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

Bacterial genomic Medium Kit

Product Number: DNK1301

Shipping and Storage

1. When the ambient temperature is low, some detergent ingredients in Buffer NLF will precipitate and become turbid or precipitate. It can be heated in a 37°C water bath for a few minutes, and then gently shaken to restore clarity. Do not shake violently to avoid excessive foam formation.
2. Buffer PP may experience precipitation and precipitation, and can be re dissolved in a water bath at 37°C for a few minutes. If it cannot be completely dissolved, it will not affect the effectiveness of use. Simply take the upper solution.
3. 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	DNK1301 20Preps	DNK1302 50Preps
Buffer NLF	RT	90ml×2	250ml×2
Buffer PP	RT	60ml	150ml
Buffer DD	RT	10 ml	20ml
RNaseA(10mg/ml)	-20°C	500μl	1ml

Description

This kit is used for rapid extraction of genomic DNA from various bacteria. Bacterial samples are added to Buffer NLF (or lysozyme or other alcohols to aid in cell wall lysis). Firstly, the cells are lysed under the action of strong detergents to release genomic DNA. Then, RNase A is added to remove RNA, followed by selective precipitation of buffer PP to remove protein. Finally, pure genomic DNA is precipitated in isopropanol and re dissolved in DNA solution.

Features

1. No need to use toxic reagents such as phenol.
2. Fast and simple, the operation of a single sample can generally be completed within 30 minutes.
3. The results are stable, with high yield. The typical OD260/OD280 ratio is 1.7~1.9, and the length can reach 50kb-150kb. It can be directly used for library construction, PCR, Southern blot, and various enzyme digestion reactions.

Application

Used for rapid extraction of various bacterial genomic DNA

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 3,000×g.
2. Users need to bring their own isopropanol, 70% ethanol, 0.5M EDTA and Lysozyme (for Gram positive bacteria), lysostaphin (for certain difficult to lyse Gram positive bacteria), and a water bath.
3. Preheat the required water bath before starting the experiment for later use
4. This reagent kit is a solution type and can easily increase or decrease the bacterial cell count in proportion for each treatment. Please contact us for operating manuals for other treatment volumes.

Protocol(Please read the precautions before the experiment)

1. Collect 10ml of overnight cultured bacteria and add it to a 15ml centrifuge tube.

2. Centrifuge 2,000×g for 3 minutes to allow the cells to precipitate. Discard the supernatant and disperse the cell precipitate by swirling or lightly bouncing. **For Gram positive bacteria, proceed to step 3. For Gram negative bacteria, proceed directly to step 6.**
3. Add 4.8ml of 50mM EDTA (pH 8.0) to completely resuspend cells.
4. Add 1.2ml of lysozyme (20mg/ml) and mix well.
   For the majority of Gram positive bacteria such as Bacillus subtilis, Micrococcus luteus, Arthrobacter luteus, Nocardia otitidiskaviarum, Rhodococcus rhodococcus, and Brevibacterium albidium, lysozyme can effectively lyse them. However, for certain types of Staphylococcus, 60µl of lysozyme (20mg/ml) and 60µl of lysostaphin (20mg/ml) should be added to ensure effective lysis.
5. Incubate at 37°C for 30-60 minutes. Centrifuge 2000×g for 10 minutes, discard the supernatant, blow and vortex to disperse the cell precipitate.
6. Add 9ml of Buffer NLF to the dispersed cells and gently blow to lyse the cells.
7. Incubate at 80°C for 5 minutes to lyse cells, then cool to room temperature.
8. Add 18µl of RNase A (10mg/ml) to the lysate to a final concentration of 20ug/ml. Invert the mixture and incubate at 37°C for 15-60 minutes to remove residual RNA. Then cool at room temperature for at least 5 minutes to return to room temperature.
9. Add 3ml of Buffer PP to the pyrolysis product that has been restored to room temperature, and mix continuously on a vortex oscillator for 25 seconds at high speed. After mixing, you may see some small protein clumps. Ice bath for 5 minutes.
   Due to the small volume and weight of the sample, the shear force generated by shaking and mixing with a vortex oscillator will not shear and interrupt the genomic DNA. If you shake and mix with your hands, you cannot shake vigorously up and down with your hands. You can only shake and mix with appropriate force, otherwise it will cut the genomic DNA; However, the force should not be small. It is important to ensure thorough mixing and disperse the viscous lysate. Otherwise, DNA cannot separate from protein precipitates, and during centrifugation, it will precipitate together with protein, causing DNA loss or reduced yield. In addition, insufficient mixing may also result in insufficient protein precipitation, and the final product may contaminate a large amount of protein. Therefore, it is recommended to use a vortex oscillator.
10. Centrifuge 5000×g (adjustable to increase centrifugal force as needed) for 10 minutes. At this point, you should be able to see white protein precipitates at the bottom of the tube, or you may see some protein precipitates floating on the surface of the liquid.
11. Carefully aspirate the supernatant into a new 50ml centrifuge tube.
    When aspirating the supernatant, be careful not to aspirate the protein precipitate at the bottom of the tube or floating on the liquid surface. If the protein precipitate is accidentally transferred into a new centrifuge tube, it can be centrifuged again for 5 minutes before taking the supernatant.
12. Add an equal volume of room temperature isopropanol (about 9ml), gently invert 30 times and mix well or until a cotton like (filamentous) white DNA precipitate appears.
    Note that sometimes when cotton like (filamentous) DNA is mixed upside down, it adheres to the lid or tube opening, and even if it is upside down, it does not follow, which can cause the operator to not see the sediment and mistakenly believe that DNA has not been obtained. The solution is to skip step 13, centrifuge 2000×g directly for 5 minutes, discard the supernatant, and then proceed to step 15.
13. Place the centrifuge tube vertically to allow the white DNA precipitate to naturally settle to the bottom of the tube, and then absorb as much of the supernatant as possible, being careful not to inhale the precipitate.
14. After adding 9ml of 70% ethanol, invert and rinse the DNA precipitate. Centrifuge 2000×g for 3-5 minutes, and white DNA precipitate blocks can be seen at the bottom of the tube. Discard the supernatant
15. Add 5m1 of 70% ethanol, invert and rinse the DNA precipitate several times, centrifuge 2000×g for 1 minute, pour out the supernatant (be careful not to pour out the DNA precipitate), invert and gently tap on absorbent paper a few times to control the residual ethanol. You can also use a gun to carefully suck out the residual ethanol around the bottom precipitate and the wall of the tube, and air dry the precipitate for a few minutes.

Be careful not to dry too much, otherwise DNA is extremely insoluble; Also, too much ethanol should not be left behind, otherwise ethanol may inhibit downstream reactions such as enzyme digestion.

16. Add 500μl of Buffer DD to rehydrate and dissolve the DNA precipitate. Mix the lightly flicked tube walls and incubate at 65°C for 30-60 minutes (no more than an hour). Occasionally flick the tube walls to help rehydrate the DNA, or place overnight at room temperature or 4°C to rehydrate the DNA. Occasionally invert the tube walls to aid in dissolution.
17. DNA can be stored at 2-8°C, and if stored for a long time, it can be stored at -20°C.