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Coagulated Blood Genomic DNA Kit

Product Number: DNK3102

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. Proteinase K is stored in a ready-to-use glycerol buffer and transported at room temperature. Upon receipt, it should be stored at room temperature for at least 6 months, at 4℃ for 12 months, and at -20℃ for 2 years
3. Avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air. Each solution should be covered tightly after use.

Components

Component	Storage	DNK3102 320Preps×50μl
Buffer NLY	RT	180 ml
Buffer PP	RT	70 ml
Glycogen	-20℃	0.7 ml
Buffer DA	RT	10 ml
Proteinase K	4℃	1 ml

Description

This reagent kit is exclusively developed based on the characteristics of whole blood coagulation. Buffer NLY is combined with Proteinase K to lyse and coagulate blood clots to release genomic DNA. Then, Buffer PP selectively precipitates to remove proteins. Finally, pure genomic DNA is precipitated in isopropanol with the assistance of molecular biology grade Glycogen and re dissolved in Buffer DA.

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 1 hour.
3. The results are stable, and Glycogen helps precipitate trace amounts of DNA 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.

Note

1. All centrifugation steps are completed at room temperature, and the operating speed can reach 2500×g. And equipped with a traditional desktop centrifuge that can accommodate a 50ml centrifuge tube rotating head (for small extraction, a small centrifuge can be used).
2. Users need to bring their own isopropanol, 70% ethanol, and TE buffer.
3. A typical clotting blood yield of 1ml can extract 10-30μg of genomic DNA from whole blood (individual differences in sample yield with different clotting degrees may 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.

Protocol(Please read the precautions before the experiment)

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1. Add 50μl of coagulated blood to a 1.5ml centrifuge tube or 1ml of coagulated blood to a 50ml centrifuge tube, vigorously vortex or tap the centrifuge tube with your hand to help disperse the clots.
2. Add 550μl or 11ml of Buffer NLY, blow and mix well, then add 3μl or 60μl of Proteinase K (20mg/ml), invert and mix well 25 times.
3. Leave at 55℃ for 3 hours until overnight, until all clots are completely melted.

Optional steps (generally not required): Add 3μl or 60μl RNase A (4mg/ml), add RNase A (10mg/ml) to the lysate to a final concentration of 30μg/ml, mix 25 times, and incubate at 37℃ for 15 minutes to remove residual RNA.

4. Quickly cool the cracked product to room temperature (it can be placed on ice for one minute).
5. Add 200μl or 4ml of Buffer PP and shake continuously at high speed on a vortex oscillator for 25 seconds. After mixing, you may see some small protein clumps.

Note: The liquid should rotate and oscillate, not just up and down, for the best mixing and protein precipitation effects.

6. Place on ice for 5 or 10 minutes.
7. Centrifuge 13000-16000×g for 5 minutes or 2500×g (adjust and increase centrifugal force as needed) for 10 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.
8. Carefully aspirate the supernatant into a new 1.5ml centrifuge tube or 50ml centrifuge tube.

Note: When aspirating the supernatant, be careful not to aspirate the protein precipitate at the bottom of the tube or 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.

9. Add 600μl of room temperature isopropanol and 1μl of Glycogen solution or 12ml of room temperature isopropanol and 20μl of Glycogen solution, gently invert 50 times and mix until cotton like (filamentous) white DNA precipitate appears.

Note: Filamentous precipitates may only be visible when handling large sample volumes, and are often not visible when handling small sample volumes or poor storage quality.

10. Centrifuge 13000-16000×g for 1 minute or 2500×g for 3 minutes, at which point a white DNA precipitate should be visible at the bottom of the tube.
11. Be careful to discard the supernatant, invert it and gently tap on absorbent paper a few times to control residual ethanol (be careful not to lose sediment).
12. Add 600μl or 12ml of 70% ethanol, invert and rinse the DNA precipitate several times.
13. Centrifuge 13000-16000×g for 1 minute or 2500×g for 1 minute, pour out the supernatant (the precipitate is very loose, be careful not to pour out the DNA precipitate), invert it, and gently tap a few times on absorbent paper 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.

14. Add 20μl or 250-400μl Buffer DA (customers can also choose TE Buffer or other suitable buffer according to their needs) to rehydrate and dissolve the DNA precipitate. Gently tap the tube wall and mix well. It can be left at 65℃ for 30-60 minutes (not more than one hour), or left overnight at room temperature or 4℃ to rehydrate the DNA. Occasionally tap the tube wall to help rehydrate the DNA.
15. DNA can be stored at 2-8℃, and if it needs to be stored for a long time, it can be placed at -20℃.