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Blood DNA Max kit

Product Number: DNK0401

Shipping and Storage

1. When the ambient temperature is low, some detergent ingredients in Buffer NLY will precipitate and become turbid or precipitate. It can be heated in a 37℃ water bath for a few minutes 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 by taking a water bath at 37℃ 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	DNK0401 16 Preps×10ml	DNK0402 32 Preps×10ml	DNK0403 96 Preps×10ml
10x Buffer ELY	RT	50 ml	100 ml	300 ml
Buffer NLY	RT	180 ml	180 ml×2	250 ml×4
Buffer PP	RT	55 ml	110 ml	330 ml
Buffer DA	RT	15 ml	30 ml	90 ml

Description

This kit uses several rapid steps to extract genomic DNA based on the characteristics of whole blood. Firstly, Buffer ELY cleaves red blood cells without DNA, while Buffer NLY cleaves white blood cells to release genomic DNA. Then, Buffer PP selectively precipitates to remove proteins. Finally, pure genomic DNA is precipitated in isopropanol and re dissolved in Buffer DA.

Features

1. The Buffer ELY formula selected from over a dozen formulas is fast and complete in cracking.
2. No need to use toxic reagents such as phenol.
3. Fast and simple, the operation of a single sample can generally be completed within 30 minutes.
4. The results are stable and the yield is high (typical yield of 10ml whole blood can extract 150-500μg). The OD260/OD280 typical 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

Suitable for rapid extraction of genomic DNA from whole blood of various animals

Note

1. All centrifugation steps are completed at room temperature, with a rotational speed of up to 2500×g, and equipped with a traditional desktop centrifuge that can accommodate a 50ml centrifuge tube rotating head
2. Users need to bring their own isopropanol and 70% ethanol.
3. A typical yield of 10ml of whole blood can extract 150-500μg of genomic DNA (the number of white blood cells in different samples, especially in disease samples, may vary greatly, so individual differences in yield may also be significant).
4. This reagent kit is a solution type and can easily increase or decrease the total blood volume (20μl-10ml) for each treatment in proportion.
5. This test kit can be used for whole blood with various anticoagulants, such as EDTA, citric acid, and heparin for anticoagulation. Due to the difficulty in dispersing and resuspending the white blood cell precipitates of heparin anticoagulant, which affects the

lysis effect, it is recommended to use non heparin anticoagulants to collect blood samples.

6. For optimal results, it is best to use fresh blood specimens or specimens stored at 4℃ for less than 3 days. Do not use specimens that have been repeatedly freeze-thawed more than 3 times, otherwise it will seriously reduce production.

Protocol(Please read the notes before the experiment)

1. Suck 30ml 1×Buffer ELY (needs to be diluted to 1×)to a 50ml centrifuge tube.

Note: Before use, 10×Buffer ELY should be diluted 10 times with deionized water to 1×.

2. Invert the anticoagulant whole blood (return to room temperature before use) and mix well. Take 10ml and add it to the centrifuge tube containing Buffer ELY in the previous step. Invert 6-8 times and gently flick the tube wall upside down to ensure thorough mixing.
3. Let it stand at room temperature for 10 minutes (during which it should be flipped upside down and lightly bounced, mixed several times to help lyse red blood cells).
4. Centrifuge 2500×g for 2 minutes, discard the red supernatant, and carefully aspirate as much supernatant as possible (be careful not to aspirate cell clusters at the bottom of the tube), leaving intact white blood cell clusters at the bottom of the tube and approximately 10μl of residual supernatant.

Note: After centrifugation, white white blood cell clusters should be seen at the bottom of the tube, and there may also be some red blood cell fragments and white blood cell clusters together. However, if most of the red blood cell clusters are seen, it indicates that red blood cell lysis is not sufficient. An appropriate amount of Buffer ELY should be added to resuspend the fine cell clusters and repeat steps 3 and 4.

5. Vortex oscillation until the white blood cell clusters are fully resuspended and dispersed.

Note: The resuspension and dispersion of white blood cells are crucial for the next step of lysis. Adding Buffer NLY without dispersing white blood cells can cause them to not fully lyse and form visible clumps.

6. Add 10ml of Buffer NLY to resuspended white blood cells, blow up and down to lyse white blood cells, or vigorously vortex for 10 seconds to help lyse white blood cells.

Optional steps, generally not required: add RNase A (10mg/ml) to the lysate to a final concentration of 30μg/ml, mix 25 times upside down, incubate at 37 ℃ for 15 minutes to remove residual RNA, then cool back to room temperature.

7. After adding 3.33ml Buffer PP, oscillate continuously at high speed on a vortex oscillator and mix well for 25 seconds. After mixing, you may see some small protein clumps.
8. 2500×g (centrifugal force can be adjusted and increased as needed) Centrifuge for 5 minutes. At this point, you should be able to see dark brown protein precipitates at the bottom of the tube, or you may see some protein precipitates floating on the surface of the liquid.
9. Carefully aspirate the supernatant (approximately 10ml) into a new 50ml centrifuge tube.

Note: When aspirating the supernatant, be careful not to aspirate onto the bottom of the tube and protein precipitates floating on the surface of the liquid. If the protein precipitate is accidentally transferred into a new centrifuge tube, it can be centrifuged again for 2 minutes before taking the supernatant.

10. Add an equal volume of room temperature isopropanol (10ml), gently invert 30 times, and mix well or until a cotton like (filamentous) white DNA precipitate appears.
11. Centrifuge 2000g for 3 minutes, and white DNA precipitates can be seen at the bottom of the tube. Discard the supernatant.
12. Add 10ml of 70% ethanol, invert and rinse the DNA precipitate several times, centrifuge 2,000×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.

Note: Do not 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.

13. Add 600μl of Buffer DA (if a high concentration is required, the amount of Buffer DA can be reduced as needed) to dissolve the DNA precipitate again. Gently flick the tube wall and mix well, allowing it to incubate at 65 ℃ for 30-60 minutes (not more than

one hour). During this time, gently flick the tube wall to help rehydrate the DNA. DNA can also be rehydrated by staying overnight at room temperature or 4 ℃.

14. DNA can be stored at 2-8 ℃, and if it needs to be stored for a long time, it can be placed at -20 ℃.