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Blood and Tissue DNA Mini Kit,50 preps

Product Number: DNK0701

Shipping and Storage

1. Buffer TL、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. 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	DNK0701 50 Preps	DNK0702 100 Preps
Balance Buffer	RT	5ml	10ml
Buffer TL	RT	11ml	20ml
Buffer CB	RT	11ml	20ml
Buffer IR	RT	25ml	50ml
Buffer WB	RT	13ml	25ml
Buffer EB	RT	15ml	15ml
Proteinase K	4°C	1ml	1ml×2
Adsorption column AC	RT	50	100
Collection tube (2ml)	RT	50	100

Description

The unique Buffer CB/Proteinase K rapidly lyse cells and use silica gel membrane centrifugation columns to specifically adsorb DNA, without the need for toxic reagents such as phenol chloroform or time-consuming alcohol precipitation, maximizing the removal of proteins and other inhibitory impurities. Suitable for efficiently extracting genomic DNA from various materials such as animal tissue cells, mouse tails, insects, 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, nor do steps such as ethanol precipitation.
2. Fast and simple, single sample operation can generally be completed within 30 minutes.
3. Multiple column rinsing ensures high purity, with a typical OD_{260}/OD_{280} ratio of 1.7-1.9 and a length of up to 30kb-50kb. It can be directly used for PCR, Southern blot, and various enzyme digestion reactions.

Application

Suitable for extracting genomic DNA from tissues/cells/mouse tails/insects.

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 liquid pre-treatment column is completed. Follow the subsequent operating steps.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a rotational speed of up to 13000rpm.
2. Self prepared ethanol, isopropanol, 1×PBS (phosphate buffer, optional), and RNase A (optional) are required.
3. Before the experiment, preheat the required water bath to 70 ℃ for later use.

Protocol(Please read the precautions before the experiment)

Note:1)Before the first use, please add the specified amount of anhydrous ethanol to the Buffer WB and mix thoroughly. 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!

2)**Preparation of equilibrium solution pre-treatment adsorption column for backup:** The use of equilibrium solution pre-treatment of silica gel membrane adsorption column is a necessary step, and the specific method can be found in the previous section "About the use of equilibrium solution"

1. Tissue culture cells

1.1. Collect approximately 10^5-10^6 suspended cells into a 1.5ml centrifuge tube; For adherent cells, they should be digested with trypsin before being blown down and collected.

1.2. Centrifuge at 13000rpm for 10 seconds to allow cells to precipitate. Discard the supernatant, leaving behind cell clusters.

1.3. Add 200μl of 1×PBS to resuspend and wash the cells, centrifuge at 13000rpm for 10 seconds, and allow the cells to precipitate. Completely discard the supernatant and resuspend the cell precipitate in 180μl of 1×PBS.

1.4. Add 20μl of Proteinase K, mix thoroughly, and then add 200μl of Buffer CB. **Immediately shake and mix thoroughly**, and let it stand at 70℃ for 10 minutes.

Optional steps: If there is a large amount of RNA residue that needs to be removed, 5μl of RNase A (100mg/ml) solution can be added before adding 200μl of Buffer CB, shaken well, and left at room temperature for 5-10 minutes.

1.5. After cooling, add 100μl of isopropanol and immediately mix thoroughly through vortex oscillation. At this point, flocculent precipitation may occur.

1.6. Add the previous mixture (including possible precipitates) to an adsorption column AC, centrifuge at 13000 rpm for 60 seconds (with the adsorption column placed in the collection tube), and discard the waste liquid from the collection tube.

1.7. **Continue to step 4 of the operation.**

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

2.1. 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 the tissue into a 1.5ml centrifuge tube containing 180μl of tissue buffer TL and mix well with a large caliber gun.

2.2. Join 20μl of Proteinase K, **immediately vortex shake and thoroughly mix.**

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

Optional steps: If there is a large amount of RNA residue that needs to be removed, 5μl of RNase A (100mg/ml) solution can be added before adding 200μl of Buffer CB, shaken well, and left at room temperature for 5-10 minutes.

2.4. Add 200μl Buffer CB, **immediately shake thoroughly with vortex** and let stand at 70 ℃ for 10 minutes.

2.5. After cooling, add 100μl of isopropanol and **immediately mix thoroughly through vortex** oscillation. At this point, flocculent precipitation may occur.

2.6. Suck the mixture with a 1ml gun tip, add the mixture to an adsorption column AC, centrifuge at 13000rpm for 60 seconds (the adsorption column is placed in the collection tube), and pour out the waste liquid in the collection tube.

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

2.7. **Continue to step 4 of the operation.**

3. Animal tissue (rat tail)

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

3.2. Join 20μl of Proteinase K (20mg/ml), **immediately vortex oscillate and thoroughly mix.**

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

Optional steps:If there is a large amount of RNA residue that needs to be removed, 5μl of RNase A (100mg/ml) solution can be added before adding 200μl of Buffer CB, 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.

3.4. Add 200μl Buffer CB and 100μl isopropanol, immediately vortex shake and mix thoroughly.

3.5. **Centrifuge** at 13000rpm **for 5 minutes**, add the supernatant to an adsorption column AC, (the adsorption column is placed in the collection tube) Centrifuge at 13000rpm for 1min, and discard the waste liquid in the collection tube.

3.6. **Continue to step 4 of the operation.**

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

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

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

6. Repeat step 5.

7. Place 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 prevent residual ethanol from inhibiting downstream reactions in Buffer WB.

8. 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 the Buffer EB in a water bath at 80-100℃ can increase production). Leave it at room temperature for 3-5 minutes and centrifuge at 13000rpm 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. Small volume reduces DNA elution efficiency and reduces DNA production.

9. DNA can be stored at -20℃, and if it needs to be stored for a long time, it can be stored at -70℃.