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Clinical DNA Mini Kit

Product Number: DNK2501

Shipping and Storage

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

Components

Component	Storage	DNK2501 50 Preps
Buffer ML	RT	11 ml
Buffer CB	RT	15 ml
Buffer IR	RT	25 ml
Buffer WB	RT	13 ml
Poly Carrier	-20℃	200μl
Buffer EB	RT	10 ml
Protease K	4℃	1ml
Ultratrace DNA centrifuge column	RT	50

Description

This reagent kit adopts a specially designed ultra trace DNA adsorption column and a unique buffer system, which is particularly suitable for isolating and purifying genomic DNA from trace samples such as blood, forensic materials, dry blood points, medical swabs, chewing gum, urine, etc. After various sources of samples are lysed and digested, DNA selectively adsorbs onto the silica matrix membrane in a highly dissociated salt state (especially equipped with Poly Carrier, which can easily capture trace amounts of nucleic acids from the system). Then, impurities such as salt, cellular metabolites, proteins, etc. are removed through a series of rapid rinsing centrifugation steps. Finally, pure genomic DNA is eluted from the silica matrix membrane using low salt Buffer EB. The purified DNA is free of impurities and PCR inhibitors, making it directly suitable for PCR analysis.

Features

1. The special ultra trace 5μg centrifugal column design can achieve a minimum of 6μl elution, greatly increasing the concentration of DNA.
2. No toxic reagents such as phenol are required, and no steps such as ethanol precipitation are required.
3. Time saving, simple, and single sample Protocol can generally be completed within 20 minutes.
4. Equipped with Poly Carrier for fully collecting special trace amounts of DNA.
5. Multiple column washes ensure high purity, the extracted DNA has high purity, stable and reliable quality, and can be used for various routine Protocols, including PCR, enzyme digestion, sequencing, Southern hybridization, etc.

Application

Suitable for isolating and purifying genomic DNA from trace samples such as blood, forensic materials, dry blood points, and medical swabs.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm.
2. Preheat the required water bath to the desired temperature before starting the experiment. Some samples require 1M DTT preparation.
3. Buffer CB and Buffer IR contain irritating compounds. Wear latex gloves during Protocol to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, rinse with plenty of water or physiological saline.
4. Poly Carrier:

   Usage of Poly Carrier: If the initial processing volume is small (such as less than 10μl of whole blood and forensic samples), we recommend using Poly Carrier. If a large amount of DNA production is expected, users can choose whether to add Poly Carrier according to their needs. When using, add 2μl Poly Carrier storage solution to the Buffer CB required for each sample extraction, and mix the Buffer CB and Poly Carrier solution completely upside down (Buffer CB is easy to form foam, do not use vortex oscillation to mix). You can also add the required Poly Carrier to the total required Buffer CB according to the number of samples and mix well for later use. The mixture is stable at room temperature for 24 hours.

Protocol(Please read the precautions before the experiment)

Tip:Before the first use, please add anhydrous ethanol to Buffer WB according to the label instructions, mix well, and mark the added ethanol in the box in a timely manner to avoid multiple additions!

1. Blood samples

1.1. Take 1-50μl of blood into a 1.5ml centrifuge tube.

1.2. Add Buffer ML to make up for 100μl.

1.3. Add 10μl of Protease K (20mg/ml) solution, mix well, then add 100μl of Buffer CB, immediately vortex and shake thoroughly, and let it stand at 70℃ for 10 minutes.

If the sample size is less than 10μl, it is recommended to add 1μl Poly Carrier storage solution to 100μl Buffer CB.

1.4. After cooling, add 50μl of anhydrous ethanol, immediately vortex and shake thoroughly, and let it stand at room temperature for 3 minutes.

If the surrounding environment is above 25℃, ethanol needs to be pre cooled on ice before being added.

1.5. Follow step 7 of the Protocol.

2. Dry blood point

2.1. Punch 3mm (1/8 inch) diameter blood card small pieces (with dry blood dots on top) using a punching machine, and place up to 3 3mm diameter blood card small pieces into a 1.5ml centrifuge tube.

Generally, blood should be dried on specific paper or blood cards, such as 903 paper or IsoCode paper (Schleicher&Schuell), BloodstainCard or FTA Card (Whatman), Guthrie test cards, or comparable blood cards

2.2. Add 180μl Buffer ML.

2.3. Add 20μl of Protease K (20mg/ml) solution and immediately vortex and shake thoroughly to mix well.

2.4. Shake at 900rpm on a 56℃ track shaker for 1 hour.

If there is no heated track shaker available, it can be done on a water bath or heating block, with vortex oscillation every 10 minutes for 10 seconds to assist in cracking.

2.5. Add 200μl Buffer CB and immediately vortex and mix thoroughly.

If only one 3mm blood card tablet is processed, it is recommended to add 2μl Poly Carrier storage solution to 200μl Buffer CB.

2.6. Shake on a 70℃ track shaker at 900rpm for 10 minutes.

If there is no heated track shaker available, it can be done on a water bath or heating block, with vortex oscillation every 3 minutes for 10 seconds to assist in cracking.

2.7. Follow step 7 of the Protocol.

3. Organizational samples

3.1. Fresh or thawed tissue is ground into fine powder in liquid nitrogen or cut into small pieces with a dissecting knife (cutting into small pieces can increase yield). Take<10mg and transfer it into a 1.5ml centrifuge tube containing 180µl Buffer ML, and mix well with a large caliber gun.

3.2. Add 20µl of Protease K and immediately vortex and mix thoroughly.

3.3. Place the lysate in a 56℃ water bath for 1-3 hours or until the tissue is completely digested, gently shaking it a few times during this period to aid in lysis.

3.4. Add 200µl Buffer CB and immediately vortex and mix thoroughly.

If the sample volume is small, it is recommended to add 2µl Poly Carrier storage solution to 200µl Buffer CB.

3.5. After cooling, add 200µl of anhydrous ethanol, immediately vortex and shake thoroughly, and let it stand at room temperature for 5 minutes.

3.6. If the surrounding environment is above 25℃, ethanol needs to be pre cooled on ice before being added.

3.7. Follow step 7 of the Protocol.

4. Chewing gum

4.1. Cut 30mg of chewing gum into small pieces and place them in a 1.5ml centrifuge tube. Add 280µl of Buffer ML and 20µl of Protease K. Immediately vortex and shake thoroughly to mix well.

4.2. Shake at 900rpm on a 56℃ track shaker for at least 3 hours.

If there is no heated track shaker available, it can be done on a water bath or heating block, with vortex oscillation every 10 minutes for 10 seconds to assist in cracking.

4.3. Add 200µl Buffer CB (2µl Poly Carrier) and immediately vortex oscillate thoroughly to mix well.

4.4. Shake on a 70℃ track shaker at 900rpm for 1 hour.

If there is no heated track shaker available, it can be done on a water bath or heating block, with vortex oscillation every 10 minutes for 10 seconds to assist in cracking.

4.5. After cooling, add 200µl of anhy drous ethanol and immediately vortex and shake thoroughly to mix well.

4.6. Centrifuge at the highest speed (about 13000rpm) for 1 minute, take the supernatant and add it to an adsorption column. (Place the adsorption column into the collection tube) Centrifuge at 13000rpm for 30-60 seconds, and discard the waste liquid in the collection tube.

4.7. Follow step 8 of the Protocol.

5. Forensic materials

5.1.1. Cut 1cm^2 of cigarette butts or filter tip outer layer paper, cut into 6 small pieces and place them in a 1.5ml centrifuge tube. Add 300µl Buffer ML and 20µl Protease K. Immediately vortex and shake thoroughly to mix well. Continue with step 5.2.

5.1.2. Cut 0.5-2.5 cm^2 envelopes or stamps, cut them into small pieces and place them in a 1.5ml centrifuge tube. Add 300µl Buffer ML and 20µl Protease K. Immediately vortex and shake thoroughly to mix well. Continue with step 5.2.

5.1.3. Starting from the hair follicles at the root of the hair, cut a length of 0.5-1 cm of hair and place it in a 1.5ml centrifuge tube. Add 280µl Buffer ML and 20µl Protease K and 20µl 1M DTT solution, and immediately vortex and shake thoroughly to mix well. Continue with step 5.2.

5.1.4. Cut the nails into small pieces and place them in a 1.5ml centrifuge tube. Add 280µl Buffer ML, 20µl Protease K, and 20µl 1M DTT solution. Immediately vortex and shake thoroughly to mix well. Continue with step 5.2.

5.1.5. Cut approximately 0.5cm^2 of envelope material contaminated with blood, saliva, and semen into small pieces and place them in a 1.5ml centrifuge tube. Add 300µl Buffer ML and 20µl Protease K (if it is semen, an additional 20µl 1M DTT solution needs to be added), immediately vortex and shake thoroughly to mix well. Follow step 5.2.

5.2. Shake at 900rpm on a 56℃ track shaker for 1 hour.

If there is no heated track shaker available, it can be done on a water bath or heating block, with vortex oscillation every 10 minutes for 10 seconds to assist in cracking.

Generally, hair lysis can be completed within 1 hour, but if it is not complete, the time can be extended. It is recommended to crack difficult to crack substances such as nails overnight. The insoluble substances that have not been cracked will be removed by centrifugation in subsequent step 5.5.

5.3. Add 200µl Buffer CB (2µl Poly Carrier) and immediately vortex oscillate thoroughly to mix well.

5.4. Shake on a 70℃ track shaker at 900rpm for 1 hour.

5.5. Centrifuge at the highest speed (about 13000rpm) for 1 minute, take the supernatant and add it to an adsorption column. (Place the adsorption column into the collection tube) Centrifuge at 13000rpm for 30-60 seconds, and discard the waste liquid in the collection tube.

5.6. Follow step 8 of the Protocol.

6. Micro cut samples (including formalin fixed micro cut samples)

6.1. Add 15µl Buffer ML to a 0.2 ml centrifuge tube and place the micro cut sample.

6.2. Add 10µl of Protease K and immediately vortex and mix thoroughly.

6.3. 56℃ water bath for 3 hours (formalin sample for 16 hours) until complete cracking, with occasional inverted vortices in between.

6.4. Add 15µl Buffer ML, then 50µl Buffer CB (add 1µl PolyCarrier), immediately vortex and shake thoroughly to mix well.

6.5. After cooling, add 50µl of anhydrous ethanol, immediately vortex and shake thoroughly, and let it stand at room temperature for 5 minutes.

If the surrounding environment is above 25℃, ethanol needs to be pre cooled on ice before being added.

6.6. Follow step 7 of the Protocol.

7. Add the previous mixture (including possible precipitates) into an adsorption column, centrifuge at 13000 rpm for 30-60 seconds (with the column placed in a collection tube), and discard the waste liquid from the collection tube.

8. Add 500µl Buffer IR, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.

9. Add 600µl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.

10. Add 600µl Buffer WB, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.

11. Put the adsorption column back into the empty collection tube, centrifuge at 12000 rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.

12. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 10µl -25µl Buffer EB to the middle of the adsorption membrane (Buffer EB is better preheated in a 75-90℃ water bath), leave at room temperature for 2-3 minutes, and centrifuge at 12000 rpm for 1 minute.

Reducing the buffer EB volume during elution can increase DNA concentration, but the minimum buffer EB volume should not be less than 6µl.

Optional: Re adding the first buffer EB to the adsorption column for secondary elution can increase the concentration by about 10%.

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