase A (optional).
3. Lysozyme (optional, for Gram positive bacteria).
4. Preheat the required water bath to 37°C or 70°C before starting the experiment..

Protocol(Please read the precautions before the experiment)

Tip: Before the first use, add anhydrous ethanol according to the instructions on the labels of the rinsing solution WB and protein removal solution PE bottle, mix thoroughly, and mark the box with a hook indicating that ethanol has been added in a timely manner to avoid multiple additions!

1. Take 0.5-2 milliliters of culture medium (up to a maximum of 2 × 109 cells), 13, 000 rpm, centrifuge for 30 seconds, discard all supernatant as much as possible, and collect bacterial cells.

   The initial processing capacity can be adjusted according to bacterial density, cell type, and expected yield, but the maximum adsorption capacity of the centrifugal adsorption column is 30μg genomic DNA. If the bacterial cells exceed the maximum adsorption capacity, it will seriously reduce the yield.
2. Add 180μL of lysis buffer GY to the bacterial sediment, shake or blow until the bacterial cells are completely suspended.

   Note: For hard-to-lyse Gram-positive bacteria, Step 2 may be skipped, and lysozyme can be added for cell lysis. Either of the following two protocols can be selected:

   (1): Add 150μL Lysis Buffer GY, vortex or pipette up and down until the bacterial pellet is fully resuspended. Add 30μL lysozyme (50mg/mL), mix well, and incubate at 37°C for no less than 30 min.

   (2): If only lysozyme powder is available, dissolve the powder directly in Buffer GY before use to prepare lysozyme-containing lysis buffer (final lysozyme concentration: 10–20mg/mL). Add 180μL Buffer GY (pre-supplemented with lysozyme), vortex or pipette up and down until the bacterial pellet is fully resuspended. Incubate at 37°C for no less than 30 min.
3. Add 20μL of proteinase K (20 mg/ml) solution, mix thoroughly, then add 200μL of binding solution CB, **immediately vortex and shake thoroughly**, and let it stand at 70°C 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 binding solution CB, shaken and mixed, and left at room temperature for 5-10 minutes.
4. **Column equilibrium:** Add 100μL of equilibrium solution to the adsorption column AC, centrifuge at 13000 rpm for 1 minute, discard the filtrate, and set aside for later use.

   The equilibrium solution can enhance the adsorption capacity of silica gel membrane for nucleic acids. Please use the adsorption column treated on the same day.
5. After cooling, add 100μL of isopropanol (which can also be replaced by anhydrous ethanol), **immediately vortex and shake thoroughly to mix well**. At this time, flocculent precipitation may appear.

   It is very important to immediately vortex or blow thoroughly in the above steps, as insufficient mixing can seriously reduce yield. If necessary, vortex oscillation can be used to mix if the sample is viscous and difficult to mix.
6. Add the mixture from the previous step (including any possible precipitates) to an adsorption column AC (place the adsorption column in a collection tube), centrifuge at 13,000 rpm for 30-60 seconds and discard the waste liquid from the collection tube.
7. Add 500μL of protein removal solution PE **(please check if anhydrous ethanol has been added first!)**, Centrifuge at 13,000 rpm for 30 seconds and discard the waste liquid.
8. Add 600μL of rinse solution WB **(please check if anhydrous ethanol has been added first!)**, Centrifuge at 13,000 rpm for 30 seconds and discard the waste liquid.
9. Repeat step 8 once.
10. 10. Place the adsorption column AC back into the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove the rinse solution as much as possible to prevent residual ethanol in the rinse solution from inhibiting downstream reactions.

11. 11. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 50-100μL of elution buffer EB to the middle of the adsorption membrane (the elution buffer can be preheated in an 80-100°C water bath to increase yield). Let it stand at room temperature for 2-4 minutes. Centrifuge at 13,000 rpm for 1 minute.
    The solution obtained from the first elution can be re added to the centrifuge column and left at room temperature for 2 minutes, Centrifuge at 13,000 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.
12. DNA can be stored at -20°C, and if it needs to be stored for a long time, it can be stored at -70°C.