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Bacterial Genomic DNA Kit(liquid form)

Product Number: DNK1201

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	DNK1201 50 Preps	DNK1202 100 Preps	DNK1203 200 Preps
Buffer NLF	RT	30 ml	60 ml	120 ml
Buffer PP	RT	10 ml	20 ml	40 ml
Buffer DD	RT	10 ml	15ml	30 ml
RNaseA(10mg/ml)	-20°C	150μl	250μl	400μl

Description

This kit is used for rapid extraction of genomic DNA from various bacteria. After adding bacterial samples to Buffer NLF (or using lysozyme or other enzymes to help break down cell walls), the cells are first lysed under strong detergent action to release genomic DNA. RNase A is then added to remove RNA, followed by selective precipitation of buffer PP to remove proteins. Finally, pure genomic DNA is precipitated in isopropanol and re dissolved in Buffer DD.

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 and the yield is high. The typical ratio of OD260/OD280 is 1.7~1.9, and the length can reach 50-150kb. It can be directly used for library construction, PCR, Southern blot, and various alcohol cleavage 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 13000rpm.
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)

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1. Collect 1ml of overnight cultured bacteria and add to a 1.5ml centrifuge tube.
2. Centrifuge at 9000rpm for 30 seconds 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 480µl 50mM EDTA to completely resuspend cells.
4. Add 120µl of lysozyme (20mg/ml in 10mM Tris HCl, pH 8.0) 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 at 12000 rpm for 2 minutes, discard the supernatant, and disperse the cell precipitate by vortex or light flick.
6. Add 600µl 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 1.8µl of RNase A (10mg/ml) to the lysate to a final concentration of 30µg/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. After adding 200µl Buffer PP to the pyrolysis product that has returned to room temperature, **shake continuously at high speed on a vortex oscillator and mix well for 25 seconds**. 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 at 13000rpm for 5 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 1.5ml 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 2 minutes before taking the supernatant.
12. Add an equal volume of room temperature isopropanol (about 600µl), 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 at 12000 rpm for 1 minute, 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 1ml of 70% ethanol, invert and rinse the DNA precipitate. Centrifuge at 12000rpm for 1 minute, and white DNA precipitate blocks can be seen at the bottom of the tube. Discard the supernatant
15. Add 0.5m1 of 70% ethanol, invert and rinse the DNA precipitate several times, centrifuge at 12000rpm 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. Join 100μL Buffer DD rehydrates and dissolves DNA precipitation by gently tapping the tube wall. It can be incubated at 65°C for 30-60 minutes (not more than an hour), and occasional light tapping of the tube wall can help rehydrate DNA. It can also be left overnight at room temperature or 4°C to rehydrate DNA, with occasional flipping of the tube wall 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.