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Soil DNA Fast Kit

Product Number: DNK2701

Shipping and Storage

1. Buffer LYS 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. After restoring clarity and transparency, it can be used by cooling to room temperature. Be careful not to shake vigorously to avoid the generation of a large number of bubbles.
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	DNK2701 50 Preps
Buffer SUS	RT	25 ml
Buffer LYS	RT	6 ml
Buffer S1	RT	15 ml
Buffer S2	RT	15 ml
Buffer S3	RT	30 ml
Buffer IR	RT	25 ml
Buffer WB	RT	13 ml
Buffer EB	RT	10 ml
Proteinase K	4℃	1 ml
Adsorption column AC and collection tube	RT	50

Description

The soil genomic DNA extracted by ordinary handheld or reagent kits often fails due to the presence of strong inhibitors of PCR, such as humic acid and palmitic acid impurities. In addition, the use of severe glass bead impacts to rupture bacterial cells often leads to DNA cleavage and degradation. Our company has developed soil genomic DNA with independent intellectual property rights through long-term research and development. By using patented humic acid and palmitic acid removal reagents combined with specially treated purification columns, these impurities can be maximally removed. At the same time, multiple column washes are added to ensure that the obtained DNA has extremely high purity. In addition, the unique extraction and lysis system can quickly lyse cells (walls) and inactivate intracellular nucleases, It does not require the use of glass beads to break walls, effectively ensuring the integrity of genomic DNA.

Features

1. Our company's unique patented formula and purification column can effectively remove impurities such as humic acid.
2. Without the need for glass bead breaking, the integrity of genomic DNA is effectively ensured, with a length of up to 30kb-50kb. It can be directly used for PCR, Southern blot, and various enzyme digestion reactions.
3. Strong compatibility, suitable for various types of soils, including difficult to extract soils such as silt.
4. Multi step removal of various impurities and inhibitors ensures extremely high purity, with a typical OD260/OD280 ratio of 1.7-1.9.
5. No toxic reagents such as phenol are required, and no steps such as ethanol precipitation are required.
6. Fast and simple, the operation of a single sample can generally be completed within 60 minutes.

Application

Suitable for rapid extraction of various soil genomic DNA

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 water bath to 37 ℃ or 70 ℃ as needed before starting the experiment.
3. Buffer S3 and Buffer IR contain irritating compounds. When operating, wear latex gloves 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.

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 Buffer S3, mix well, and mark the added ethanol in the box in a timely manner to avoid multiple additions!

1. Take 0.2 grams of soil and place it in a centrifuge tube. Crush it with a toothpick or gun tip and add 0.5ml of Buffer SUS. Stir with the gun tip and briefly vortex to help resuspend.

   If it is expected that the soil contains a large number of difficult to lyse bacteria, such as Gram positive bacteria, 10mg of lysozyme can be added to 0.5ml of Buffer SUS first, beaten and mixed well, and then added again, followed by step 2.

   1.1. **Optional steps:** Incubate at 37 ℃ for 30 minutes, invert and mix several times every 10 minutes.

   For bacteria that are difficult to lyse, such as soil with abundant Gram positive bacteria, and samples with lysozyme added in the previous step, this step needs to be added to help with the lysis.
2. Add 20ul of protease K and briefly vortex to help mix well.

   2.1. **Optional steps:** To increase production, oscillate at 37 ℃ for 10 minutes or vortex for 2 minutes. (Note that vortex oscillations may shear DNA)
3. Add 120ul Buffer LYS, briefly vortex and mix well, incubate at 65 ℃ for 30 minutes, invert and mix several times during the process.

   The 65 ℃ incubation time can be extended or shortened according to specific sample types and yields to achieve optimal yield and purity, and can be adjusted within the range of 10 minutes to 2 hours.

   3.1. **Optional steps:** Freeze at -70 ℃, melt at 65 ℃, repeat 3 times.

   For difficult to lyse fungi such as soil with abundant Gram positive bacteria content, this step can be added to assist in lysis.
4. After inverting and mixing, centrifuge at 13000 rpm for 2 minutes, carefully transfer the supernatant to a new centrifuge tube (record the volume of supernatant).
5. Add 1/3 (one-third) volume of Buffer S1, invert several times, vortex for 5 seconds, mix well, and leave on ice for 5 minutes.
6. Centrifuge at 13000 rpm for 5 minutes, carefully transfer the supernatant to a new centrifuge tube (record the volume of supernatant).
7. Add 1/3 (one-third) volume of Buffer S2, invert a few times, briefly vortex and mix well, then leave on ice for 5 minutes.

   This step is mainly to further remove PCR inhibitors such as humic substance to improve purity, but it will reduce some yield. If high yield is required or the extracted DNA is not used for PCR, this step can be omitted to increase yield. If it is expected that the soil composition is complex and there are many PCR inhibitory substances, increasing the amount of Buffer S2 (such as adding an equal volume of Buffer S2) can improve purity, but attention should also be paid to significantly reduce yield.

8. Centrifuge at 13000 rpm for 5 minutes, carefully transfer the supernatant to a new centrifuge tube (record the volume of supernatant).
9. Add 1.5 times the volume of Buffer S3 (please check if anhydrous ethanol has been added first!), invert a few times, and briefly vortex and mix well.
10. Add 700ul of the previous mixture (including possible precipitates) to an adsorption column AC, place the column in a collection tube, centrifuge at 12000rpm for 30-60 seconds, and discard the waste liquid in the collection tube. Repeat until all the mixture is added.
11. Add 500ul Buffer IR, centrifuge at 12000 rpm for 30 seconds, and discard the waste liquid.
12. Add 600ul Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000 rpm for 30 seconds, and discard the waste liquid.
13. Add 600ul Buffer WB, centrifuge at 12000 rpm for 30 seconds, and discard the waste liquid.
14. Put the adsorption column AC back into the empty collection tube, centrifuge at 13000 rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.
15. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 100ul Buffer EB to the middle of the adsorption membrane (pre heating the elution Buffer in a 65-70 ℃ water bath is more effective). Leave it 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 50ul. If the volume is too small, it will reduce the DNA elution efficiency and DNA production.
16. DNA can be stored at 2-8 ℃, and if it needs to be stored for a long time, it can be placed at -20 ℃.