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

Stool DNA Mini Kit

Product Number: DNK2301

Shipping and Storage

1. Buffer ASL or Buffer CB may precipitate and precipitate at low temperatures. It can be fully dissolved again by taking a water bath at 70℃ for a few minutes, restoring 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. 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	DNK2301 50 Preps
Balance Buffer	RT	5ml
Buffer ASL	RT	70 ml
Buffer AB	RT	5 ml
Buffer CB	RT	11 ml
Buffer IR	RT	25 ml
Buffer WB	RT	13 ml
Buffer EB	RT	15 ml
Proteinase K	4°C	1 ml
Adsorption column AC	RT	50
Collection tube (2ml)	RT	50

Buffer AB upgrade, can be stored at room temperature for more convenient use.

Description

The conventional DNA purification method cannot effectively remove a large number of inhibitory factors present in feces, leading to the failure of downstream experiments, such as PCR not being able to amplify the required fragments. This reagent kit adopts a DNA adsorption column and a new unique solution system, which can effectively remove various inhibitory factors that affect downstream experiments (such as PCR) in animal feces, and efficiently recover genomic DNA from feces. Animal fecal samples were resuspended with special Buffer ASL and treated at 70 ℃ for 5 minutes to lyse bacteria; Centrifuge to remove insoluble impurities, and Protein K digestion to further remove proteins and impurities; Then, the genomic DNA is selectively adsorbed onto the silica matrix membrane in a highly dissociated salt state. Through a series of rapid rinsing centrifugation steps, Buffer IR and Buffer WB remove impurities such as cellular metabolites and proteins. Finally, the pure genomic DNA is eluted from the silica matrix membrane by low salt Buffer EB.

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 40 minutes.
4. Multiple column washes ensure high purity, with a typical OD260/OD280 ratio of 1.7~1.9, which can be directly used for PCR, Southern blot, and various enzyme digestion reactions.

Application

Suitable for rapid extraction of various fecal 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 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 70℃ for later use before starting the experiment.
3. 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.
4. 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 the pH is greater than 7.5, as low pH can affect 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, pH 8.0). However, EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.
5. PCR may be inhibited by excessive DNA (generally greater than 1μg), and using the minimum amount of washed DNA (diluted appropriately) can actually result in better amplification. Generally, the volume of washed DNA added should not exceed 10% of the total PCR reaction volume. We suggest adding BSA (bovine serum albumin) with a final concentration of 0.1μg/μl to the PCR reaction system to achieve the best amplification effect.

Protocol(Please read the precautions before the experiment)

Tip: **Before the first use, please add 60ml anhydrous ethanol to 15ml 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 multiple additions!

1. Collect approximately 200-220mg of feces into a 2ml centrifuge tube and place on ice.
   If it is a frozen specimen, it should not be thawed before adding Buffer ASL, otherwise DNA is prone to degradation.
2. Add 1.4ml of Buffer ASL and vortex continuously for 1 minute or until completely mixed and uniform.
   Pay attention to complete vortex mixing, otherwise it will seriously reduce production.
3. Incubate the resuspended substance at 70℃ for 5 minutes.
   This heating step can increase DNA production by 3-5 times and help lyse bacteria and parasites. For certain difficult to lyse cells (such as Gram positive bacteria), it can be raised to 95℃.
4. Vortex oscillation for 15 seconds, leave at room temperature for 1 minute. Centrifuge at maximum speed for 1 minute to precipitate fecal particles.

5. Transfer 900μl of supernatant to a 1.5ml centrifuge tube, add 100μl of Buffer AB, immediately vortex and shake for 1 minute or until completely mixed and uniform, and leave at room temperature for 1 minute. Centrifuge at maximum speed for 3 minutes to remove impurities.
6. Transfer all supernatants to a 1.5ml centrifuge tube and centrifuge at maximum speed for 3 minutes.
7. Transfer 210μl of supernatant to a 1.5ml centrifuge tube, add 20μl Protein K solution, mix well, add 200μl Buffer CB, vortex for 15 seconds, and mix well. Incubate at 70℃ for 10 minutes.

   If the yield is low, more supernatant can be transferred and the corresponding proportion of protease K and buffer CB, as well as the subsequent use of isopropanol, can be increased.

   Balance Buffer pretreatment adsorption column backup: Using Balance Buffer pretreatment silica gel membrane adsorption column is a necessary step. For specific methods, please refer to the previous section "Use of balance buffer"
8. After cooling, add 100μl of isopropanol and vortex mix well.
9. Add the solution obtained from the previous step and any possible precipitates into an adsorption column AC, and centrifuge at 13000rpm for 30 seconds (with the adsorption column placed in the collection tube). Discard the waste liquid in the collection tube.
10. Add 500μl Buffer IR, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
11. Add 600μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
12. Add 600μl Buffer WB, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
13. 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.
14. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 100-150μl Buffer EB to the middle of the adsorption membrane (Buffer EB can be preheated in a 65-70℃ water bath in advance), leave at room temperature for 2 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.
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℃.

Problems and Solutions

Problem	Possible cause	Suggestion
Buffer EB has no DNA or low yield	Improper sample storage	The sample should be stored at 4℃ or -20℃
	Insufficient vortex homogenization in Buffer ASL	The sample undergoes sufficient vortices in ASL until uniform
	Insufficient mixing with Buffer CB	The supernatant and Buffer CB immediately interrupted the vortex mixing thoroughly
	Forgot to add isopropanol before installing the centrifuge column	Remember to add isopropanol
	Insufficient DNA elution	Leave at room temperature for 5 minutes before elution
	Forgot to add anhydrous ethanol to Buffer WB before first use	Add the specified amount of anhydrous ethanol to Buffer WB.
A260/A280 ratio abnormally high	Excessive residual RNA	Buffer EB with RNase A, room temperature for 10-30 minutes.
Abnormal downstream reaction	BSA was not added to the PCR reaction system	Add BSA with a final concentration of 0.1μg/μl to the PCR reaction system.

of DNA	Excessive use of DNA in downstream reactions	If too much DNA is used, it may inhibit the PCR reaction, thus reducing the amount of washed DNA used
	Non specific amplification bands	The amount of target DNA in Buffer EB is too low and the background DNA is too high, so hot start PCR polymerase can be considered
	Difficulty in cell lysis for certain purposes, insufficient results in low yield	The temperature incubation time of the cracking solution can be increased to 95℃
	Not enough DNA washed off	Check the possible reasons above
After the initial centrifugation step 4, there was not much supernatant observed	Insufficient centrifugal force	You can try centrifuging at 14000rpm for 5 minutes