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FFPE Tissue DNA Kit

Product Number: DNK3201

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. 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℃ for at least 6 months, at 4℃ for 12 months, and at -20℃ for 2 years.
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	DNK3201 50 times	DNK3202 100 times	DNK3203 200 times
Balance Buffer	RT	5 ml	10 ml	20 ml
Buffer FTL	RT	11 ml	20 ml	40 ml
Buffer CB	RT	11 ml	20 ml	40 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	15ml×2
Proteinase K	-20℃	1.5ml	3ml	6ml
Adsorption column AC	RT	50	100	200
Collection tube (2ml)	RT	50	100	200

Description

Formalin fixed or paraffin embedded tissues are rapidly lysed by unique Buffer FTL heat treatment and Proteinase K to release genomic DNA. The genomic DNA is then selectively adsorbed onto the silica matrix membrane in a high dissociation salt state in a centrifuge column, followed by a series of rapid rinsing centrifugation steps. Buffer IR and Buffer WB remove impurities such as cell metabolites and proteins, Finally, low salt Buffer EB elutes pure genomic DNA from the silica matrix membrane.

Features

1. The silicon matrix membrane inside the centrifugal adsorption column is entirely made of specially designed adsorption membranes from world-renowned imported companies, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawback of unstable membrane quality in domestic reagent kits.
2. No toxic reagents such as phenol are required, and no steps such as ethanol precipitation are required.
3. Fast and simple, the operation of a single sample can generally be completed within 30 minutes.
4. Multiple column washes ensure high purity, with a typical OD260/OD280 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 rapid extraction of DNA from various formalin fixed and paraffin embedded tissues

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 steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm.
2. You need to prepare ethanol (with different concentrations of 100%/80%/60%/40%) or xylene.
3. Preheat the required water bath to 37℃ before the experiment for later use.
4. Buffer CB and Buffer IR contain irritating compounds, and latex gloves should be worn during operation to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, rinse with plenty of water or physiological saline.
5. Buffer EB does not contain chelating agent EDTA and does not affect downstream enzyme cleavage, linking, and other reactions. Water can also be used for elution, but it should be ensured that pH is greater than 7.5 and pH is lower than 5, which affects elution efficiency. Wash DNA with water and store it at -20℃. If DNA needs to be stored for a long time, it can be eluted with TE buffer (10mM Tris HCl, 1mM EDTA, pH8.0). However, EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.

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!

1. Put the tissue slices into a centrifuge tube and soak them in xylene for about 30 minutes for dewaxing (the specific time is adjusted according to the thickness of the slices).
2. Put the slices into 100% ethanol/80% ethanol/60% ethanol/40% ethanol/deionized water in sequence, soak in each liquid for 10 seconds, and rehydrate the slices.

   When just adding 100% ethanol, you should see the slices turn white.
3. Under a microscope, use a blade to cut off the target tissue for DNA extraction and place it in a pre weighed 1.5ml centrifuge tube. Weigh again and calculate the weight of the sliced tissue.
4. Add 200μl of Buffer FTL to 25-50mg of tissue, and then add 20μl of Proteinase K solution (20mg/ml). Immediately mix well and leave in a 37℃ water bath overnight.
5. Add another 10μl of Proteinase K solution (20mg/ml), mix well, and then take a water bath at 55℃ for 1-2 hours.
   After this step, coarse tissue particles should not be seen anymore.
6. Add 200μl Buffer CB, immediately vortex and shake for 20 seconds, mix thoroughly, and then let it sit in a 70℃ water bath for 10 minutes.

   Balance Buffer pre-treatment adsorption column backup: The use of Balance Buffer pre-treatment of silicone membrane adsorption column is a necessary step, please refer to the previous section "Use of Balance Buffer" for specific methods
7. After cooling, add 100μl of isopropanol and immediately vortex for 30 seconds to mix thoroughly. At this time, flocculent precipitation may occur.
8. 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 discard the waste liquid in the collection tube.

   If there is insoluble tissue that may block the gun head, the gun head can be gently rubbed on absorbent paper to remove

the insoluble material; If there is a small amount of mixture sucked up, the gun tip and insoluble substances can be discarded together. This method is to remove insoluble substances to avoid clogging the centrifuge column.

9. Add 500μl Buffer IR, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
10. Add 600μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
11. Add 600μl Buffer WB, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
12. 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.
13. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 100μl Buffer EB to the middle of the adsorption membrane (Buffer EB is better preheated in a 65-70℃ water bath), leave at room temperature for 3-5 minutes, and centrifuge at 12000 rpm for 1 minute. Add the obtained solution back into the centrifugal adsorption column, let it stand at room temperature for 2 minutes, and centrifuge at 12000 rpm for 1 minute.

    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.
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℃.

Appendix: Another Dewaxing Method

1. Place the target tissue slice into a centrifuge tube, add 1ml of 100% xylene, and vortex for 10 seconds. Instantly centrifuge and immerse the entire tissue in xylene.
2. Melt paraffin in a 50℃ water bath for 3 minutes, centrifuge at the highest speed at 20-25℃ for 2 minutes, and collect tissue to the bottom of the tube.
3. Be careful to use a pipette to remove the supernatant xylene and be careful not to aspirate the sediment.
4. Add 1ml of anhydrous ethanol, vortex oscillate, centrifuge at maximum speed for 2 minutes, and carefully discard the supernatant ethanol.
5. Add 1ml of anhydrous ethanol, repeat step 4, and absorb all ethanol as much as possible.
6. Dry ethanol at room temperature or 37℃ for 10 minutes or until all ethanol evaporates.

Problems and Solutions

Problem	Possible cause	Suggestion
Low DNA production	The tissue block is too large, and Proteinase K digestion is incomplete	Grind the tissue with liquid nitrogen or cut it into small pieces as much as possible, or extend the Proteinase K digestion time overnight or add an additional 20μl of Proteinase K digestion for 1-2 hours on top of the original digestion.
	Proteinase K is invalid	After receiving Proteinase K, pack and freeze according to the amount used each time to avoid repeated freeze-thaw cycles.
	Incomplete cracking or insufficient mixing with isopropanol	After adding Buffer CB and Proteinase K, immediately blow or vortex mix well; After adding isopropanol, immediately blow or vortex mix before adding to the adsorption column. If it is too viscous, vortex shake for 15 seconds to fully mix.
The tissue DNA has degraded	Nuclease activity in the organization leads to degradation	Store the sample properly at -20℃ before processing, and do not handle too much.
No DNA extracted	Forgot to add anhydrous ethanol in	In the first experiment, add a specified amount of

	Buffer WB	anhydrous ethanol to the Buffer WB.
Low yield of washed DNA	There is a significant amount of ethanol remaining in the centrifuge column or the bottom is accidentally stained with ethanol	Make sure to follow step 12, otherwise residual ethanol will affect the elution efficiency.
	Using water or other non optimal liquids instead of Buffer EB	Carefully read the precautions 5 and step 13, and only use Buffer EB for elution.
A260 absorption value abnormally high	Some silicon-based membrane components are washed off together, interfering with the absorbance value	Centrifuge the washed genomic DNA solution at 13000rpm for another minute, and carefully remove the supernatant for use.
DNA downstream enzyme cleavage cannot be cleaved or incomplete	Some silicon-based membrane components were eluted together, inhibiting the enzyme cleavage reaction	Centrifuge the washed genomic DNA solution at 13000rpm for another minute, and carefully remove the supernatant for use.
	The residual ethanol in the centrifuge column or the accidental presence of ethanol at the bottom inhibited the enzyme digestion reaction	Make sure to follow step 7, then let it air dry for a few minutes to allow the residual ethanol to evaporate.