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

Genomic Fast DNA Kit

Product Number: DNK1001

Shipping and Storage

1. Buffer TL, Buffer CB, or Buffer IR may precipitate and precipitate at low temperatures. They can be re dissolved in a water bath at 37°C for a few minutes, restored to clarity and transparency, and then cooled to room temperature before use.
2. Proteinase K is stored in a ready to use glycerol buffer and transported at room temperature. After receipt, store at room temperature not exceeding 25°C for at least 6 months, at 4°C for 12 months, and at -20°C 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.

Components

Component	Storage	DNK1001 50 Preps	DNK1002 100 Preps	DNK1003 200 Preps
Balance Buffer	RT	5 ml	10 ml	20 ml
Buffer TL	RT	11 ml	20 ml	40 ml
Buffer CB	RT	15 ml	30 ml	60 ml
Buffer IR	RT	25 ml	50 ml	100 ml
Buffer WB	RT	13 ml	25 ml	50 ml
Buffer EB	RT	15 ml	15 ml	20 ml
Proteinase K	4°C	1 ml	1ml ×2	1ml ×4
Adsorption column AC	RT	50	100	200
Collection tube (2ml)	RT	50	100	200

Description

The unique Buffer CB/Proteinase K rapidly lyses cells and uses a silica gel membrane centrifuge column to specifically adsorb DNA, without the need for toxic reagents such as phenol and chloroform, or time-consuming alcohol precipitation, maximizing the removal of proteins and other inhibitory impurities. Suitable for efficiently extracting genomic DNA from various materials (whole blood, animal tissue cells, mouse tail, Escherichia coli, etc.). The extracted DNA can be directly used for experiments such as enzyme digestion, PCR, Southern Blot, and virus detection.

Features

1. There is no need to use toxic reagents such as phenol or time-consuming ethanol precipitation.
2. Fast and simple, the Protocol of a single sample can generally be completed within 30 minutes.
3. Multiple column washes ensure high purity, with a typical yield of 200 μ l of whole blood capable of extracting 3-6 μ g of genomic DNA. The typical ratio of OD260/OD280 is 1.7-1.9, with a length of 30kb-50kb. It can be directly used for PCR, Southern blot, and various enzyme digestion reactions.
4. The red blood cell lysis fluid formula selected from over a dozen formulas is fast and complete, and customers can choose to purchase it according to their needs.

Application

Suitable for anticoagulating whole blood/tissue/cells/mouse tail/bacteria and other genomic DNA.

Use of balance buffer

1. Description

During the long-term placement of nucleic acid adsorption silica gel membrane columns, they react with charges/dust in the air and affect their nucleic acid binding ability.After pre-treatment with equilibrium solution, the silica gel column can greatly reduce the hydrophobic groups of the silica gel membrane in the column and improve the binding ability of nucleic acids.Thus improving the recovery efficiency or yield of silicone columns. The equilibrium solution is a strong alkaline solution. If accidentally touched, please clean it with a large amount of tap water.After use, the bottle cap needs to be tightly closed to avoid contact with air. Store at room temperature. During storage, there may be precipitation formation. Please heat to 37℃ to completely eliminate the precipitation.

2. Protocol

Take a new silica gel membrane adsorption column and install it in a collection tube, and suck 100μl of equilibrium solution into the column.Centrifuge at 13000rpm for 1 minute, discard the waste liquid from the collection tube, and reposition the adsorption column in the collection tube. At this point, the Balance Buffer pre-treatment column is completed. Follow the subsequent operating steps.

Note

1. All centrifugation is completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm.
2. Self prepared ethanol, isopropanol, 1× PBS (phosphate buffer, optional), RNase A (optional) lysozyme (for Gram positive bacteria, optional) are required.
3. There may be significant differences in the number of white blood cells in different samples, especially in disease samples, so individual differences in yield may also be significant.
4. Preheat the required water bath to 70°C for later use before starting the experiment.
5. 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 precautions before the experiment)

Tip: Before the first use, please add the specified amount of anhydrous ethanol to Buffer WB and mix well. After adding, please mark the box with a check mark indicating that ethanol has been added in a timely manner to avoid adding it multiple times!

Balance Buffer pre-treatment adsorption column backup:

The pre-treatment of silica gel membrane adsorption column with equilibrium solution is a necessary step, and the specific method can be found in the previous section "Use of balance buffer"

1. Whole blood

1.1. Take 200μl of fresh, frozen or mixed with various anticoagulants of blood and place it in a 1.5ml centrifuge tube.

Note:1) If the starting amount of whole blood is less than 200μl, supplement with 1×PBS to 200μl. If the starting amount is between 200μl-300μl, subsequent Protocols need to increase the reagent dosage proportionally. If the starting amount is between 300μl-1ml, red blood cell lysis Protocol needs to be performed first (see the appendix after this manual).

2)If the processed blood sample is anticoagulant blood from poultry, birds, amphibians, or lower level organisms, and the red blood cells are nucleated cells, the processing amount is only 5-20μl, and 1×PBS can be added to make up for 200μl before proceeding with the subsequent steps.

1.2. Add 20μl Proteinase K, mix well, then add 200μl Buffer CB, immediately vortex and shake well, and let it stand at 70°C for 10 minutes. The solution strain is clear (but the color is slightly black).

Optional steps, generally not required: If there is a lot of RNA residue and RNA needs to be removed, you can add 5μl of RNase A (100mg/ml) solution before adding 200l of Buffer CB, shake well, and let it stand at room temperature for 5-10 minutes.

1.3. After cooling, add 100μl of isopropanol (or anhydrous ethanol can be used as a substitute, the same below), immediately vortex and shake thoroughly to mix well. At this time, flocculent precipitation may occur.

Note: It is very important to immediately vortex or blow thoroughly in the above steps. Insufficient mixing seriously reduces production. If necessary, if the sample is viscous and difficult to mix, vortex oscillation can be used for 15 seconds to mix.

1.4. Add the previous mixture (including possible precipitates) to an adsorption column AC, and centrifuge at 13000rpm for 1

minute (with the adsorption column placed in the collection tube). Discard the waste liquid in the collection tube.

1.5. Follow step 6 of the Protocol.

2. Tissue cultured cells

2.1. Collect approximately 10^5-10^6 suspended cells into a 1.5ml centrifuge tube; For adherent cells, they should be digested with trypsin first and then collected by blowing them down.

2.2. Centrifuge at 130000 rpm for 10 seconds to allow the cells to precipitate. Absorb and discard the supernatant, leaving behind cell clusters.

2.3. Add 200μl of 1× PBS and resuspend to wash the cells. Centrifuge at 130000 rpm for 10 seconds to allow the cells to precipitate. Completely aspirate the supernatant and resuspend the cell precipitate in 180μl 1× PBS.

2.4. Add 20μl Proteinase K, mix well, then add 200μl Buffer CB, immediately vortex and shake well, and let it stand at 70°C for 10 minutes.

Optional steps: If there is a lot of RNA residue and RNA needs to be removed, you can add 5μl of RNase A (100mg/ml) solution before adding 200μl of Buffer CB, shake well, and let it stand at room temperature for 5-10 minutes.

2.5. After cooling, add 100μl of isopropanol and immediately vortex to mix thoroughly. At this time, flocculent precipitation may occur.

2.6. Add the previous mixture (including possible precipitates) to an adsorption column AC, and centrifuge at 130000 rpm for 1 minute (with the adsorption column placed in the collection tube). Discard the waste liquid in the collection tube.

2.7. Follow step 6 of the Protocol.

3. Animal and plant tissues (such as mouse liver and brain or plant leaves)

3.1. Cut 20-50mg of fresh or thawed tissue into small pieces using a dissecting knife (cutting into small pieces can increase yield) or grind the tissue into fine powder in liquid nitrogen, transfer it into a 1.5ml centrifuge tube containing 180μl Buffer TL, and use a large caliber gun to blow and mix well.

3.2. Add 20μl Proteinase K and immediately vortex and mix thoroughly.

3.3. Place the lysate in a 55°C 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.

Optional steps: If there is a lot of RNA residue and RNA needs to be removed, 5μl of RNase A (100mg/ml) solution can be added after completing step 3.3, shaken well, and left at room temperature for 5-10 minutes.

3.4. Add 200μl of Buffer CB, immediately vortex and shake thoroughly, and let it stand at 70°C for 10 minutes.

3.5. After cooling, add 100μl of isopropanol and immediately vortex to mix thoroughly. At this time, flocculent precipitation may occur.

3.6. Add the previous mixture (including possible precipitates) to an adsorption column AC, and centrifuge at 130000 rpm for 1 minute (with the adsorption column placed in the collection tube). Discard the waste liquid in the collection tube.

Note: If there is insoluble tissue that may block the suction head, the suction head can be gently rubbed on absorbent paper to remove the insoluble material; If there is a small amount of mixture sucked up, the suction head and insoluble substances can be discarded together. This method is to remove insoluble substances to avoid clogging the centrifuge column.

3.7. Follow step 6 of the Protocol.

4. Animal tissue (rat tail)

4.1. Cut the 0.2-0.5cm tip of the mouse tail (i.e. 20-50mg) into small pieces (be sure to cut the tip within the range of 0-2cm, otherwise the cracking effect will not be good), or grind it into fine powder in liquid nitrogen, transfer it into a 1.5ml centrifuge tube containing 180μl Buffer TL, and use a large caliber gun to blow and mix well.

4.2. Add 20μl Proteinase K and immediately vortex and mix thoroughly.

4.3. Place the lysate in a 55°C water bath for 3 hours or until the tissue is completely digested, gently shaking it several times during this period to aid in lysis.

Optional steps: If there is a lot of RNA residue and RNA needs to be removed, 5μl of RNase A (100mg/ml) solution can be added after completing step 4.3, shaken well, and left at room temperature for 5-10 minutes.

Optional: Use a large diameter suction head to pump the cracked product 2-3 times to assist in cracking.

4.4. Add 200µl Buffer CB and 100µl isopropanol, immediately vortex and shake thoroughly to mix well.

4.5. Centrifuge at 13000rpm for 5 minutes, add the supernatant to an adsorption column AC, (place the adsorption column into the collection tube) Centrifuge at 13000rpm for 1 minute, and discard the waste liquid in the collection tube.

4.6. Follow step 6 of the Protocol.

Note: It is very important to immediately vortex or blow thoroughly in the above steps. Insufficient mixing seriously reduces production. If necessary, if the sample is viscous and difficult to mix, vortex oscillation can be used for 15 seconds to mix.

5. Bacteria

5.1. Take 0.5-2ml of culture medium (maximum of 2×10^9 cells), centrifuge at 10000rpm for 30 seconds, and discard the supernatant as much as possible. Collect the bacterial cells.

Note: The initial processing capacity can be adjusted based on bacterial density, cell type, and expected yield, but the maximum adsorption capacity of the centrifugal adsorption column is 30µg genomic DNA. If the bacterial excess exceeds the maximum adsorption capacity, it will seriously reduce yield.

5.2. Add 200µl of 1× PBS and resuspend, centrifuge at 10000rpm for 30 seconds, and discard the supernatant. Shake or blow the cells thoroughly and resuspend them in 180µl 1× PBS.

Note: For Gram positive bacteria that are difficult to break walls, skip step 5.2 and add lysozyme for wall breaking treatment. The specific method is to add 180µl buffer (20mM Tris, pH 8.0; 2 mM Na2 EDTA; 1.2% Triton X-100; add final concentration of 20mg/ml lysozyme before use (lysozyme must be dissolved in dry powder in the buffer for preparation, otherwise it will cause lysozyme to be inactive), and treat at 37℃ for more than 30 minutes.

5.3. Add 20µl Proteinase K solution, mix well, then add 200µl Buffer CB, immediately vortex and shake thoroughly, and let it stand at 70°C for 10 minutes.

Optional steps: If there is a lot of RNA residue and RNA needs to be removed, you can add 5µl of RNase A (100mg/ml) solution before adding 200µl of Buffer CB, shake well, and let it stand at room temperature for 5-10 minutes.

5.4. After cooling, add 100µl of isopropanol and immediately vortex to mix thoroughly. At this time, flocculent precipitation may occur.

5.5. Add the previous mixture (including possible precipitates) to an adsorption column AC, centrifuge at 13000rpm for 1 minute (with the adsorption column placed in the collection tube), and discard the waste liquid in the collection tube.

5.6. Follow step 6 of the Protocol.

6. Add 500µl of Buffer IR, centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.

7. Add 600µl of Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.

8. Repeat step 7 once.

9. Put the adsorption column AC back into the empty collection tube, centrifuge at 13000rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.

10. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 100µl of Buffer EB to the middle of the adsorption membrane (Preheating Buffer EB in a water bath at 80-100°C can increase yield). Leave it at room temperature for 3-5 minutes and centrifuge at 13000 rpm for 1 minute.

Note:1) The solution obtained from the first elution can be re added to the centrifuge column, left at room temperature for 2 minutes, and centrifuged at 13000 rpm for 1 minute. It can increase the concentration by about 10%.

2)The larger the elution volume, the higher the elution efficiency. If a higher DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 50µl. If the volume is too small, it will reduce the DNA elution efficiency and DNA production.

11. DNA can be stored at -20°C, and if stored for a long time, it can be stored at -70°C.

Appendix (using 300µl, 1ml whole blood as an example of red blood cell lysis Protocol):

1. Take 900µl of buffer ELY into a 1.5ml centrifuge tube or 3ml of buffer ELY into a 15ml centrifuge tube. (Buffer ELY can be purchased from our company)

2. Invert the anticoagulant whole blood (return to room temperature before use) and mix well. Take 300μl of whole blood and 1ml of whole blood and add them to the 1.5ml and 15ml centrifuge tubes mentioned above, 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 and gently mixed several times to help lyse red blood cells).
4. Centrifuge at 13000 rpm for 20 seconds (for 1.5ml centrifuge tubes) or 2000-3000rpm for 5 minutes (for 15ml centrifuge tubes), 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 a complete white blood cell cluster at the bottom of the tube and approximately 10 liters of residual supernatant.
5. 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. Red blood cell lysis solution should be added to resuspend the cell clusters and repeat steps 3 and 4.
6. Add 200μl of 1× PBS vortex oscillation to resuspend white blood cell clusters and fully disperse them.
7. Due to the difficulty in dispersing and resuspending the white blood cell precipitates of heparin anticoagulant, which affects the subsequent experimental lysis effect, it is recommended to use non heparin anticoagulants to collect blood samples.
8. Now we can extract whole blood genomic DNA according to the Protocol.