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 PE	RT	16 ml
Buffer WB	RT	Add anhydrous ethanol according to the instructions on the bottle label before the first use 13 ml Add anhydrous ethanol according to the instructions on the bottle label before the first use
Buffer EB	RT	15 ml
Proteinase K	4°C	1 ml
Adsorption column AC	RT	50
Collection tube (2ml)	RT	50

This reagent kit can be stored at room temperature for 12 months without affecting its effectiveness.

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. No toxic reagents such as phenol are required, and no steps such as ethanol precipitation are required.
2. Fast and simple, the operation of a single sample can generally be completed within 40 minutes.
3. 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.

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 bring your own isopropanol (for the steps of adding isopropanol, it is recommended to use isopropanol first, and ethanol can also be used as a substitute without isopropanol). RNase A (optional).
3. Preheat the required water bath to 70℃ for later use before starting the experiment.

Protocol(Please read the precautions before the experiment)

Tip: **Before the first use, please add 60ml anhydrous ethanol to 15ml Buffer WB and Buffer PE** 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 13000 rpm 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 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.
7. Column equilibrium: Add 100μl of equilibrium solution to the adsorption column, centrifuge at 13000 rpm for 1 minute, discard the filtrate, and set aside for later use.
   The equilibrium solution can enhance the adsorption capacity of silica gel membrane for nucleic acids. Please use the adsorption column treated on the same day.
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 PE (please check if anhydrous ethanol has been added first!), centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
11. Add 600μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 13000rpm for 30 seconds, and discard the waste liquid.
12. Add 600μl Buffer WB, centrifuge at 13000rpm 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 30μl. If the volume is too small, it will reduce the DNA elution efficiency and DNA production.

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